

The neurobiology of sensing respiratory gases for the control of animal behavior

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Abstract Aerobic metabolism is fundamental for almost all animal life. Cellular consumption of oxygen (O₂) and production of carbon dioxide (CO₂) signal metabolic states and physiologic stresses. These respiratory gases are also detected as environmental cues that can signal external food quality and the presence of prey, predators and mates. In both contexts, animal nervous systems are endowed with mechanisms for sensing O₂/CO₂ to trigger appropriate behaviors and maintain homeostasis of internal O₂/CO₂. Although different animal species show different behavioral responses to O₂/CO₂, some underlying molecular mechanisms and pathways that function in the detection of respiratory gases are fundamentally similar and evolutionarily conserved. Studies of *Caenorhabditis elegans* and *Drosophila melanogaster* have identified roles for cyclic nucleotide signaling and the hypoxia inducible factor (HIF) transcriptional pathway in mediating behavioral responses to respiratory gases. Understanding how simple invertebrate nervous systems detect respiratory gases to control behavior might reveal general principles common to nematodes, insects and vertebrates that function in the molecular sensing of respiratory gases and the neural control of animal behaviors.

Keywords oxygen, carbon dioxide, *C. elegans*, *Drosophila*, respiratory gases, animal behaviors

Introduction

The appearance of life on earth caused dramatic changes in atmospheric O₂ and CO₂ concentrations. The atmosphere of pre-biotic earth had little O₂ and abundant CO₂ (Lenton, 2003; Maina, 1998). The appearance of primitive single-celled organisms with a capacity for photosynthesis increased atmospheric O₂, and in the presence of high O₂ concentrations emerged more complex multicellular organisms that are capable of aerobic respiration. During aerobic respiration, glucose and O₂ are metabolized to generate CO₂, H₂O and the universal currency of cellular energy, ATP. Over billions of years of evolution, O₂ has become essential for most forms of animal life, and CO₂ has become a near-ubiquitous metabolic by-product of cellular respiration (Lenton, 2003; Maina, 1998).

Changes either in internal or external concentrations of CO₂ and O₂ can carry crucial biologic information. Because O₂ and CO₂ are fundamentally involved in metabolism, an organism experiences changes in internal concentrations of these two gases as cellular and organismal metabolism changes. Organisms that rely on aerobic respiration must avoid environments with low O₂ and high CO₂ concentrations. Also, changes in the environmental concentrations of respiratory gases can signal the presence of predators, mates, prey and food quality (Guerenstein and Hildebrand, 2008; Luo et al., 2009; Scott, 2011). Accordingly, animals have evolved mechanisms for sensing both internal and external O₂/CO₂ concentrations to initiate and execute appropriate behavioral responses for optimal survival and reproduction. For example, large vertebrates have developed sophisticated neuronal circulatory and respiratory motor systems for controlling internal concentrations of O₂/CO₂. Animals small enough to exchange respiratory gases by passive diffusion do not require a respiratory motor program. In its absence, however, the only way such animals can control internal concentrations of respiratory gases is to move to

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environments with desirable concentrations of respiratory gases.

Adaptive respiratory movements and foraging behaviors triggered by sensing respiratory gases are examples of acute behavioral responses to O₂ and CO₂ that are mediated by gas-sensing neurons. Animal nervous systems also display homeostatic responses to long-term changes in environmental and internal O₂ concentrations. For example, neurons in both vertebrates and invertebrates can respond to prolonged hypoxia by regulating the expression of genes that modulate neuronal survival and excitability (Bickler and Donohoe, 2002; Powell-Coffman, 2010). In general, acute and homeostatic responses to respiratory gas stimuli recruit distinct mechanisms. Acute responses invoke a sensory cascade leading to rapid activation of a specific motor program (Luo et al., 2009; Scott, 2011). By contrast, homeostatic responses invoke changes in gene expression that leads to *de novo* protein synthesis and modification of cell metabolism, cell physiology, and in the nervous system changes in synapses and circuits.

Studies of genetically tractable model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* have discovered molecular mechanisms by which neurons sense acute and chronic changes in O₂ and CO₂ to control behavior. Because many of the molecular mechanisms uncovered by these studies are present in vertebrates, these studies might elucidate pathways that function in the neuronal control of the respiratory motor program and in homeostatic responses of the brain to hypoxia and hypercapnia in diverse organisms.

Diverse mechanisms of CO₂ sensing by invertebrate and vertebrate neurons

Although CO₂ was long known to exert effects on neuronal physiology and activity, studies of invertebrate sensory neurons were the first to clearly identify receptor-type proteins that mediate the effects of CO₂ on neurons. The olfactory system of *D. melanogaster* contains neurons that are highly specific to CO₂ and that drive an innate avoidance behavior (Suh et al., 2004). Subsequently, two receptor-like proteins, Gr21a and Gr63a, were shown to be necessary for activation of these olfactory neurons (Jones et al., 2007). Moreover, expression of Gr21a and Gr63a sufficed to convert olfactory sensory neurons into CO₂-sensors, suggesting that these two proteins constitute a heteromeric receptor for CO₂ or a CO₂ metabolite. Subsequent to the discovery of CO₂-sensing neurons in the insect olfactory system, CO₂-sensing neurons were discovered in the insect gustatory system (Fischler et al., 2007). Interestingly, these neurons promote ingestive behaviors, indicating that while atmospheric CO₂ is an aversive stimulus, aqueous CO₂ *i.e.* carbonation is appetitive. The molecular receptors mediating activation of CO₂-sensing gustatory neurons have not yet been identified.

D. melanogaster Gr21a and Gr63a proteins are part of an insect-specific family of putative odorant and gustatory receptor proteins that might constitute ligand-gated ion channels. Homologs are found in other insect species, such as the mosquito. In mosquitoes, Gr21a/Gr63a receptors are expressed in maxillary palps, not antennae, where they likely mediate attraction behavior to CO₂ emitted by warm-blooded hosts (Jones et al., 2007). No homolog of the Gr21a/Gr63a receptor exists in mammals. CO₂-sensing neurons of animals in non-insect phyla must, therefore, use different mechanisms to detect external and internal CO₂. Studies of another invertebrate model organism, the nematode *C. elegans*, have revealed such a distinct mechanism. *C. elegans*, like *D. melanogaster*, detect CO₂ as an aversive environmental cue (Bretscher et al., 2008; Hallem and Sternberg, 2008). *C. elegans* have sensory neurons specialized for the detection of CO₂, the BAG neurons (Hallem and Sternberg, 2008; Hallem et al., 2011), and these neurons are required for acute CO₂ avoidance behavior. In addition to acute avoidance of CO₂ stimuli, *C. elegans* can navigate down a CO₂ gradient over longer periods of time. Navigation in a CO₂ gradient does not strictly depend on the BAG neurons, and requires the function of multiple types of sensory neuron, which display distinct physiologic responses to CO₂ stimuli (Bretscher et al., 2008). Behavioral responses of *C. elegans* to CO₂, therefore, are triggered either by specific activation of the BAG sensory neurons, or by activating a distributed neural circuit comprising multiple types of sensory neuron.

All *C. elegans* sensory neurons known to function in CO₂-sensing use transduction pathways that generate cyclic nucleotides as second messengers to activate cyclic nucleotide-gated ion channels. In this regard, these *C. elegans* sensory neurons are similar to vertebrate olfactory sensory neurons and photoreceptors. The BAG neurons, which mediate acute CO₂ avoidance, require a receptor-type guanylate cyclase, GCY-9, for CO₂ detection (Hallem et al., 2011). GCY-9 is one of many integral membrane receptor-type proteins with cyclase homology domains encoded by the *C. elegans* genome (Yu et al., 1997). Recently, expression of GCY-9 was shown to be sufficient to confer CO₂ sensitivity upon sensory neurons, suggesting that it acts as a receptor in BAG neurons for CO₂ or a CO₂ metabolite (Brandt et al., 2012). The molecular mechanisms of CO₂-sensing by *C. elegans* BAG neurons might be conserved between nematodes and mammals; the rodent olfactory system contains CO₂-sensitive neurons that are marked by expression of a receptor-type cyclase, GC-D (Hu et al., 2007). On the basis of *in vitro* studies of its enzyme activity, GC-D has been proposed to act as a receptor for bicarbonate, a CO₂ metabolite (Guo et al., 2009; Sun et al., 2009). If GC-D, as is GCY-9 in nematode sensory neurons, is necessary and sufficient for CO₂ detection by olfactory neurons, there might be an evolutionarily ancient and conserved role for receptor-type cyclases in CO₂ sensation.

C. elegans BAG neurons and vertebrate CO₂-sensing

neurons might also use related cell-fate specification pathways. BAG neurons require a conserved transcription factor, ETS-5, to promote expression of BAG-neuron-specific genes and to sense CO₂ (Guillermin et al., 2011; Brandt et al., 2012). A likely mammalian homolog of ETS-5, Pet1, is likewise required for specification of serotonergic neurons in the vertebrate brain stem (Hendricks et al., 1999). *In vitro* some of these neurons are activated by CO₂ (Richerson, 2004), and *in vivo* serotonergic neurons of the brainstem are required for the respiratory chemoreflex (Hodges et al., 2008; Ray et al., 2011). In *C. elegans* BAG neurons, ETS-5 directly regulates expression of the GCY-9 cyclase; it is possible that Pet1 likewise regulates the expression of a CO₂ receptor in a subset of brainstem neurons.

Hydration of CO₂ generates carbonic acid and causes acidosis. In some instances, CO₂ is detected via pH-sensing mechanisms. Studies of the rodent gustatory system revealed that, like insect gustatory neurons, some taste-receptor neurons of mammals are activated by aqueous CO₂ *i.e.* carbonation (Chandrashekar et al., 2009). Carbonated solutions activate acid-sensitive sour-taste neurons, and this activation requires a cell-surface-tethered isoform of carbonic anhydrase, an enzyme that catalyzes the reaction of CO₂ with water to generate free protons (Chandrashekar et al., 2009).

Another example of CO₂-sensing via acidosis is in the triggering by CO₂ of an innate anxiety behavior of rodents: freezing. Rodent freezing behavior requires a central brain structure, the amygdala, which was recently shown to be acid-sensing (Ziemann et al., 2009). Exposure to a high-CO₂ environment results in large changes in the pH of the amygdala *in vivo* and subsequent activation of amygdala neurons. The molecular basis of the acid-sensitivity of amygdala has been identified; amygdala neurons express acid-sensitive ASIC channels that depolarize neurons upon activation. Although the ethological relevance of CO₂-evoked freezing behavior is unclear, a relationship between internal CO₂ levels and anxiety behaviors has been previously observed in the clinic; subjects diagnosed with anxiety disorders are prone to experiencing panic attacks in response to a respiratory CO₂ challenge (Papp et al., 1993). The intrinsic sensitivity of the amygdala to acid stimuli might explain this connection between CO₂ and panic attacks in human subjects, and might provide a mechanistic basis for a 'suffocation-alarm' theory of panic disorders (Klein, 1993; Ziemann et al., 2009).

Respiratory centers of the vertebrate brain are also activated by acidosis. Multiple types of brainstem neurons are activated by acid stimuli, including serotonergic neurons and neurons of the retrotrapezoid nucleus (Richerson, 2004; Spyer, 2009). In other chemosensitive brainstem areas, acidosis caused by increased CO₂ levels activates pH-sensitive glia, which release ATP and trigger neuronal activity (Gourine et al., 2005; Gourine et al., 2010). How distinct brainstem cell-types detect acidosis, how the detection of CO₂ by distinct brainstem circuits is integrated in *in vivo*, and

whether CO₂ regulates brainstem chemosensitive neurons in a pH-independent manner remain to be determined.

O₂ sensing by invertebrate neurons

Behavioral studies of *C. elegans* have also led to the discovery of mechanisms by which neurons detect O₂. *C. elegans* is a free-living nematode species that inhabits soils and microbe-rich environments in which O₂ levels are usually far below the ambient level of 21% (Anderson and Ultsch, 1987; Félix and Braendle, 2010). Under laboratory conditions, *C. elegans* prefers O₂ concentrations of 5% to 10%, and navigates in an O₂ gradient to this preferred concentration range (Gray et al., 2004; Chang and Bargmann, 2008). Avoidance of high O₂ concentrations by *C. elegans* requires four O₂-sensing neurons: URXL/R, AQR and PQR. These neurons, like the CO₂-sensing BAG neurons, use cyclic nucleotide signaling. O₂-sensing neurons of *C. elegans* require both cyclic nucleotide-gated ion channels and a guanylate cyclase comprising subunits encoded by the genes *gcy-35* and *gcy-36*. The GCY-35/GCY-36 cyclase is a cytoplasmic heme-containing enzyme that directly interacts with molecular oxygen (Gray et al., 2004). Expression of the GCY-35/GCY-36 cyclase in BAG neurons confers upon BAG neurons the ability to respond to hyperoxic stimuli (Zimmer et al., 2009), indicating that this enzyme is sufficient to mediate O₂ detection when expressed in sensory neurons.

Genetic studies of hyperoxia avoidance by *C. elegans* have identified another gene that functions in O₂ sensing. The neuronal globin GLB-5 functions in O₂-sensing neurons and is required for behavioral discrimination between similar, high concentrations of O₂ (Gray et al., 2004; McGrath et al., 2009; Persson et al., 2009). Animals carrying a polymorphism in the *glb-5* locus cannot discriminate between 20% and 21% O₂, likely as a consequence of loss of *glb-5* function. GLB-5 is required in O₂-sensing neurons, where it is required for their physiologic activation by small increases in O₂. Like the GCY-35/GCY-36 cyclase, GLB-5 contains a heme prosthetic group that directly interacts with O₂. How GLB-5 changes the function of O₂-sensing neurons to permit their activation by small changes in atmospheric O₂ levels remains to be determined. The *C. elegans* genome encode more than 30 related globin genes, many of which are expressed by neurons, suggesting that many *C. elegans* circuits, even those not directly regulated by O₂-sensing neurons, might be modulated by O₂.

In addition to hyperoxia-avoidance behaviors, *C. elegans* displays an acute avoidance response to hypoxia (Zimmer et al., 2009). This response requires the CO₂-sensing BAG neurons and yet another guanylate cyclase, this one comprising GCY-31 and GCY-33 subunits. The GCY-31/GCY-33 cyclase is related to the GCY-35/GCY-36 cyclase and contains a heme group; unlike the GCY-35/GCY-36 cyclase, O₂ binding is thought to inhibit the activity of the GCY-31/

GCY-33 cyclase. Indeed, related invertebrate cyclases have been shown to be directly inhibited by O₂ (see below).

Like studies of *C. elegans*, behavioral genetic studies of *D. melanogaster* have discovered roles for cytoplasmic guanylate cyclases with heme domains as molecular O₂ sensors that control acute behavioral responses to changes in O₂ levels (Morton, 2011). Three *Drosophila* GC subunits — Gcy89-Da, Gcy89-Db, and Gcy88E - can constitute a cyclase that directly binds O₂ (Morton, 2004; Huang et al., 2007). These cyclase subunits are related to *C. elegans* GCY cyclases, and like their *C. elegans* counterparts, these *D. melanogaster* cyclases function in behavioral responses to changes in environmental O₂ (Vermehren-Schmaedick et al., 2010). *D. melanogaster* O₂-sensing neurons express different combinations of cyclase subunits and mediate responses to different O₂ stimuli. Gcy89-Da-expressing neurons are required for responses to O₂ downshifts from 16% to 11%; Gcy89-Db-expressing neurons mediate responses to O₂ up shift from 21% to 30% (Vermehren-Schmaedick et al., 2010). In both *C. elegans* and *Drosophila*, the O₂-responding properties of neurons are largely determined by the expression of different cyclases, which are regulated in distinct ways by molecular O₂. Other cell-intrinsic factors, which have not been defined, also contribute to the differential roles of O₂-sensing neurons in O₂-dependent behaviors (Zimmer et al., 2009; Vermehren-Schmaedick et al., 2010).

C. elegans show robust behavioral responses to O₂ changes ranging from 5% to 21%. O₂ levels below 5%, which are ethologically relevant to wild strains of *C. elegans*, can also have dramatic influences on animal behavior and physiology (Anderson and Ultsch, 1987; Powell-Coffman, 2010). Prolonged anoxia (0% O₂) causes *C. elegans* to enter a behavioral state of “suspended animation” characterized by drastically reduced metabolic rates and locomotion speeds (Padilla et al., 2002). Brief exposure of *C. elegans* to anoxia and subsequent restoration of O₂ levels (5%, 10%, or 20%) elicit robust locomotive behavioral responses that are independent of the known O₂-sensing neurons (URXL/R, AQR, PQR) and the O₂ sensors GCY-31/GCY-33 and GCY-35/GCY-36 (Ma et al., 2012). The molecular and cellular O₂ sensors and the mechanisms for these anoxia/reoxygenation-induced behaviors are unknown.

Modulation of *C. elegans* behaviors by chronic hypoxia

Aerotaxis behavior of *C. elegans* can be modified by prior experience of hypoxia (Chang and Bargmann, 2008). Wild-type animals normally prefer O₂ concentrations at around 10%. By contrast, animals that have been cultivated in hypoxic conditions (48 h at 0.5% O₂) prefer lower O₂ concentration at around 8%. This modification of *C. elegans* O₂ preference by hypoxia experience requires the proline hydroxylase EGL-9 (Chang and Bargmann, 2008), which

uses molecular O₂ as a substrate for the hydroxylation of target proteins. The canonical target of EGL-9 in hypoxia pathways is the transcription factor HIF (Epstein et al., 2001), which is hydroxylated and degraded under normoxic conditions. Under hypoxic conditions, EGL-9 cannot efficiently hydroxylate HIF resulting in its accumulation and the activation of HIF target genes (Epstein et al., 2001). The *C. elegans* HIF homolog, *hif-1*, is partly required for the hypoxia-induced change in aerotaxis behavior, and the hydroxylase EGL-9 is completely required indicating that HIF-1 is not the sole substrate of EGL-9 required for modification of aerotaxis behavior. Surprisingly, HIF-1 activation changes what neurons are required for hyperoxia avoidance, suggesting that the underlying aerotaxis neural circuit undergoes “reorganization” by hypoxia experience (Chang and Bargmann, 2008).

Chronic hypoxia and the HIF pathway also change a gustatory circuit in *C. elegans*. Under normoxic conditions, *C. elegans* exhibits chemotaxis behavior in response to sodium chloride (NaCl) in a manner that requires the ASE chemosensory neurons (Bargmann et al., 1993). Prolonged hypoxia enhances NaCl chemotaxis through the HIF-1-dependent upregulation of TPH-1, a biosynthetic enzyme for the neural modulator serotonin, in neurons that are not required for sensory processing under normoxic conditions (Pocock and Hobert, 2010). This remarkable finding demonstrates that hypoxia can regulate the neurotransmitter identity of neurons and demonstrates a specific mechanism by which hypoxia modifies neural circuits and behavior.

The *C. elegans* locomotory response to restoration of high O₂ levels after brief exposure of anoxia (the so-called “O₂-ON response”) is also modulated by prior exposure to hypoxia (Ma et al., 2012). Unlike naïve animals, which robustly accelerate during the O₂-ON response, animals exposed to 0.5% O₂ for 24 h, followed by 2 h of recovery at room air, show a suppressed O₂-ON response. This hypoxia-induced suppression of the O₂-ON response requires both HIF-1 and EGL-9. Also required is a cysteine synthase or sulfhydrylase-like protein CYSL-1, which was identified from a screen for EGL-9 regulators. CYSL-1 functions by sequestering EGL-9 and thereby inhibiting EGL-9-mediated hydroxylation of HIF-1 during hypoxia. Interestingly, the interaction between EGL-9 and CYSL-1 is modulated by the gas hydrogen sulfide (H₂S), which accumulates under hypoxic conditions because of reduced oxidation (Olson, 2011a, b). Prior experience of hypoxia might produce preconditioning effects that modulate the O₂-ON response in response to anoxia/reoxygenation-induced cellular signals, analogous to alleviation of the reperfusion injury response by hypoxic preconditioning in mammals (Semenza, 2011a). In this context, EGL-9 acts as a homeostatic O₂ sensor to control a transcriptional pathway to enable behavioral state changes in a hypoxia experience-dependent manner. The underlying mechanisms that trigger CYSL-1 regulation of the O₂-sensing EGL-9 hydroxylase and how unidentified HIF-1 targets

modify the acute locomotive O₂-ON behavioral response await further investigation.

Mechanisms of O₂ sensing by mammalian carotid bodies

Changes in environmental O₂ can induce rapid behavioral responses in mammals, notably responses in the respiratory motor program. Hypoxia, for example, causes a rapid increase in the intensity and frequency of breaths, the hypoxic ventilatory response (HVR). In mammals, O₂ levels in blood are sensed by chemoreceptors in the carotid body, a specialized tissue located near the bifurcation of the carotid artery that is innervated by fibers of the glossopharyngeal nerve (Prabhakar, 2005; Teppema and Dahan, 2010). Physiologic responses of O₂-sensitive cells of the carotid body have been extensively studied. Neuron-like cells of the carotid body release ATP in response to hypoxic stimuli, exciting neurons that express purinergic receptors and project to respiratory centers of the brainstem (Prabhakar, 2005; Teppema and Dahan, 2010). The molecular nature of the O₂ sensor that functions in carotid body is not known. A list of candidates includes: O₂-sensitive potassium channels, AMP-activated protein kinase (AMPK), plasma membrane bound NADPH oxidase hemoxygenases and mitochondrial complex III (Prabhakar, 2005; Olson and Whitfield, 2010; Peng et al., 2010; Olson, 2011a).

Emerging lines of evidence support the hypothesis that O₂-sensing by the carotid body recruits signaling by a gas messenger, hydrogen sulfide (H₂S) (Olson et al., 2006; Li et al., 2010; Peng et al., 2010). Under normoxic conditions, endogenous H₂S is produced by several thiol metabolic enzymes, including cystathionine γ -lyases (CSE) and cystathionine-beta-synthases (CBS), but is constantly oxidized, mainly in mitochondria, and remains at very low levels (Olson, 2011a; Singh et al., 2009). Under hypoxic conditions, H₂S levels increase rapidly, and increases in H₂S might depolarize O₂-sensing cells of the carotid body either through inhibition of ATP-sensitive potassium channels or through activation of L-type Ca²⁺ channels (Li et al., 2010; Olson, 2011a; Peng et al., 2010). Although genetic and pharmacological evidence supports the hypothesis that CBS or CSE-biosynthesized H₂S mediates O₂ sensing, many questions remain. It is not known how endogenous H₂S at physiologic concentrations is oxidized under normoxic conditions, how H₂S activates carotid body cells under hypoxic conditions, and to what extent H₂S might interact with other proposed O₂-sensing mechanisms to coordinate the hypoxic response. Nevertheless, the proposed role of H₂S in O₂ sensing by mammalian carotid bodies might have an interesting parallel in invertebrates; as described above, hypoxia experience recruits H₂S signaling to modulate the suppression of the acute O₂-ON behavioral response in *C. elegans* (Ma et al., 2012). Given that H₂S biosynthetic enzymes are

evolutionarily conserved (Kimura, 2010; Vozdek et al., 2012) and hypoxia can trigger rapid and large increases of H₂S accumulation (Olson, 2011b), acute O₂ sensing mediated by H₂S might operate in both vertebrates and invertebrates, including *C. elegans* and *D. melanogaster*. Furthermore, prolonged or chronic hypoxia modulates the effects of hypoxia on the respiratory motor program via the mammalian EGLN/HIF pathway (Teppema and Dahan, 2010), underscoring the importance the evolutionarily conserved homeostatic transcriptional EGLN/HIF pathway to mediate the plasticity of acute behavioral responses to O₂ level changes in diverse animal species.

Conclusions and future directions

Studies of many organisms have identified molecular mechanisms required for sensing respiratory gases and generating behavioral responses. Some of the gas-sensing mechanisms discovered in *C. elegans* neurons and *D. melanogaster* neurons are fundamentally similar and have likely been conserved through evolution (Fig. 1). These studies show that gas-sensing neurons of different animals can mediate different behavioral responses to changes in respiratory gases, reflecting adaptation of a fundamental sensory system to different ecological niches.

Cyclic nucleotide signaling and the HIF transcriptional pathway have emerged as conserved major mediators of acute and homeostatic responses, respectively, to modulate diverse animal behaviors (Fig. 1). In both *C. elegans* and *D. melanogaster*, cyclic nucleotide signaling systems mediate gas-sensing by neurons that drive acute behavioral responses to environmental changes of O₂/CO₂ rapidly. In vertebrates, soluble guanylate cyclases are well characterized receptors in both non-neuronal cells and neurons for the signaling gas nitric oxide (Potter, 2011). A role for cyclic nucleotide signaling in CO₂ sensation was suggested by recent studies of the rodent olfactory system (Hu et al., 2007). It remains to be determined whether other modes of gas-sensing by vertebrate neurons are mediated by cyclic nucleotide signals, and whether vertebrate cyclases themselves play a central role as gas receptors as they do in invertebrate model organisms.

In both vertebrates and invertebrates, the HIF transcriptional pathway functions in homeostatic responses to changes in O₂. Unlike cyclic nucleotide signaling, HIF mediates transcriptional responses to chronic changes in O₂ by regulating gene expression. In the HIF pathway, the sensor for molecular O₂ is an evolutionarily conserved HIF hydroxylase, which uses O₂ as a substrate to hydroxylate HIF and target it for proteosomal degradation. HIF hydroxylases have low affinities for O₂ ($K_m = 100\text{--}250\ \mu\text{M}$) (Ehrismann et al., 2007; Ward, 2008) rendering them particularly suitable for sensing ambient O₂ levels and driving homeostatic hypoxic adaptation in nearly all

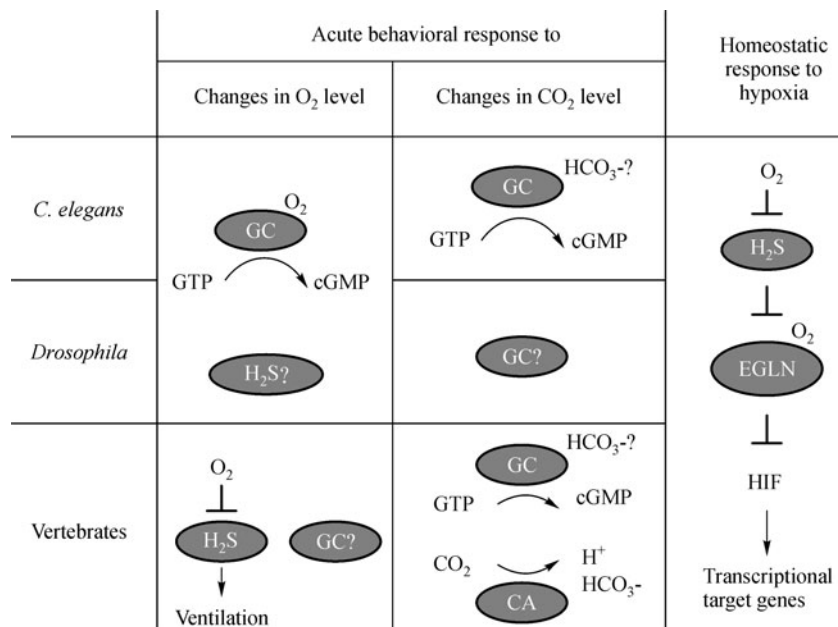


Figure 1 Schematic molecular mechanisms of sensing O₂ or CO₂ levels to direct acute behavioral or homeostatic responses in *C. elegans*, *D. melanogaster* and vertebrates. In both *C. elegans* and *Drosophila*, sensing changes in O₂ levels to direct acute behavioral responses is mediated by atypical nucleotide guanylate cyclases (GCs) that can bind O₂, which regulates the enzymatic activity of GCs to convert GTP to cyclic GMP. In vertebrates, sensing reduction in O₂ levels to direct ventilation responses in the carotid body appears to be mediated by H₂S, which increases in levels upon hypoxia. There are also acute behaviors that require mechanisms independently of cyclic nucleotides in *C. elegans* and it remains unknown whether vertebrates also use GCs to modulate ventilation. Sensing changes in CO₂ levels in both *C. elegans* and vertebrates appears to be mainly mediated by GCs and/or adenylate cyclases (ACs); whether it is also the case in *Drosophila* remains to be seen. The perception of carbonation in mammals uses pH-sensing mechanisms via carbonic anhydrase (CA)-generated protons. In all animal species examined so far, an evolutionarily conserved transcriptional pathway mediates the homeostatic response to hypoxia. The O₂-sensing hydroxylase EGLN family proteins is modulated by antagonizing actions of O₂ and H₂S to inhibit HIF transcription factors, which ultimately direct adaptive responses to hypoxia by the transcriptional regulation of its numerous target genes.

metazoans, from humans to the simplest animal *Trichoplax adhaerens* (Loenarz et al., 2011; Semenza, 2011b). In vertebrates, the HIF pathway is best understood as driving changes to cell metabolism and cell physiology in response to hypoxia. Studies of invertebrate models have revealed roles for HIF in remodeling neuronal circuits and mediating adaptive behavioral responses to chronic hypoxia. Whether the HIF function plays a similar role in the mammalian brain remains to be determined. If so, studies of HIF-mediated remodeling of neural circuits might not only elucidate mechanisms of adaptive behavioral responses to hypoxia but might also serve as powerful and general models for understanding molecular and neural circuit mechanisms that drive behavioral plasticity.

Much progress has been made in understanding the molecular basis of behavioral controls by O₂/CO₂; many challenges remain. First, studies of invertebrate models have provided evidence for the existence of gas sensors whose molecular identities remain to be determined (Morton, 2011; Ma et al., 2012). Second, we need to understand at both the molecular and neural circuit levels how the initial signals generated by the gas-sensing neurons are translated into

motor programs, and how those programs are modulated by experience to generate behavioral plasticity. Genetically tractable model organisms, including *C. elegans* and *Drosophila*, will continue to play crucial roles in advancing our understanding of the mechanisms underlying O₂/CO₂ -sensing and biologic responses to them. Finally, since defective or abnormal responses to O₂ and CO₂ are critically involved in a wide variety of human diseases (Quaegebeur and Carmeliet, 2010; Semenza, 2011b), including blood diseases, behavioral disorders, neurodegeneration and cancer, it is an important and rewarding challenge to translate the knowledge learned from O₂ and CO₂-related basic biology into clinically useful interventions and medicines in the future to benefit human health.

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