Hypoxia and the HIF-1 transcriptional pathway reorganize a neuronal circuit for oxygen-dependent behavior in *Caenorhabditis elegans*

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Rapid behavioral responses to oxygen are generated by specialized sensory neurons that sense hypoxia and hyperoxia. On a slower time scale, many cells respond to oxygen through the activity of the hypoxia-inducible transcription factor HIF-1. Here, we show that in the nematode *Caenorhabditis elegans*, prolonged growth in hypoxia alters the neuronal circuit for oxygen preference by activating the *hif-1* pathway. Activation of *hif-1* by hypoxia or by mutations in its negative regulator *egl-9*/prolyl hydroxylase shifts behavioral oxygen preferences to lower concentrations and eliminates a regulatory input from food. At a neuronal level, *hif-1* activation transforms a distributed, regulated neuronal network for oxygen preference into a smaller, fixed network that is constitutively active. The *hif-1* pathway acts both in neurons and in gonadal endocrine cells to regulate oxygen preference. These results suggest that physiological detection of hypoxia by multiple tissues provides adaptive information to neuronal circuits to modify behavior.

chemosensation | neural plasticity

Oxygen availability is a powerful motivator of behavior. Many animals sense internal oxygen levels (1), and organisms that live in soil and water environments with variable oxygen levels can detect external oxygen as well (2, 3). In the soil nematode *Caenorhabditis elegans*, specialized chemosensory neurons sense environmental oxygen gradients to guide migration to intermediate oxygen levels (7–14% O_2). The oxygen sensors in these neurons have been proposed to be soluble guanylate cyclase homologs (sGCs) that bind directly to molecular oxygen (4, 5).

Avoidance of hyperoxia (>14% O_2) is mediated by a distributed network of *C. elegans* sensory neurons. Two sets of neurons that express sGCs are largely redundant for hyperoxia avoidance (6). In addition to the sGC neurons, hyperoxia avoidance depends on the activity of nociceptive neurons and serotonergic neurons that sense stress (6, 7). The distributed and partly redundant function of these neurons generates subtle gradations in oxygen preference.

C. elegans oxygen preference is regulated by food context and by naturally-occurring genetic variation. The standard wild-type strain, N2, does not avoid hyperoxia in the presence of bacterial food (4). Food suppresses hyperoxia avoidance by signaling through the G protein-coupled neuropeptide receptor NPR-1 and the TGF-β homolog DAF-7 (4, 6). Natural isolates of *C. elegans* with low *npr-1* activity display hyperoxia avoidance on food, accumulate at the border of a bacterial lawn, and aggregate into social feeding groups (4, 8). Hyperoxia avoidance is a component of all of these behaviors; lawn borders and aggregates are attractive in part because they have lower, more favorable oxygen concentrations (4, 7). As expected from these linked activities, common genes are required for hyperoxia avoidance on food and for social feeding behavior.

Along with the rapid neuronal pathways for oxygen detection, the slower hypoxia-inducible factor-1 (HIF-1) transcriptional pathway monitors internal oxygen levels to regulate metazoan metabolism, vascular biology, cell survival, and cell proliferation (9). In normoxia, where HIF-1 is inactive, the HIF-1α subunit of the HIF-1 transcription factor is modified by prolyl hydroxylases (PHDs) that use oxygen as a cofactor (reviewed in ref. 10). Modified HIF-1α is recognized by the von Hippel-Lindau tumor suppressor protein (VHL), which targets HIF-1α for degradation. In hypoxia, PHD activity is reduced, and unmodified HIF-1α is stable and transcriptionally active. The HIF-1 pathway is conserved in *C. elegans*, where the genes encoding homologs of PHDs, VHL, and HIF-1α are named *egl-9*, *vhl-1*, and *hif-1*, respectively (11, 12). As in other animals, *C. elegans* HIF-1 protein accumulates to high levels after 4–8 h of hypoxia (1% or 0.1% O_2) but disappears within minutes in normoxia because of the actions of EGL-9 and VHL-1 (11, 12). Unlike Hif-1α knockout mice, which die embryonically (13, 14), *C. elegans hif-1* mutants are viable in normoxia, and embryonic lethal only in hypoxia (1% O_2) (11).

How do physiological and behavioral pathways for oxygen homeostasis interact? A study in ref. 5 showed that *C. elegans* strains with low *npr-1* activity shift their oxygen preferences after a few hours in hypoxia, but the wild-type N2 strain does not. Here, we show that longer-term exposure to hypoxia changes the behavioral response of wild-type *C. elegans*, causing it to prefer lower oxygen levels and avoid hyperoxia in the presence of food. This modified preference results from activation of the *hif-1* pathway in both neurons and nonneuronal cells, and leads to a striking reorganization of the neuronal circuit for oxygen preference.

Results

Cultivation in Hypoxia Modifies Oxygen Preference Through the *egl-9*/*hif-1* Pathway. To examine long-term consequences of growth in hypoxia, wild-type animals were cultivated for 2 days at 1% O_2, where *C. elegans* metabolic rates are approximately half of normal (11, 15), and then tested for their oxygen preference in a gas-phase linear gradient of 0–21% O_2. The behavior of animals in these gradients is called aerotaxis and reflects both hyperoxia and hypoxia avoidance (4). Wild-type animals grown in standard laboratory conditions (normoxia) distributed themselves through the chamber with a median preferred oxygen concentration ≈10%, whereas animals cultivated in hypoxia shifted their oxygen preference to a lower median preferred oxygen concentration near 8% (Fig. 1 *A* and *B*). This shift in oxygen preference was similar to, but weaker

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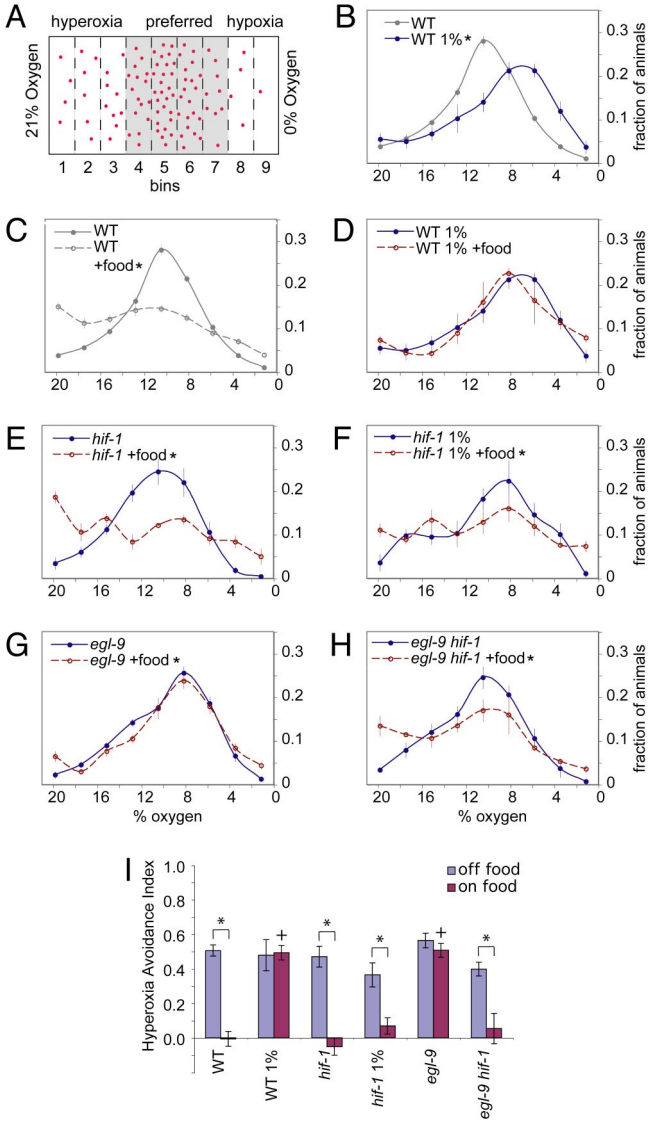

Fig. 1. Cultivation in hypoxia modifies oxygen preference through the *egl-9/hif-1* pathway. (*A*) Diagram showing typical distribution of *C. elegans* (dots) in an aerotaxis assay. Preferred area is shaded. (*B–H*) Aerotaxis of wild-type animals and *egl-9/hif-1* pathway mutants grown in normoxia or in 1% O_2 for 2 days as adults. Asterisks denote distributions different at $P < 0.01$ by χ^2 analysis. (*I*) The hyperoxia avoidance index, defined as [avg(fraction in 5–14% O_2) − avg(fraction in 14–21% O_2)]/[avg(fraction in 5–14% O_2) + avg(fraction in 14–21% O_2)]. Asterisks indicate values different from the same genotype and condition without food at $P < 0.05$ by *t* test. Crosses indicate values different from normoxia-grown wild-type on food at $P < 0.01$ by Dunnett test. In all panels, $n \geq 3$ assays per genotype, 80–100 animals/assay; error bars denote standard error of mean (SEM).

than, the shift in *npr-1* mutants grown in hypoxia (5) [supporting information (SI) Fig. S1].

The addition of bacterial food to the assay uncovered a second difference between wild-type animals grown in normoxia and hypoxia. Animals cultivated in normoxia did not avoid high oxygen levels in the presence of food (Fig. 1*C*), but animals cultivated in hypoxia had similar oxygen preferences whether food was present or absent (Fig. 1*D*). We conclude that cultivation in hypoxia eliminates the food regulation of aerotaxis.

Because of its known role in physiological adaptation to hypoxia, the *hif-1* transcriptional pathway was considered as a possible mediator of the altered oxygen response. Animals mutant for *hif-1* were cultivated in normoxia or hypoxia and tested for oxygen preferences in the absence or presence of food. Whether cultivated in normoxia or hypoxia, *hif-1* null mutants behaved like wild-type animals in normoxia: They avoided hyperoxia in the absence of food but not in its presence (Fig. 1 *E* and *F*). Null mutants in *egl-9*/PHD, the negative regulator of *hif-1*, had a complementary defect. These mutants with constitutively high HIF-1 levels behaved like wild-type animals grown in hypoxia, and avoided hyperoxia in the presence or absence of food (Fig. 1*G*). *egl-9 hif-1* double mutants closely resembled *hif-1* single mutants, indicating that *egl-9* acts through *hif-1* to change behavioral responses to oxygen (Fig. 1*H*).

These results demonstrate that hypoxia regulates aerotaxis through the *hif-1* pathway. When the *hif-1* pathway is active (in chronic hypoxia or in *egl-9* mutants), animals avoid hyperoxia in the presence of food. When the *hif-1* pathway is inactive (in normoxia or in *hif-1* mutants), food suppresses hyperoxia avoidance. As expected for a slow transcriptional pathway, *hif-1* activity was required for changes in oxygen preference after 2 days of hypoxia, but it was not required for changes in wild-type or *npr-1* mutants after 6 h of hypoxia (Fig. S1). Like cultivation in hypoxia, *hif-1* activation in *egl-9* mutants also caused a small but significant shift in the median preferred oxygen concentration (Fig. S1), but, for further analysis, we focused on its strong effect on regulation by food. This effect can be summarized in a hyperoxia avoidance index that compares the relative accumulation of animals at preferred oxygen concentrations (5–14% O_2) versus hyperoxia (14–21% O_2) (Fig. 1*I*; see also *Methods*). The 5–14% preferred interval was chosen to capture both the normal preference for 10% O_2 and the shift to 8% O_2 observed in hypoxic animals.

***egl-9* Activity in Neurons and Nonneuronal Cells Regulates Oxygen Preference.** *hif-1* and *egl-9* are coexpressed in virtually all cells in *C. elegans* (11, 16) (Fig. 2*A*) and are expected to act in the same cells. To determine where the *hif-1* pathway acts to regulate oxygen preference, we performed tissue-specific rescue of *egl-9* mutants, monitoring hyperoxia avoidance on food. We chose to rescue *egl-9* because *egl-9* mutants were defective when grown in normoxia, whereas *hif-1* defects were only apparent after cultivation in hypoxia (Fig. 1).

Expression of an *egl-9* cDNA from its endogenous promoter rescued food regulation of hyperoxia avoidance in *egl-9* mutants, as did expression of the *egl-9* cDNA from the *unc-31* promoter (Fig. 2 *A* and *B* and Fig. S2). The *unc-31* promoter is expressed in all neurons and in secretory cells of the somatic gonad: the spermatheca, vulval muscles, and uv1 uterine-vulval cells (17). *egl-9* aerotaxis behavior was not rescued when the *egl-9* cDNA was expressed only in all neurons or in sGC or serotonergic subsets of neurons that regulate oxygen preferences (Fig. 2 *A* and *B*). Negative results were also obtained with *egl-9* expression in body wall and vulval muscles, uterine cells, uv1 uterine-vulval cells, pharyngeal muscles, pharyngeal marginal cells, pharyngeal gland cells, XXX endocrine cells, and hypodermis (Fig. 2 *A* and *B*). However, expression of *egl-9* both in neurons and in the uv1 cells (*H20+tdc-1* promoters) rescued food regulation of hyperoxia avoidance, unlike either *H20::egl-9* or *tdc-1::egl-9* alone (Fig. 2 *A* and *B*).

egl-9 mutants were first isolated based on defective egg-laying behavior and retention of late-stage eggs in the uterus (18). Egg-laying in *egl-9* mutants was rescued by the same transgenes that rescued oxygen-dependent behaviors: *egl-9::egl-9*, *unc-31::egl-9*, and *H20::egl-9+tdc-1::egl-9* transgenes (Fig. 2*A*). An involvement of uv1 gonadal cells in egg laying was further supported by genetic mosaic analysis of *egl-9* expressed from its own promoter (*SI Methods*). These rescue experiments indicate that *egl-9/hif-1* act in at least two sets of cells to regulate egg

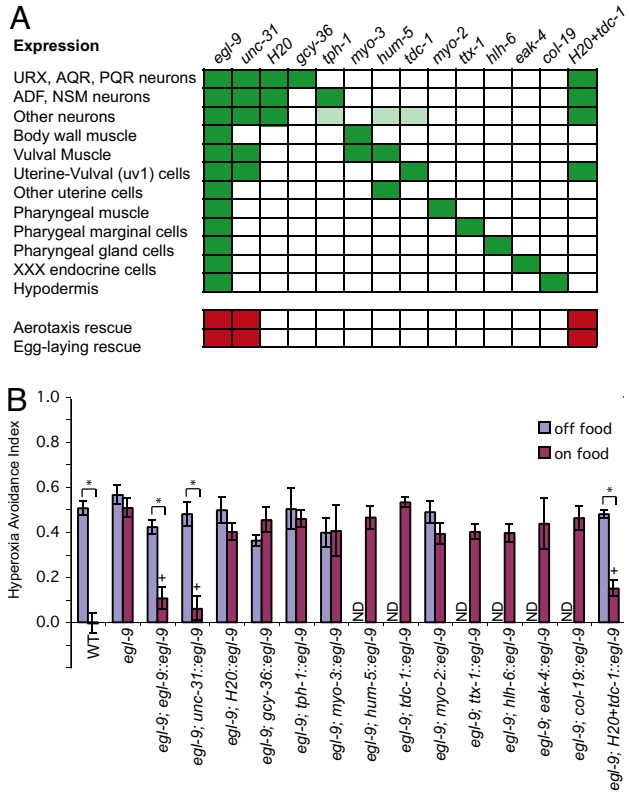

Fig. 2. *egl-9*/PHD activity in neurons and nonneuronal secretory cells regulates hyperoxia avoidance on food. (*A*) Expression patterns of *egl-9* transgenes under the listed promoters and rescue of aerotaxis and egg-laying. Colored boxes indicate expression of the transgene or rescue of behavior. (See also *SI Methods*.) (*B*) Hyperoxia avoidance index. ND, not determined. Asterisks indicate values different from the same genotype without food at $P < 0.05$ by *t* test. Crosses indicate rescued lines with values different from *egl-9* in the presence of food at $P < 0.01$ by Dunnett test. Aerotaxis assays shown in Fig. S2.

laying and hyperoxia avoidance: neurons and nonneuronal uv1 cells of the somatic gonad.

The *egl-9*/*hif-1* Pathway Alters Neuronal Requirements for Hyperoxia Avoidance. Studies have defined four groups of sensory neurons that promote hyperoxia avoidance (4, 6, 7) (Fig. 3*A*). The four groups are (*i*) URX, AQR, and/or PQR, which express sGCs (the URX set); (*ii*) SDQ, ALN, and/or PLN, which express sGCs (the SDQ set); (*iii*) ASH, which expresses transient receptor potential vanilloid (TRPV) channels; and (*iv*) ADF, which expresses TRPV channels and synthesizes serotonin. Hyperoxia avoidance requires at least one set of sGC-expressing neurons and at least one class of the TRPV-expressing neurons.

To determine which neurons were affected by the *egl-9*/*hif-1* pathway, we systematically crossed *egl-9* into mutants that affect these sensory neurons, and tested the double mutants for their aerotaxis behavior on and off food. *egl-9* mutants were expected to resemble animals grown in hypoxia (12) (Fig. 1) and therefore could also shed light on the effects of environmental hypoxia on wild-type behaviors.

To our surprise, the neuronal requirements for hyperoxia avoidance in *egl-9* mutants were very different from those of wild-type animals (Fig. 3*B* and Fig. S3). Only the URX set of neurons was important for hyperoxia avoidance, with other sensory neurons making negligible contributions. Notably, a transgene that kills the URX set of neurons disrupted hyperoxia avoidance in *egl-9* mutants, but not in wild-type animals (Fig. 3 *C* and *E*). *egl-9* mutants required the sGC *gcy-36*, which acts in the URX set, but were only slightly affected by *gcy-35,* which acts in both the URX set and the SDQ set (4–6) (Fig. 3*E*). These sGC requirements are consistent with a greater role for the URX set, and a diminished role for the SDQ set, in *egl-9* mutants.

The TRPV channel genes *osm-9* and *ocr-2* are required for ASH and ADF function and for hyperoxia avoidance in wild-type animals (6, 7). By contrast, *osm-9* or *ocr-2* were not required in an *egl-9* background (Fig. 3 *D* and *E*), suggesting that ASH and ADF are unimportant in this context.

Hyperoxia avoidance in wild-type N2 animals is potentiated by the neurotransmitter serotonin, which is produced by the ADF neurons (6). Mutants in the tryptophan hydroxylase enzyme gene *tph-1* are defective in serotonin synthesis in ADF neurons and four other classes of *C. elegans* serotonergic neurons (19). Like N2 animals, *egl-9* mutants required *tph-1* for hyperoxia avoidance (6) (Fig. 3 *F* and *H*). However, unlike *tph-1* single mutants, *tph-1;egl-9* double mutants were not rescued by expression of *tph-1* in ADF neurons (6) (Fig. 3 *G* and *H*). Instead, transgenic expression of *tph-1* in only the NSM neurons resulted in small improvements in hyperoxia avoidance that were increased by expression in both NSM and ADF neurons together (Fig. 3 *G* and *H*). These results suggest that the essential serotonin sources differ in wild-type and *egl-9* backgrounds (Fig. 3 *A* and *B*).

In summary, induction of the *hif-1* pathway in *egl-9* mutants changes the neuronal requirement for hyperoxia avoidance from a multicellular network of sGC-expressing and TRPV-expressing neurons to a smaller network of neurons. In *egl-9* mutants, the essential elements are sGC-expressing URX, AQR, and/or PQR neurons and serotonin signaling from NSM neurons (Fig. 3*B*).

Cultivation in Hypoxia Alters the Neural Circuit for Oxygen Preference. The initial analysis of *egl-9* mutants suggested that they resembled wild-type animals after long-term cultivation in hypoxia (Fig. 1). To strengthen the connection between *egl-9* mutants and the hypoxia response, we asked whether cultivation in hypoxia could shift the genetic requirements for aerotaxis in the same way as an *egl-9* mutation. These experiments focused on the most striking differences between wild-type and *egl-9*, which were the differential requirements for the URX set of neurons (required in *egl-9* but not in wild-type) and for *osm-9*/TRPV neurons (required in wild-type but not in *egl-9*). After cultivation in hypoxia, wild-type animals lacking the URX set of neurons were profoundly defective in hyperoxia avoidance (Fig. 4 *A* and *C*). Conversely, cultivation in hypoxia suppressed the hyperoxia avoidance defect of *osm-9* mutants (Fig. 4 *B* and *C*). The altered behaviors in these strains suggests that growth in hypoxia changes the neuronal requirements for hyperoxia avoidance, increasing the contribution of the URX set of neurons and diminishing the requirement for TRPV neurons. These changes are similar to the changes in *egl-9* mutants, supporting the hypothesis that chronic hypoxia changes the aerotaxis circuit by activating *hif-1*.

***egl-9*/*hif-1*, *npr-1*, and *daf-7*/TGF-β Pathways Regulate Oxygen Preferences Independently.** Like *egl-9* mutants, animal with mutations in the neuropeptide receptor *npr-1* or the neuronal TGF-β homolog *daf-7* avoid hyperoxia in the presence of food (4, 6). This similarity motivated us to examine possible relationships among the *egl-9*/*hif-1* pathway and the *npr-1* and *daf-7* pathways.

In *npr-1* mutants, deleting either the URX set of neurons or the TRPV channels restores food regulation of hyperoxia avoidance (6). However, the same genetic manipulations did not restore food regulation to *egl-9* mutants (Fig. 5*A* and Fig. S4). Conversely, a *hif-1* mutation that restored food regulation to *egl-9* did not restore food regulation to *npr-1* (Fig. 5*B*). These results suggest that *egl-9* and *npr-1* mutants use different mechanisms to maintain hyperoxia avoidance on food.

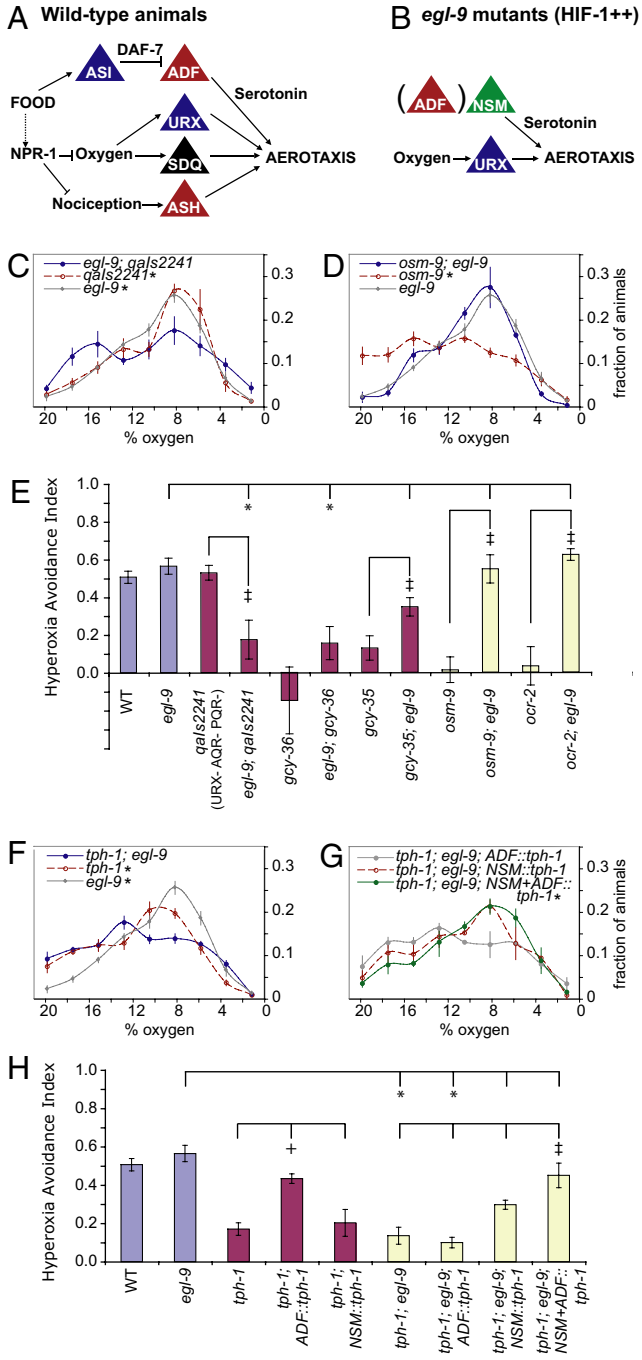

Fig. 3. Induction of the *egl-9/hif-1* pathway alters neuronal requirements for hyperoxia avoidance. (*A*) A distributed network of sensory neurons generates hyperoxia avoidance in wild-type N2 animals (6). URX, AQR, and PQR neurons (URX set) and SDQ, ALN, and BDU neurons (SDQ set) express sGC homologs and are likely oxygen sensors. ASH and ADF neurons express the TRPV channels *osm-9* and *ocr-2*; ADF produces serotonin. In the presence of food, hyperoxia avoidance is suppressed by the activity of the neuropeptide receptor NPR-1 and TGF-β homolog DAF-7. (*B*) Summary of results in *C–H*. Aerotaxis of *egl-9*/PHD mutants requires URX, AQR, and PQR sensory neurons but not TRPV channel-expressing neurons. The NSM neurons are a more important source of serotonin than ADF neurons. (*C* and *D*) Effects of *qaIs2241* and *osm-9* on *egl-9* aerotaxis off food. The *qaIs2241* transgene kills URX, AQR, and PQR neurons (6). Asterisks denote distributions different from the double mutant at $P < 0.01$ by χ^2 analysis. (*E*) Hyperoxia avoidance index off food. Asterisks indicate double mutants different from *egl-9* controls at $P < 0.01$ by Dunnett test. Double crosses indicate values different from the designated single mutant at $p < 0.05$ by Bonferroni *t* test. Data for some single mutants from ref. 6. (*F* and *G*) Effects of *tph-1* on *egl-9* aerotaxis off food and rescue of *tph-1*. ADF promoter was *srh-142*; NSM promoter was *ceh-2*; and serotonergic neurons other than ADF and NSM were not tested. Asterisks denote distributions different from *tph-1; egl-9* at $P < 0.01$ by χ^2 analysis. (*H*) Hyperoxia avoidance of *tph-1* strains. Asterisks indicate values different from *egl-9* control at $P < 0.01$ by Dunnett test. Crosses and double-crosses indicate values different from nonrescued strain at $P < 0.05$ by Bonferroni *t* test. Additional aerotaxis assays shown in Fig. S3.

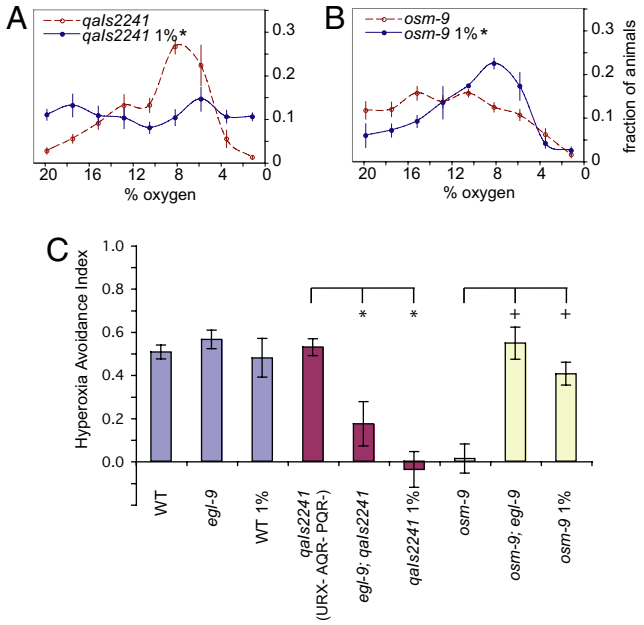

Fig. 4. Cultivation in hypoxia alters the neural circuit for oxygen preference. (*A* and *B*) Aerotaxis of *qaIs2241* (URX, AQR, and PQR killed) (*A*) and *osm-9* (*B*) animals grown in 1% O_2 for 2 days as adults. Asterisks denote distributions different at $P < 0.01$ by χ^2 analysis. (*C*) Hyperoxia avoidance index. Asterisks indicate values different from *qaIs2241* at $P < 0.05$ by Bonferroni *t* test. Crosses indicate values different from *osm-9* at $P < 0.05$ by Bonferroni *t* test.

Additional results supported the conclusion that *egl-9/hif-1* regulation of oxygen responses is distinct from and parallel to regulation by *npr-1*. Like the respective single mutants, *egl-9;npr-1* double mutants exhibited strong hyperoxia avoidance that was not regulated by food (4, 6) (Fig. 5*B*). However, *egl-9;npr-1* double mutants preferred even lower oxygen concentrations than either single mutant, consistent with parallel functions (Fig. 5*C*). They also showed enhanced aggregation on food compared to *egl-9* and *npr-1* single mutants (data not shown).

Another gene that regulates oxygen responses on food is the TGF-β homolog *daf-7* (6). In *daf-7* mutants, food regulation of hyperoxia avoidance can be restored by a mutation in the downstream SMAD transcription factor *daf-3* (6, 20). *daf-3* did not affect *egl-9* hyperoxia avoidance on food (Fig. 5*D*), indicating that *egl-9* does not function through the *daf-7*/TGF-β pathway. We also examined a known transcriptional target of *daf-7*/TGF-β pathway, the serotonin biosynthetic enzyme *tph-1*. In *daf-7* mutants, *tph-1* expression in ADF neurons is increased, and this increase stimulates hyperoxia avoidance in the presence of food (6). By contrast, no changes in *tph-1*::*GFP* expression were observed in ADF or NSM neurons of *egl-9* mutants, *hif-1* mutants, or wild-type worms grown in hypoxia (Fig. S5). Thus, the *egl-9/hif-1* and *daf-7/daf-3* transcriptional pathways regulate hyperoxia avoidance by different mechanisms.

Transcriptional targets of the *hif-1* pathway have been identified through microarray analysis (21, 22), but none have known

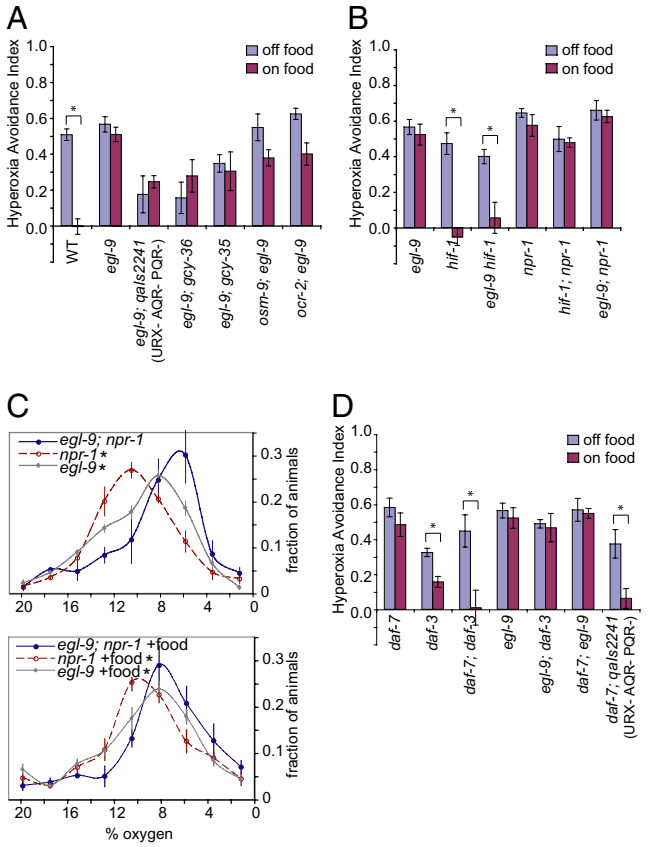

Fig. 5. *egl-9*/*hif-1*, *npr-1*, and *daf-7*/TGF-β pathways act independently to modulate oxygen preference. (*A*, *B*, and *D*) Hyperoxia avoidance index and food regulation in *egl-9*/sensory mutants (*A*), *egl-9*/*npr-1* mutants (*B*), and *egl-9*/*daf-7* pathway mutants (*D*). Asterisks indicate values different from the same genotype without food at $P < 0.05$ by *t* test. Some data published in ref. 6 are included for clarity. (*C*) Aerotaxis of *egl-9;npr-1* double mutants. Asterisks indicate distributions different from double mutants at $P < 0.01$ by χ^2 analysis. Additional aerotaxis assays shown in Fig. S4.

effects on oxygen preference. By screening additional transcriptional reporters in *egl-9* mutants, we identified several regulated genes in relevant cell types. Decreased expression of *sGC*::*GFP* transcriptional reporters in URX neurons was reported in animals grown at 1% O_2 (5); we found that expression of four *sGC*::*GFP* transcriptional reporters was decreased in URX neurons of *egl-9* mutants (Fig. S5). *egl-9* mutants also had reduced *daf-7*::*GFP* fluorescence in ASI neurons and reduced expression of the catecholaminergic biosynthetic enzyme *tdc-1* in uv1 gonadal cells (Fig. S5). Although these molecular changes could contribute to the behavior, neither sGCs, *daf-7,* or *tdc-1* are sufficient to explain the effects of hypoxia and *hif-1* (Fig. 3, Fig. 5, and data not shown).

Discussion

After long-term cultivation in hypoxia, *C. elegans* prefers lower oxygen concentrations and avoids hyperoxia in the presence of food. Our work provides three insights into this process. First, these changes occur through activation of the *hif-1* transcriptional pathway, which couples physiological and behavioral adaptations to environmental oxygen. Second, the *hif-1* pathway acts both in neurons and in uv1 cells of the somatic gonad to modify oxygen preferences, suggesting that uv1 senses hypoxia through *egl-9*/*hif-1* and signals to the nervous system, which also senses hypoxia. Third, the neuronal control of oxygen preference changes dramatically after cultivation in hypoxia. Even in the absence of food, where the behavioral responses appear similar, neuronal contributions are different.

The use of HIF-1 to modify oxygen preferences provides an elegant integrated solution to match physiological needs and behavioral priorities. *C. elegans* adapts to a range of environmental conditions by deploying HIF-1 in hypoxia and antioxidative enzymes in hyperoxia (11, 23). Antagonistic physiological adaptations to hypoxia and hyperoxia provide a rationale for the enhanced avoidance of hyperoxia after cultivation in hypoxia. Normoxia-grown animals can tolerate high oxygen; hypoxia-grown animals may be more sensitive to high oxygen due to HIF-1-dependent physiological changes. We speculate that other physiological pathways will drive specialized behavioral adaptations by tuning the relevant neural circuits.

Transcriptional modulation by the *egl-9*/*hif-1* pathway provides adaptive information to mammalian sensory behaviors as well. In mice, reduced HIF-1 activity causes abnormal ventilatory adaption to chronic hypoxia, in part because of defects in the oxygen-sensing carotid body (24). Acute ventilatory responses to hypoxia appear normal in HIF-1-deficient mice, but the primary regulators of the hypoxia response in HIF-1 mutants are vagal afferents instead of the carotid body (24). Thus, HIF-1-dependent transcriptional regulation of circuits may be a general feature of oxygen sensation.

In *C. elegans*, *egl-9* acts both in neurons and in uv1 cells of the somatic gonad to detect hypoxia. The uv1 cells express neuropeptides, catecholamines, and the secretory machinery for their release and therefore could generate long-range signals to the nervous system (17, 25–27). Oxygen diffusion through *C. elegans* tissues is expected to be rapid, but the gonad may have special needs to monitor hypoxia. Reproductive activity is associated with the highest oxygen consumption in the *C. elegans* life cycle (28, 29), and embryos are preferentially susceptible to hypoxia-induced lethality (11). The action of the *hif-1* pathway in the somatic gonad may drive appropriate behavior at the crucial period during which eggs are produced and laid.

The molecular targets of *egl-9*/*hif-1* that regulate oxygen preference are unknown. The cell fates of the oxygen-sensing neurons appear unaffected by *egl-9*/*hif-1* genes (Fig. S5 and data not shown). Because the transcriptional pathway acts in several cell types, it is likely to have multiple relevant targets, perhaps including sGCs, *daf-7*, and *tdc-1*. At least one target in uv1 is predicted to affect intercellular signaling after chronic hypoxia.

The most striking effect of the *egl-9*/*hif-1* pathway is the reorganization of neuronal contributions to oxygen preference. Hypoxia simplifies the circuit, eliminating food regulation and the contributions of several sensory neurons. These changes may increase the reliability of the behavior but decrease its flexibility. Even for elements of the circuit that seem similar, closer examination reveals differences in normoxia and hypoxia. Serotonin is important for hyperoxia avoidance in both contexts, but its neural source is different. In *egl-9* mutants, NSM pharyngeal neurons, rather than ADF neurons, are the most important source of serotonin. NSM neurons integrate feeding state or starvation into egg-laying, locomotion, feeding, and male mating behaviors (30–32). Similarly, NSM may monitor food intake and other signals in the pharynx to modulate hyperoxia avoidance.

egl-9, *npr-1*, and *daf-7* mutants avoid hyperoxia in the presence of bacterial food, and all exhibit the oxygen-dependent behaviors of aggregation and bordering on bacterial lawns (4, 6, 8, 33). However, each strain achieves hyperoxia avoidance on food by regulating a different combination of neurons. Analogous studies have demonstrated that different combinations of neurons mediate locomotion and olfactory avoidance behaviors in *C. elegans* depending on whether animals are starved or well fed (31, 34). It is generally assumed that outwardly similar behaviors have a common neural basis. Our results lead to the surprising

conclusion that this is not always true, even in the simple nervous system of *C. elegans*. A change in the environment can uncover alternative circuits within the apparently fixed anatomy of the nervous system.

Materials and Methods

Nematode Growth and Strains. Strains were cultured under standard conditions (35) with *Escherichia coli* HB101; under these conditions, they are exposed to ≈12–21% O_2 (4). For growth in hypoxia, animals were transferred onto fresh culture plates in a gas-tight hypoxic chamber at 1% O_2 (Coy Laboratory Products). Wild-type animals were *C. elegans* Bristol strain N2. Mutants in the *hif-1* pathway were *hif-1(ia4)* and *egl-9(sa307)* (11, 16). The *qaIs2241* transgene kills URX, AQR, and PQR neurons (6). A complete strain list is included in *SI Methods*. In all cases, null alleles or strong loss-of-function alleles were used.

Behavioral Assays. Aerotaxis assays were performed by placing animals on nematode growth medium (NGM) agar in custom-made microfluidic devices fabricated from polydimethylsiloxane with a gas-phase linear gradient from 0 to 21% O_2, and monitoring animals' accumulation in nine bins across the gradient after 25 min (4, 6) (see *SI Methods*). For all genotypes and conditions, at least three independent assays of 80–100 animals each were performed on different days. Primary data are in Dataset S1.

For assays on food, thin bacterial lawns of *E. coli* HB101 were made by seeding NGM plates for overnight growth at 37°C and returning them to room temperature for at least 1 h before assay. Animals grown in 1% O_2 were assayed immediately upon removal from the hypoxic chamber.

The hyperoxia avoidance index used in this study differs from that used in ref. 6 to normalize the index for the preference shift toward lower oxygen in hypoxia-reared wild-type animals and *egl-9* mutants (Fig. 1), but is the same index used in ref. 4. It varies from −1.0 (all animals in hyperoxic regions, >14% O_2) to + 1.0 (all animals in normoxic regions, 5–14% O_2) with 0 representing no preference between normoxia and hyperoxia (equal densities of animals in hyperoxic and normoxic regions). Some data published in ref. 6 were recalculated by using the modified hyperoxia avoidance index for presentation here.

Statistical Analysis. Statistical analysis of aerotaxis was conducted as described in ref. 6. The overall distributions of animals in the aerotaxis assay were first compared by χ^2 analysis (9 bins per aerotaxis assay × 2 strains, yielding 8 degrees of freedom). For controls, such as N2 or *egl-9*, that were compared to multiple mutants, $P < 0.01$ was used as the level of significance. Most results were significant at $P < 0.001$ (Dataset S2). Any stated differences in a secondary measure, such as hyperoxia avoidance index or median preferred oxygen concentration, also passed this first test. In the second stage of analysis, unpaired *t* tests were used to compare hyperoxia avoidance indices or median preferred oxygen concentrations. ANOVA plus Bonferroni *t* tests or Dunnett tests were used to correct for multiple comparisons (Statview). Further analysis of sources of variance was conducted by principal components analysis (*SI Methods* and Fig. S6).

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