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

Dengke K. Ma[1] and **Niels Ringstad**[2]

Dengke K. Ma: dkma@mit.edu; Niels Ringstad: Niels.Ringstad@med.nyu.edu

[1]Department of Biology, and McGovern Institute for Brain Research, MIT, Cambridge, MA 02139, USA

[2]Department of Cell Biology and the Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, New York University Langone Medical Center, New York NY 10016

Abstract

Aerobic metabolism is fundamental for almost all animal life. Cellular consumption of oxygen (O_2) and production of carbon dioxide (CO_2) signal metabolic states and physiological 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 O_2/CO_2 to trigger appropriate behaviors and maintain homeostasis of internal O_2/CO_2. Although different animal species show different behavioral responses to O_2/CO_2, 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 O_2 and CO_2 concentrations. The atmosphere of pre-biotic Earth had little O_2 and abundant CO_2 (Lenton, 2003; Maina, 1998). The appearance of primitive single-celled organisms with a capacity for photosynthesis increased atmospheric O_2, and in the presence of high O_2 concentrations emerged more complex multicellular organisms that are capable of aerobic respiration. During aerobic respiration, glucose and O_2 are metabolized to generate CO_2, H_2O and the universal currency of cellular energy, adenosine-5′-triphosphate (ATP). Over billions of years of evolution, O_2 has become essential for most forms of animal life, and CO_2 has become a near-ubiquitous metabolic by-product of cellular respiration (Lenton, 2003; Maina, 1998).

Correspondence to: Dengke K. Ma, dkma@mit.edu; Niels Ringstad, Niels.Ringstad@med.nyu.edu.

Changes either in internal or external concentrations of CO_2 and O_2 can carry crucial biological information. Because O_2 and CO_2 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 O_2 and high CO_2 concentrations. Also, changes in the environmental concentrations of respiratory gases can signal the presence of predators, mates and prey, as well as food quality (Guerenstein and Hildebrand, 2008; Luo et al., 2009; Scott, 2011). Accordingly, animals have evolved mechanisms for sensing both internal and external O_2/CO_2 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 O_2/CO_2. Animals small enough to exchange respiratory gases by passive diffusion do not require a respiratory motor organ. In its absence, however, the only way such animals can control internal concentrations of respiratory gases is to move to environments with desirable concentrations of respiratory gases.

Adaptive respiratory movements and foraging behaviors triggered by respiratory gases are examples of acute behavioral responses to O_2 and CO_2 that are mediated by gas-sensing neurons. Animal nervous systems also display homeostatic responses to long-term changes in environmental and internal O_2 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 can invoke a sensory cascade leading to activation of a specific motor program (Luo et al., 2009; Scott, 2011). By contrast, homeostatic responses can invoke changes in gene expression that leads to *de novo* protein synthesis and modification of cell metabolism, cell physiology, and neural 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 O_2 and CO_2 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.

Mechanisms of CO_2 sensing by invertebrate and vertebrate neurons

Although CO_2 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 CO_2 on neurons. The olfactory system of *D. melanogaster* contains neurons that are highly specific to CO_2 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 CO_2-responders, suggesting that these two proteins might constitute a heteromeric receptor for CO_2 or a CO_2 metabolite. Subsequent to the discovery of CO_2-responding neurons in the insect olfactory system, CO_2-responding neurons were discovered in the insect gustatory system (Fischler et al., 2007). Interestingly, these neurons promote ingestive behaviors, indicating that while atmospheric CO_2 is an aversive stimulus, aqueous CO_2 (carbonation) is appetitive. The molecular receptors mediating activation of CO_2-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 CO_2 emitted by warm-blooded hosts (Jones et al., 2007). No homolog of the Gr21a/Gr63a receptor exists in mammals. CO_2-sensing neurons of animals in non-insect phyla must, therefore, use different mechanisms to detect external and internal CO_2. Studies of another invertebrate model organism, the nematode *C. elegans*, have revealed such a distinct mechanism.

C. elegans, like *D. melanogaster*, detect CO_2 as an aversive environmental cue (Bretscher et al., 2008; Hallem and Sternberg, 2008). *C. elegans* have sensory neurons specialized for the detection of CO_2, the BAG neurons (Hallem et al., 2011; Hallem and Sternberg, 2008), and these neurons are required for acute CO_2 avoidance behavior. In addition to acute avoidance of CO_2 stimuli, *C. elegans* can navigate to a preferred CO_2 concentration in a CO_2 gradient over longer periods of time. Navigation in a CO_2 gradient does not strictly depend on the BAG neurons, and requires the function of multiple types of sensory neuron, which display distinct physiological responses to CO_2 stimuli (Bretscher et al., 2008). Behavioral responses of *C. elegans* to CO_2, 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 CO_2-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 CO_2 avoidance, require a receptor-type guanylate cyclase, GCY-9, for CO_2 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 CO_2 sensitivity to other sensory neurons, suggesting that it acts as a receptor in BAG neurons for CO_2 or a CO_2 metabolite (Brandt et al., 2012). The molecular mechanism of CO_2-sensing by *C. elegans* BAG neurons might be conserved between nematodes and mammals; the rodent olfactory system contains CO_2-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 CO_2 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 CO_2 detection by olfactory neurons, there might be an evolutionarily ancient and conserved role for receptor-type cyclases in CO_2 sensation.

C. elegans BAG neurons and vertebrate CO_2-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 CO_2 (Brandt et al., 2012; Guillermin et al., 2011). A likely mammalian homolog of ETS-5, Pet1, is likewise required for specification of serotonergic neurons in the vertebrate brainstem (Hendricks et al., 1999). *In vitro* some of these neurons are activated by CO_2 (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 CO_2 receptor in a subset of brainstem neurons (Brandt et al., 2012).

Hydration of CO_2 generates carbonic acid and causes acidosis. In some instances, CO_2 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 CO_2 *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 CO_2 with water to generate free protons (Chandrashekar et al., 2009).

Another example of CO_2-sensing via acidosis is in the triggering by CO_2 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-CO_2 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 the amygdala has been identified: amygdala neurons express acid-sensitive ASIC channels that depolarize neurons upon activation. Although the ethological relevance of CO_2-evoked freezing behavior is unclear, a relationship between internal CO_2 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 CO_2 challenge (Papp et al., 1993). The intrinsic sensitivity of the amygdala to acid stimuli might explain this connection between CO_2 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 CO_2 levels activates pH-sensitive glia, which release ATP and trigger neuronal activity (Gourine et al., 2010; Gourine et al., 2005). How distinct brainstem cell-types detect acidosis, how the detection of CO_2 by distinct brainstem circuits is integrated in *in vivo*, and whether CO_2 regulates brainstem chemosensitive neurons in a pH-independent manner remain to be determined.

O_2 sensing by invertebrate neurons

Behavioral studies of *C. elegans* have also led to the discovery of mechanisms by which neurons detect O_2. *C. elegans* is a free-living nematode species that inhabits soils and microbe-rich environments in which O_2 levels are usually far below the ambient level of 21% (Anderson and Ultsch, 1987; Felix and Braendle, 2010). Under laboratory conditions, *C. elegans* prefers O_2 concentrations of 5% to 10%, and navigates in an O_2 gradient to this preferred concentration range (Chang and Bargmann, 2008; Gray et al., 2004). Avoidance of high O_2 concentrations by *C. elegans* requires four O_2-sensing neurons: URXL/R, AQR and PQR. These neurons, like the CO_2-sensing BAG neurons, use cyclic nucleotide signaling. O_2-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 O_2 (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 O_2 detection when expressed in sensory neurons.

Genetic studies of hyperoxia avoidance by *C. elegans* have identified another gene that functions in O_2 sensing. The neuronal globin GLB-5 functions in O_2-sensing neurons and is required for behavioral discrimination between similar, high concentrations of O_2 (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% O_2, likely as a consequence of

loss of *glb-5* function. GLB-5 functions in O_2-sensing neurons, where it is required for their physiological activation by small increases in O_2. Like the GCY-35/GCY-36 cyclase, GLB-5 contains a heme prosthetic group that directly interacts with O_2. How GLB-5 changes the function of O_2-sensing neurons to permit their activation by small changes in atmospheric O_2 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 O_2-sensing neurons, might be modulated by O_2.

In addition to hyperoxia-avoidance behaviors, *C. elegans* displays an acute avoidance response to loss of O_2 (Zimmer et al., 2009). This response requires the CO_2-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, O_2-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 O_2 (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 O_2 sensors that control acute behavioral responses to changes in O_2 levels (Morton, 2011). Three *Drosophila* GC subunits - Gcy89-Da, Gcy89-Db, and Gcy88E - can constitute a cyclase that directly binds O_2 (Huang et al., 2007; Morton, 2004). 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 O_2 (Vermehren-Schmaedick et al., 2010). *D. melanogaster* O_2-sensing neurons express different combinations of cyclase subunits and mediate responses to different O_2 stimuli. Gcy89-Da-expressing neurons are required for responses to an O_2 downshift from 16% to 11%; Gcy89-Db-expressing neurons mediate responses to an O2 upshift from 21% to 30% (Vermehren-Schmaedick et al., 2010). In both *C. elegans* and *Drosophila*, the O_2-responding properties of neurons are largely determined by the expression of different cyclases. Other cell-intrinsic factors, which have not been defined, also contribute to the differential roles of O_2-sensing neurons in O_2-dependent behaviors (Vermehren-Schmaedick et al., 2010; Zimmer et al., 2009).

C. elegans show robust behavioral responses to O_2 changes ranging from 5% to 21%. O_2 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% O_2) 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 O_2 levels (5%, 10%, or 20%) elicit robust locomotive behavioral responses that are independent of the known O_2-sensing neurons (URXL/R, AQR, PQR) and the O_2 sensors GCY-31/GCY-33 and GCY-35/GCY-36 (Ma et al., 2012). The molecular and cellular O_2 sensors and the mechanisms for these anoxia/reoxygenation-induced behaviors are unknown.

Modulation of *C. elegans* behaviors by chronic hypoxia

The aerotaxis behavior of *C. elegans* can be modified by prior experience of hypoxia (Chang and Bargmann, 2008). Wild-type animals normally prefer O_2 concentrations around 10%. By contrast, animals that have been cultivated in hypoxic conditions (48 hours at 0.5% O_2) prefer lower O_2 concentrations around 8%. This modification of *C. elegans* O_2 preference by hypoxia experience requires the proline hydroxylase EGL-9 (Chang and Bargmann,

2008), which uses molecular O_2 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* is attracted to sodium chloride (NaCl), and this attraction requires the ASE chemosensory neurons (Bargmann et al., 1993). Prolonged hypoxia enhances NaCl chemotaxis through the HIF-1-dependent up-regulation 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 O_2 levels after brief exposure of anoxia (the so-called "O_2-ON response") is also modulated by prior exposure to hypoxia (Ma et al., 2012). Unlike naïve animals, which robustly accelerate when O_2 is restored to normal levels, animals exposed to 0.5% O_2 for 24 hours, followed by 2 hours of recovery at room air, show a suppressed O_2-ON response. This hypoxia-induced suppression of the O_2-ON response requires both HIF-1 and EGL-9. This suppression of behavior also requires 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 (H_2S), which accumulates under hypoxic conditions because of reduced oxidation (Olson, 2011a, b). Prior experience of hypoxia might produce preconditioning effects that modulate the O_2-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 O_2 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 O_2-sensing EGL-9 hydroxylase and how unidentified HIF-1 targets modify the acute locomotive O_2-ON behavioral response await further investigation.

Mechanisms of O_2 sensing by mammalian carotid bodies

Changes in environmental O_2 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, O_2 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, 2006; Teppema and Dahan, 2010). Physiological responses of O_2-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, 2006; Teppema and Dahan, 2010). The molecular nature of the O_2

sensor that functions in carotid body is not known. A list of candidates includes: O_2-sensitive potassium channels, AMP-activated protein kinase (AMPK), plasma membrane bound NADPH oxidase, heme oxygenases and mitochondrial complex III (Olson, 2011a; Olson and Whitfield, 2010; Peng et al., 2010; Prabhakar, 2006).

Emerging lines of evidence support the hypothesis that O_2-sensing by the carotid body recruits signaling by a gas messenger, hydrogen sulfide (H_2S) (Li et al., 2010; Olson et al., 2006; Peng et al., 2010). Under normoxic conditions, endogenous H_2S 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, H_2S levels increase rapidly, and increases in H_2S might depolarize O_2-sensing cells of the carotid body either through inhibition of ATP-sensitive potassium channels or through activation of L-type Ca^{2+} channels (Li et al., 2010; Olson, 2011a; Peng et al., 2010). Although genetic and pharmacological evidence supports the hypothesis that CBS or CSE-biosynthesized H_2S mediates O_2 sensing, many questions remain. It is not known how endogenous H_2S at physiological concentrations is oxidized under normoxic conditions, how H_2S activates carotid body cells under hypoxic conditions, and to what extend H_2S might interact with other proposed O_2-sensing mechanisms to coordinate the hypoxic response. Nevertheless, the proposed role of H_2S in O_2 sensing by mammalian carotid bodies might have an interesting parallel in invertebrates; as described above, hypoxia experience recruits H_2S signaling to modulate the suppression of the acute O_2-ON behavioral response in *C. elegans* (Ma et al., 2012). Given that H_2S biosynthetic enzymes are evolutionarily conserved (Kimura, 2010; Vozdek et al., 2012) and hypoxia can trigger rapid and large increases of H_2S accumulation (Olson, 2011b), acute O_2 sensing mediated by H_2S 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). These findings underscore the importance of the evolutionarily conserved homeostatic EGLN/HIF pathway in mediating the plasticity of acute behavioral responses to O_2 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 (Figure 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 major conserved mediators of acute and homeostatic responses, respectively, to modulate diverse animal behaviors (Figure 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 O_2/CO_2 rapidly. In vertebrates, soluble guanylate cyclases are well characterized receptors in both non-neuronal cells and neurons for signaling nitric oxide gas (Potter, 2011). A role for cyclic nucleotide signaling in CO_2 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 O_2. Unlike cyclic nucleotide signaling, HIF mediates transcriptional responses to chronic changes in O_2 by regulating gene expression. In the HIF pathway, the sensor for molecular O_2 is an evolutionarily conserved HIF hydroxylase, which uses O_2 as a substrate to hydroxylate HIF and target it for proteosomal degradation. HIF hydroxylases have low affinities for O_2 (K_m = 100–250 μM) (Ehrismann et al., 2007) (Ward, 2008) rendering them particularly suitable for sensing ambient O_2 levels and driving homeostatic hypoxic adaptation in nearly all 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 HIF similarly functions 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 O_2/CO_2; many challenges remain. First, studies of invertebrate models have provided evidence for the existence of gas sensors whose molecular identities remain to be determined (Ma et al., 2012; Morton, 2011). 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 O_2/CO_2 -sensing and biological responses to them. Finally, since defective or abnormal responses to O_2 and CO_2 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 O_2 and CO_2-related basic biology into clinically useful interventions and medicines to benefit human health.

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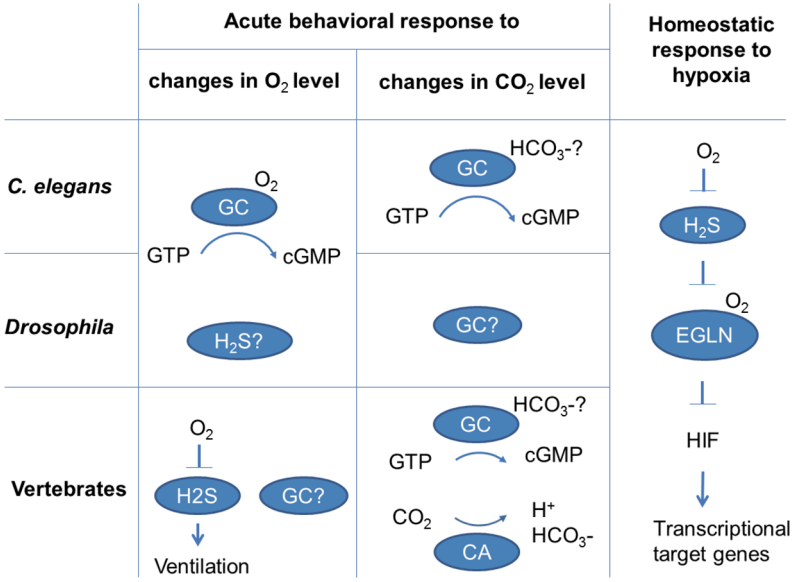

Figure 1. Schematic molecular mechanisms of sensing O_2 or CO_2 to direct acute behavioral or homeostatic responses in *C. elegans, D. melanogaster* and vertebrates

In both *C. elegans* and *Drosophila*, changes in O_2 levels directs acute behavioral responses mediated by atypical nucleotide guanylate cyclases (GCs). GCs can bind O_2, which regulates the enzymatic activity of GCs to convert GTP to cyclic GMP. In vertebrates, sensing reduction in O_2 levels directs ventilation responses in the carotid body by altering the H_2S level, which increases 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 CO_2 levels in both *C. elegans* and vertebrates appears to be mainly mediated by GCs and/or adenylate cyclases; whether this 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 O_2-sensing hydroxylase EGLN family proteins is modulated by the antagonizing actions of O_2 and H_2S to inhibit HIF transcription factors, which ultimately direct adaptive responses to hypoxia by the transcriptional regulation of its numerous target genes.