Review

# Hypoxia signaling and resistance in *C. elegans*

**Jo Anne Powell-Coffman**

Genetics, Development, and Cell Biology Department, Iowa State University, Ames, IA 50011, USA

**In normal development and homeostasis and in many disease states, cells and tissues must overcome the challenge of oxygen deprivation (hypoxia). The nematode *C. elegans* is emerging as an increasingly powerful system in which to understand how animals adapt to moderate hypoxia and survive extreme hypoxic insults. This review provides an overview of *C. elegans* responses to hypoxia, ranging from adaptation and arrest to death, and highlights some of the recent studies that have provided important insights into hypoxia signaling and resistance. Many of the key genes and pathways are evolutionarily conserved, and *C. elegans* hypoxia research promises to inform our understanding of oxygen-sensitive signaling and survival in mammalian development and disease.**

## Overview

During normal development and homeostasis, cells and tissues are often challenged by oxygen levels that are too low to provide for normal physiological functions, and this condition is termed hypoxia. The nematode *Caenorhabiditis elegans* is emerging as an increasingly powerful genetic model organism for understanding how animals adapt to and survive oxygen deprivation. In standard laboratory culture, microscopic *C. elegans* are grown on agar plates with bacterial food, and they live approximately three weeks at 20°C. The advantages of this system include short generation times, detailed descriptions of cell fates in wild-type and mutant animals, the relative ease of forward genetics and RNAi to discover genes that govern hypoxic responses and resistance, and a wealth of studies on stress and longevity upon which to build.

The study of hypoxia response in *C. elegans* has provided insights into the various strategies that organisms can employ to respond to fluctuations in environmental oxygen and to escape hypoxic death. One of the emerging themes is that *C. elegans* respond in qualitatively different ways to oxygen deprivation. The animals can adapt to moderate hypoxia, and this requires gene expression changes controlled by the hypoxia-inducible factor (HIF-1) transcription factor. *C. elegans* can survive the complete lack of oxygen (anoxia) for about a day in normal culture conditions, by entering a state of reversible arrest that has been termed 'suspended animation'. Recovery from anoxia-induced arrest does not require *hif-1*, but cell cycle checkpoints are crucial for embryonic survival in anoxia. Long-term anoxia or severe hypoxia at high temperatures is ultimately lethal (Table 1), and recent studies have identified genes and pathways that increase resistance to severe hypoxic stress.

This review provides an overview of the field and highlights some of the major discoveries that have been published in recent years. These studies have provided important insights into the pathways and processes that control hypoxia signaling and response in *C. elegans*. All aerobic organisms require oxygen to generate the ATP used to power cellular reactions, and many of the mechanisms that enable *C. elegans* to adapt to moderate hypoxia or to survive severe anoxic stress are evolutionarily conserved. This genetic model system is therefore providing new inroads to understand hypoxia response in other ecological or medical contexts.

## Moderate hypoxia: a central role for the hypoxia-inducible factor

Successful adaptation of *C. elegans* to life in 0.5% or 1% oxygen requires the hypoxia-inducible factor (HIF) [1–3]. The HIF DNA-binding complex is heterodimeric, and the alpha and beta subunits are both proteins with basic-helix-loop-helix and PAS (PER/ARNT/SIM) motifs. The stability of the HIFα subunit depends on oxygen levels. When oxygen levels are sufficiently high, proteins of the PHD/EGL-9 family of deoxygenases hydroxylate specific proline residues in HIFα. Once hydroxylated, HIFα binds an E3 ubiquitin ligase termed the von Hippel Lindau (VHL) tumor suppressor and is targeted for proteasomal degradation [4,5]. In hypoxic conditions, HIFα is stable. The homologs of human HIFα, HIFβ, PHD, and VHL are encoded by the *C. elegans hif-1*, *aha-1*, *egl-9*, and *vhl-1* genes, respectively [1,4,6], and *C. elegans hif-1* controls most hypoxia-induced changes in gene expression [7] (Figure 1).

EGL-9 and HIF-1 are well-positioned to integrate information about cellular metabolism and stress. 2-oxoglutarate and oxygen are co-substrates for the enzymatic reaction in which EGL-9 hydroxylates HIF-1 [4]. Thus, metabolites from the mitochondrial citric acid cycle might impinge upon HIF-1 stability. Reactive oxygen species (ROS) also influence the hydroxylation of HIF alpha subunits [8–11].

Several recent papers have described new roles for *C. elegans* HIF-1 in stress responses and aging [12–16]. Compared to wild-type animals, *egl-9*-deficient mutants are more resistant to heat, hydrogen cyanide, hydrogen sulfide, and certain pathogens [12,15,17–20]. In all cases so far studied, deletion of *hif-1* suppresses these *egl-9* loss-of-function phenotypes [12,15,20]. Moderate overexpression of HIF-1 causes dose-dependent increases in adult lifespan

*Corresponding author:* Powell-Coffman, J.A. (japc@iastate.edu).

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**Table 1. Different responses to hypoxic stress**

| Hypoxic challenge | Example | Major pathway shown to allow adaptive response or resistance |
|---|---|---|
| Sublethal hypoxia | 0.5% oxygen at 20–22°C | HIF-1 hypoxia-inducible factor |
| Anoxia-induced arrest | No oxygen, 20–22°C | SAN-1 cell-cycle checkpoint |
| Lethal hypoxic stress | Anoxia or severe hypoxia at high temperature (<0.3% $O_2$, 26–28°C) | DAF-2 insulin-like receptor pathway/DAF-16 forkhead transcription factor |
| | Long-term anoxia (48–72 hr) | |

and protects animals from amyloid or polyglutamine proteotoxicity [13,16]. Interestingly, in conditions that provide abundant oxygen and nutrients, *hif-1* mutants live longer than wild-type animals [14–16], but *hif-1* RNAi does not slow aging in conditions that activate the dietary restriction pathway [14]. An important goal for future studies will be to decipher the regulatory circuitry that integrates HIF-1 signaling with the other stress response pathways (Box 1).

How does HIF-1 activation result in such a broad range of phenotypes? Genome-wide microarray studies identified genes that were induced after 4 hours of moderate hypoxia, and most of these gene expression changes required *hif-1* function. Many of the genes activated by HIF-1 have predicted roles in signal transduction or in the regulation of gene expression, suggesting that HIF-1 initiates cascades of events that might act collectively to increase stress resistance and promote longevity [7]. Interestingly, some of the genes downstream of HIF-1 contribute to the formation of stress-resistant dauers, and *hif-1*-deficient animals exhibit an increased frequency of dauer formation at high temperatures [7]. The *C. elegans* homolog of the TWIST basic-helix-loop-helix transcription factor, *hlh-8*, was identified in a screen for RNAi treatments that repressed the egg-laying defects in *egl-9* loss-of-function mutants. Further, mammalian HIF-2 was shown to activate the expression of TWIST1 in HeLa cells, suggesting that this genetic interaction is ancient [21]. Future studies will further define and characterize the genes downstream of *C. elegans* HIF-1 that govern stress resistance, metabolic adaptation, and development.

In the hundreds of millions of years since nematode evolution diverged from that of mammals, some of the regulatory interactions that attenuate HIF-1 have been conserved, suggesting that animals in the wild benefit from tight regulation of HIF-1 function. Induction of HIF-1 initiates a negative feedback loop in which *egl-9* mRNA is upregulated [4,7,22]. Similarly, mammalian PHD2 is induced by mammalian HIF [22,23]. Recent studies showed that *egl-9* acts through at least two pathways to inhibit HIF-1: the VHL-1-mediated pathway for oxygen-dependent degradation of HIF-1 protein and a second pathway that has little or no requirement for EGL-9 hydroxylase activity [22,24,25]. In a second negative feedback loop, the *C. elegans rhy-1* gene is activated by HIF-1.

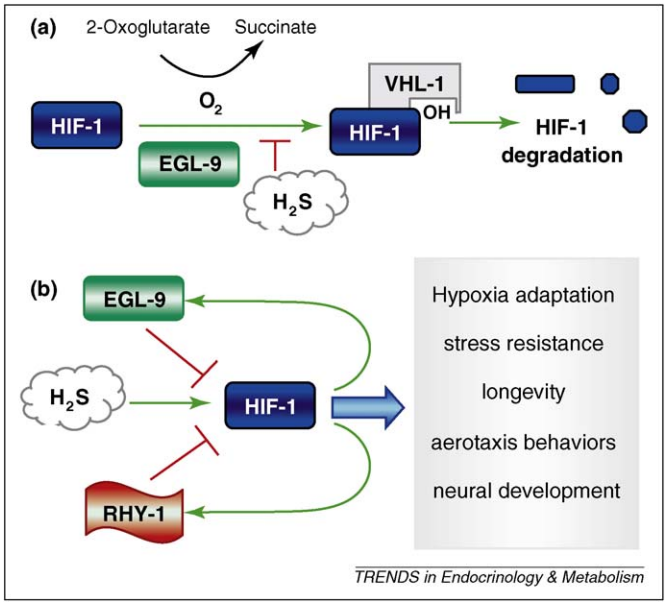


**Figure 1**. Regulation and function of *C. elegans* HIF-1. **(a)** Oxygen-dependent degradation of HIF-1 is an evolutionarily conserved process. The EGL-9 enzyme hydroxylates a proline residue in HIF-1, and this reaction requires oxygen, $Fe^{2+}$, and 2-oxoglutarate. Once hydroxylated, HIF-1 binds the VHL-1 protein and is targeted for proteasomal degradation. In hypoxia, the hydroxylation reaction is inefficient, and the HIF-1 is stable [4,5]. $H_2S$ has been shown to increase HIF-1 protein levels in a *vhl-1*-dependent manner [12]. **(b)** HIF-1-mediated transcription is also regulated by VHL-1-independent pathways. EGL-9 inhibits HIF-1 activity, and this pathway is not completely dependent upon EGL-9 hydroxylase activity [25]. The RHY-1 transmembrane protein is predicted to acetylate molecules that are not proteins, and RHY-1 inhibits HIF-1 activity. The *egl-9* and *rhy-1* mRNAs are induced by HIF-1, and this establishes negative feedback loops that attenuate HIF-1 function [24]. $H_2S$ also promotes expression of HIF-1 target genes [12]. The regulatory relationships between EGL-9, RHY-1, and $H_2S$ are not yet fully understood.

## Box 1. Outstanding Questions

***HIF-1 regulation and function***

- In addition to serving as the oxygen sensor that controls HIF-1 protein levels, the EGL-9 prolyl hydroxylase modulates HIF-1-dependent gene expression via other pathway(s). What are the *vhl-1*-independent functions of EGL-9, and are they evolutionarily conserved? Do EGL-9, RHY-1 and $H_2S$ act via common pathway(s) to control HIF-1-mediated transcription?
- How does crosstalk between HIF-1 and other stress response pathways influence hypoxia signaling and response?

***Neural circuits and integrative responses to changing levels of environmental oxygen***

- How do cells and systems integrate inputs from multiple sensors of oxygen, including HIF-1, the GCY-35 oxygen-sensitive guanylate cyclase and neural globins, to control adaptive responses?
- How does HIF-1 expression in maternal neurons modulate the hypoxia-induced arrest of embryos *in utero*?

***Surviving severe hypoxia***

- Males and sterile hermaphrodites are relatively resistant to severe hypoxic stress. How similar or different are the genetic networks that confer hypoxia resistance to males versus those in reproductive hermaphrodites?
- Genetic screens have identified several mutations or treatments that enable *C. elegans* to survive severe hypoxic stress. Which pathways or processes will prove to be most crucial for surviving oxygen deprivation, and can they function in combination to heighten resistance to hypoxic insults? How will these translate to biomedical advances that limit tissue damage and increase patient survival?

*rhy-1* encodes a transmembrane acyl transferase that inhibits HIF-1-mediated transcription [24] (Figure 1). The *vhl-1*-independent mechanism(s) by which EGL-9 and RHY-1 repress HIF-1 activity are not yet understood, and further studies are needed to determine whether these regulatory strategies are evolutionarily conserved (Box 1).

## HIF-1, neural development, and behavior

Wild-type *C. elegans* avoid both hypoxia and hyperoxia, showing a preference for oxygen levels in the range of 5 – 12% [26]. This behavior is influenced by food and by carbon dioxide levels and is regulated by GCY-35, a soluble guanylate cyclase that binds oxygen [26–28]. Overexpression of HIF-1 targets, caused by hypoxia or *egl-9* loss-of-function mutations, changes the serotonin-dependent neural circuits that modulate these behaviors [29]. Neural globin genes also influence hyperoxia avoidance [30,31]. Moderate hypoxia increases the frequency of some axonal pathfinding aberrations, and this appears to be due in part to HIF-1-mediated increases in VAB-1 receptor expression [32]. Building upon these early discoveries, *C. elegans* promises to be an exceptionally strong system for investigating the regulatory networks and neural circuits that control oxygen-sensitive development and behavior.

The field now has the tools to investigate which HIF-1-mediated responses require cell–cell signaling. Because *C. elegans* are small and do not have complex circulatory systems, one could imagine that most responses to hypoxia might be cell autonomous. Recent studies have approached this issue by expressing *hif-1* or *egl-9* from cell-type-specific promoters in animals that otherwise lack the gene function. For example, HIF-1 appears to act cell autonomously in the intestinal cells to protect against pore-forming toxins [15]. Certain neural aberrations in *hif-1* mutants can be partially restored by expressing *hif-1* in either muscle cells or in neurons, demonstrating that HIF-1 targets have both cell-autonomous and non-cell-autonomous roles in neural pathfinding and signaling [32]. Normal hyperoxia avoidance behaviors require expression of *egl-9* in both neurons and uterine-vulval (uv1) cells [29]. The role(s) of uv1 in oxygen sensing or signaling is not yet understood, but they might produce paracrine or endocrine signals that modulate behavioral responses to oxygen levels.

## Anoxia-induced suspended animation

If *C. elegans* are placed in anoxia at 20°C, they enter a state of reversible arrest. The ratio of ATP/ADP drops several-fold, and embryos cease cell cycle progression. Wild-type larvae and adults stop moving, eating, or laying eggs. Remarkably, *C. elegans* can recover from 24 hours of anoxia-induced arrest and continue development [2,33]. This process does not require *hif-1,* but the anoxic arrest and subsequent recovery of embryos is integrally linked to cell cycle control. The spindle checkpoint components *sans-1, mdf-2,* and *bub-3* are required for anoxia-induced metaphase arrest [2,34–36]. The nucleoporin gene *nnp-16* and the *cdk-1* cyclin-dependent kinase are required for late prophase arrest of embryos in anoxic conditions [37]. Thus, the process of anoxia-induced suspended animation has different genetic requirements than *hif-1*-dependent adaptation to moderate hypoxia.

For *C. elegans* embryos no longer *in utero*, 0.1% oxygen is more lethal than anoxia. This oxygen level appears to be too high to induce suspended animation, but too low to sustain cellular functions [3]. In these conditions, *C. elegans* self-fertile hermaphrodites arrest oocyte production and induce an apparent suspended animation response in animals held *in utero.* Although this response does not require *hif-1*, neuronal HIF-1 activity influences the oxygen tension at which hermaphrodites induce diapause in their embryos [38]. This ability to arrest embryonic development *in utero* might increase the fitness of *C. elegans* that venture into near-anoxic soil microenvironments in the wild.

*C. elegans* has also provided some insights into how hydrogen sulfide ($H_2S$) protects animals from hypoxic stress. The combination of $H_2S$ and low temperatures can induce a hypometabolic state in mice, a species that does not normally hibernate. Moreover, mice can recover from this suspended animation-like state [39,40]. Endogenously produced $H_2S$ is hypothesized to have multiple physiological roles. The known targets of $H_2S$ include cytochrome c oxidase and carbonic anhydrase, and $H_2S$ might have wide-ranging effects on cellular redox reactions [41]. In *C. elegans*, low levels of $H_2S$ increase heat tolerance and longevity [42]. *hif-1*-deficient animals cannot survive even very low levels of $H_2S$, whereas *egl-9* mutants are highly resistant to $H_2S$. Interestingly, $H_2S$ appears to regulate HIF-1 mediated transcription via multiple mechanisms and increases HIF-1 protein levels. In addition, when oxygen-dependent degradation of HIF-1 is disabled by a *vhl-1* mutation, $H_2S$ treatment increases and expands the expression of HIF-1 targets. This suggests that $H_2S$ modulates both HIF-1 protein levels and HIF-1 transcriptional activity (Figure 1) [12].

## Lethal hypoxic stress

Ultimately, severe oxygen deprivation is lethal. The ability of larval and adult *C. elegans* to survive and recover from anoxia-induced suspended animation drops off after 24 hours, and high temperatures expedite hypoxic death [2,43,44]. The mechanisms by which heat acclimation or heat stress modulate hypoxic death are not fully understood, but recent studies have shown that metabolic energy requirements and protein homeostasis have important roles in survival of and recovery from oxygen deprivation.

Certain loss-of-function mutations in the *daf-2* insulin-like-receptor protect *C. elegans* from severe hypoxic stress [43]. Nematodes that have been incubated at high temperatures in severe hypoxia or anoxia for 20+ hours suffer from pathologies in multiple tissues, including muscle, the nervous system, and the pharynx. Animals that are homozygous for the *daf-2 (e1370)* mutation are protected from these hypoxic injuries and remain motile for several hours in anoxia [43,44]. Loss-of-function mutations in *daf-2* result in nuclear localization of the DAF-16 forkhead transcription factor and confer resistance to a range of other stresses [45]. The *gpd-2/3* glyceraldehyde-3-phosphate dehydrogenases are targets of DAF-16 activation and are required for *daf-2*-mediated hypoxic resistance [44]. There are interesting differences in the *daf-2* mutations that confer hypoxic resistance phenotypes and those that

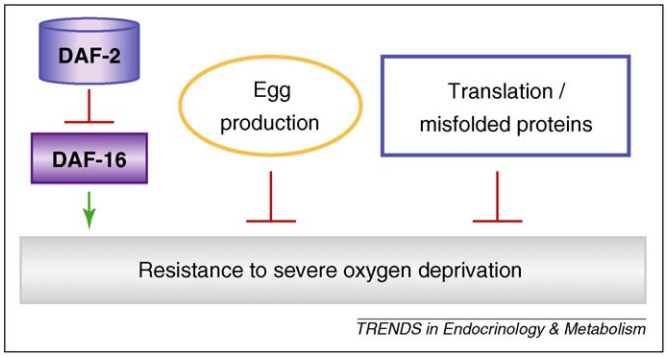


**Figure 2**. Interventions and mutations that allow *C. elegans* to survive severe hypoxic stress. Certain loss-of-function mutations in the DAF-2 insulin-like receptor protect *C. elegans* from long-term anoxia or high-temperature hypoxia, and this effect requires the DAF-16 / FOXO transcription factor [43,44]. The demands of reproduction compromise long-term anoxia resistance. Germline mutants and males are more resistant to oxygen deprivation, and these phenotypes are not dependent upon *hif-1* or *daf-16* [47]. Treatments that inhibit translation increase resistance to hypoxia. Current models suggest that this is due to both the high energy demands of protein translation and the toxic effects of misfolded proteins [48].

cause dauer formation or longevity phenotypes. These allelic differences have been used as tools to identify gene expression differences that correlate with resistance to severe hypoxia [46].

The demands of reproduction make *C. elegans* more vulnerable to hypoxic injury. Whereas most gravid hermaphrodites die after 3 days of anoxia at 20°C, these conditions are not lethal to males or to hermaphrodites incapable of producing embryos. The long-term anoxia-resistance phenotypes of germline mutants are not dependent upon *hif-1* or *daf-16* [47]. These findings support a model in which egg production compromises the ability of animals to survive severe oxygen deprivation, but certain mutations in the *daf-2* pathway can enable reproductive hermaphrodites to survive this stress (Figure 2). Future studies will continue to refine this model (Box 1).

## Slowing translation increases high-temperature hypoxia survival

A forward genetic screen for mutations that conferred resistance to severe hypoxic stress led to the discovery that slowing translation of proteins can protect *C. elegans* from hypoxic injury. A loss-of-function mutation in the arginyl-tRNA synthetase enabled *C. elegans* to survive severe hypoxic stress (<0.3% oxygen at 26.5°C for 20 hours). RNAi-mediated inactivation of most other tRNA synthetases also increased resistance to severe hypoxia, as did the translation inhibitor cyclohexamide [48]. Inhibitors of translation might protect *C. elegans* from high-temperature hypoxia by decreasing energy requirements and limiting the damage caused by misfolded proteins. These findings are especially exciting because they immediately suggest experiments to limit hypoxic injury using modulators of translation in mammalian systems.

## Crosstalk between HIF-1, mTOR, and unfolded protein response (UPR) pathways

Cancer cells respond to oxygen deprivation through at least three major pathways: HIF-1 activation, the UPR pathway initiated in the endoplasmic reticulum, and by slowing translation through regulation of the mTOR pathway [49]. Recent studies in *C. elegans* have explored the roles for the UPR pathway and mTOR signaling in the hypoxia response and have examined crosstalk between these pathways. The lifespan extension conferred by *hif-1* RNAi requires the functions of *ire-1* and *xbp-1*, major regulators of the UPR [14]. Consistent with the hypothesis that *C. elegans* HIF-1 pathways modulate the UPR, moderate hypoxia or mutation of *egl-9* activate the UPR [15,48]. Further, stabilization of HIF-1 by *vhl-1* or *egl-9* RNAi protects *C. elegans* from polyglutamine or amyloid beta toxicity [13]. Finally, loss-of-function mutations in either *xbp-1* or *ire-1* partially suppress the resistance to severe hypoxia conferred by mutation of the *rrt-1* tRNA synthase [48]. The relative importance of each of these core hypoxia response pathways depends on the severity of the hypoxic insult and developmental context, but it is increasingly clear that cells must limit proteotoxicity to survive severe oxygen deprivation.

Mutations in ceramide synthase genes also influence survival of long-term anoxia. Ceramides and sphingolipids have roles in cell signaling and cell death [50]. *C. elegans* has three ceramide synthase genes. Mutation of either *hyl-1* or *hyl-2* alters the composition of ceramides, but in different ways and with different consequences to anoxia tolerance [51]. It is not yet clear whether this affects the activity of SBP-1, the *C. elegans* sterol regulatory element binding protein (SREBP) transcription factor homolog, but it is worth noting that SBP-1 is activated by anoxia [52]. An important direction for future studies will be to understand how ceramide derivatives modulate hypoxia resistance.

## Biomedical implications and future directions

Cardiovascular disease ultimately deprives tissues of oxygen, and the consequences can be devastating. Therapies that improve hypoxia resistance are being sought for the treatment of anemia and peripheral vascular disease. Conversely, the cells at the center of a solid tumor are deprived of oxygen, and pharmaceuticals that inhibit hypoxia resistance might prove to be effective chemotherapies [53].

Studies in *C. elegans* promise to inform mammalian biology in at least three broad arenas. First, *C. elegans* genetics will continue to play an important role in the interrogation of HIF-1 regulation and function. An important next step will be to define the biochemical mechanisms by which EGL-9 represses HIF-1 activity and to identify other genes that act in this pathway. Other core elements of HIF-1 regulatory networks might also be conserved, and it will be important to determine how HIF-1 interacts with other known regulators of lifespan and stress responses to coordinate adaptive responses. Second, recent discoveries have shown that egg maturation, insulin-like receptor signaling, and protein translation all compromise *C. elegans* survival in conditions of severe oxygen deprivation. Future studies will examine and compare the downstream consequences of these interventions to better understand how they can best be translated to treatments for hypoxia-related human disease. Third, studies in *C. elegans* will examine the cellular and genetic underpinnings of suspended animation induced by short-term anoxia or by

hydrogen sulfide. If this strategy can be translated to human medicine, perhaps reversible hypometabolic arrest could provide more time for treatment of critically injured patients. In sum, studies of hypoxia signaling and response in *C. elegans* have implicated many regulatory networks and cellular processes that are conserved throughout the animal kingdom, and the field continues to advance our understanding of how animals adapt to changing levels of oxygen during development, homeostasis, and disease.

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**References**

1 Jiang, H. *et al.* (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
2 Padilla, P.A. *et al.* (2002) Dephosphorylation of cell cycle-regulated proteins correlates with anoxia-induced suspended animation in *Caenorhabditis elegans*. *Mol. Biol. Cell* 13, 1473–1483
3 Nystul, T.G. and Roth, M.B. (2004) Carbon monoxide-induced suspended animation protects against hypoxic damage in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 9133–9136
4 Epstein, A.C. *et al.* (2001) *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107, 43–54
5 Kaelin, W.G., Jr and Ratcliffe, P.J. (2008) Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* 30, 393–402
6 Powell-Coffman, J.A. *et al.* (1998) *Caenorhabditis elegans* orthologs of the aryl hydrocarbon receptor and its heterodimerization partner the aryl hydrocarbon receptor nuclear translocator. *Proc. Natl. Acad. Sci. U. S. A.* 95, 2844–2849
7 Shen, C. *et al.* (2005) Roles of the HIF-1 hypoxia-inducible factor during hypoxia response in *Caenorhabditis elegans*. *J. Biol. Chem.* 280, 20580–20588
8 Guzy, R.D. *et al.* (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metab.* 1, 401–408
9 Mansfield, K.D. *et al.* (2005) Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation. *Cell Metab.* 1, 393–399
10 Brunelle, J.K. *et al.* (2005) Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. *Cell Metab.* 1, 409–414
11 Bell, E.L. *et al.* (2007) Mitochondrial reactive oxygen species trigger hypoxia-inducible factor-dependent extension of the replicative life span during hypoxia. *Mol. Cell. Biol.* 27, 5737–5745
12 Budde, M.W. and Roth, M.B. (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
13 Mehta, R. *et al.* (2009) Proteasomal regulation of the hypoxic response modulates aging in *C. elegans*. *Science* 324, 1196–1198
14 Chen, D. *et al.* (2009) HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in *C. elegans*. *PLoS Genet.* 5, e1000486
15 Bellier, A. *et al.* (2009) Hypoxia and the hypoxic response pathway protect against pore-forming toxins in *C. elegans*. *PLoS Pathog.* 5, e1000689
16 Zhang, Y. *et al.* (2009) The HIF-1 hypoxia-inducible factor modulates lifespan in *C. elegans*. *PLoS ONE* 4, e6348
17 Gallagher, L.A. and Manoil, C. (2001) *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. *J. Bacteriol.* 183, 6207–6214
18 Darby, C. *et al.* (1999) Lethal paralysis of Caenorhabditis elegans by Pseudomonas aeruginosa. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15202–15207
19 Anyanful, A. *et al.* (2005) Paralysis and killing of *Caenorhabditis elegans* by enteropathogenic *Escherichia coli* requires the bacterial tryptophanase gene. *Mol. Microbiol.* 57, 988–1007
20 Treinin, M. *et al.* (2003) HIF-1 is required for heat acclimation in the nematode *Caenorhabditis elegans*. *Physiol. Genomics* 14, 17–24
21 Gort, E.H. *et al.* (2008) The TWIST1 oncogene is a direct target of hypoxia-inducible factor-2alpha. *Oncogene* 27, 1501–1510
22 Bishop, T. *et al.* (2004) Genetic analysis of pathways regulated by the von Hippel–Lindau tumor suppressor in *Caenorhabditis elegans*. *PLoS Biol.* 2, e289
23 Berra, E. *et al.* (2006) The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signalling. *EMBO Rep.* 7, 41–45
24 Shen, C. *et al.* (2006) The *Caenorhabditis elegans rhy-1* gene inhibits HIF-1 hypoxia-inducible factor activity in a negative feedback loop that does not include *vhl-1*. *Genetics* 174, 1205–1214
25 Shao, Z. *et al.* (2009) Two distinct roles for EGL-9 in the regulation of HIF-1-mediated gene expression in *Caenorhabditis elegans*. *Genetics* 183, 821–829
26 Gray, J.M. *et al.* (2004) Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. *Nature* 430, 317–322
27 Cheung, B.H. *et al.* (2005) Experience-dependent modulation of *C. elegans* behavior by ambient oxygen. *Curr. Biol.* 15, 905–917
28 Bretscher, A.J. *et al.* (2008) A carbon dioxide avoidance behavior is integrated with responses to ambient oxygen and food in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 105, 8044–8049
29 Chang, A.J. and Bargmann, C.I. (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
30 McGrath, P.T. *et al.* (2009) Quantitative mapping of a digenic behavioral trait implicates globin variation in *C. elegans* sensory behaviors. *Neuron* 61, 692–699
31 Persson, A. *et al.* (2009) Natural variation in a neural globin tunes oxygen sensing in wild *Caenorhabditis elegans*. *Nature* 458, 1030–1033
32 Pocock, R. and Hobert, O. (2008) Oxygen levels affect axon guidance and neuronal migration in *Caenorhabditis elegans*. *Nat. Neurosci.* 11, 894–900
33 Van Voorhies, W.A. and Ward, S. (2000) Broad oxygen tolerance in the nematode *Caenorhabditis elegans*. *J. Exp. Biol.* 203, 2467–2478
34 Nystul, T.G. *et al.* (2003) Suspended animation in *C. elegans* requires the spindle checkpoint. *Science* 302, 1038–1041
35 Hajeri, V.A. *et al.* (2005) Characterization of sub-nuclear changes in *Caenorhabditis elegans* embryos exposed to brief, intermediate and long-term anoxia to analyze anoxia-induced cell cycle arrest. *BMC Cell Biol.* 6, 47
36 Hajeri, V.A. *et al.* (2008) Genetic analysis of the spindle checkpoint genes *san-1*, *mdf-2, bub-3* and the CENP-F homologues *hcp-1* and *hcp-2* in *Caenorhabditis elegans*. *Cell Div.* 3, 6
37 Hajeri, V.A. *et al.* (2010) NPP-16/Nup50 function and CDK-1 inactivation are associated with anoxia-induced prophase arrest in *Caenorhabditis elegans*. *Mol. Biol. Cell* 21, 712–724
38 Miller, D.L. and Roth, M.B. (2009) *C. elegans* are protected from lethal hypoxia by an embryonic diapause. *Curr. Biol.* 19, 1233–1237
39 Blackstone, E. *et al.* (2005) $H_2S$ induces a suspended animation-like state in mice. *Science* 308, 518
40 Blackstone, E. and Roth, M.B. (2007) Suspended animation-like state protects mice from lethal hypoxia. *Shock* 27, 370–372
41 Szabo, C. (2007) Hydrogen sulphide and its therapeutic potential. *Nat. Rev. Drug. Discov.* 6, 917–935
42 Miller, D.L. and Roth, M.B. (2007) Hydrogen sulfide increases thermotolerance and lifespan in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 104, 20618–20622
43 Scott, B.A. *et al.* (2002) Regulation of hypoxic death in *C. elegans* by the insulin/IGF receptor homolog DAF-2. *Science* 296, 2388–2391
44 Mendenhall, A.R. *et al.* (2006) Glyceraldehyde-3-phosphate dehydrogenase mediates anoxia response and survival in *Caenorhabditis elegans*. *Genetics* 174, 1173–1187
45 Panowski, S.H. and Dillin, A. (2009) Signals of youth: endocrine regulation of aging in *Caenorhabditis elegans*. *Trends Endocrinol. Metab.* 20, 259–264
46 Mabon, M.E. *et al.* (2009) Divergent mechanisms controlling hypoxic sensitivity and lifespan by the DAF-2/insulin/IGF-receptor pathway. *PLoS One* 4, e7937

47 Mendenhall, A.R. *et al.* (2009) Reduction in ovulation or male sex phenotype increases long-term anoxia survival in a daf-16-independent manner in *Caenorhabditis elegans*. *Physiol. Genomics* 36, 167–178
48 Anderson, L.L. *et al.* (2009) Survival from hypoxia in *C. elegans* by inactivation of aminoacyl-tRNA synthetases. *Science* 323, 630–633
49 Wouters, B.G. and Koritzinsky, M. (2008) Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nat. Rev. Cancer* 8, 851–864
50 Hannun, Y.A. and Obeid, L.M. (2008) Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat. Rev. Mol. Cell Biol.* 9, 139–150
51 Menuz, V. *et al.* (2009) Protection of *C. elegans* from anoxia by HYL-2 ceramide synthase. *Science* 324, 381–384
52 Taghibiglou, C. *et al.* (2009) Essential role of SBP-1 activation in oxygen deprivation induced lipid accumulation and increase in body width/length ratio in *Caenorhabditis elegans*. *FEBS Lett.* 583, 831–834
53 Rankin, E.B. and Giaccia, A.J. (2008) The role of hypoxia-inducible factors in tumorigenesis. *Cell Death Differ.* 15, 678–685