Interactions between oxygen homeostasis, food availability, and hydrogen sulfide signaling

Nicole N. Iranon1,2 and Dana L. Miller1*

1 Department of Biochemistry, University of Washington School of Medicine, Seattle, WA, USA
2 Molecular and Cellular Biology Graduate Program, University of Washington School of Medicine, Seattle, WA, USA

*Correspondence: Dana L. Miller, Department of Biochemistry, University of Washington School of Medicine, J/UW Mailbox 357350, Seattle, WA 98195-3750 USA. e-mail: dlm16@uw.edu

The ability to sense and respond to stressful conditions is essential to maintain organismal homeostasis. It has long been recognized that stress response factors that improve survival in changing conditions can also influence longevity. In this review, we discuss different strategies used by animals in response to decreased O2 (hypoxia) to maintain O2 homeostasis, and consider interactions between hypoxia responses, nutritional status, and H2S signaling. O2 is an essential environmental nutrient for almost all metazoans as it plays a fundamental role in development and cellular metabolism. However, the physiological response(s) to hypoxia depend greatly on the amount of O2 available. Animals must sense declining O2 availability to coordinate fundamental metabolic and signaling pathways. It is not surprising that factors involved in the response to hypoxia are also involved in responding to other key environmental signals, particularly food availability. Recent studies in mammals have also shown that the small gaseous signaling molecule hydrogen sulfide (H2S) protects against cellular damage and death in hypoxia. These results suggest that H2S signaling also integrates with hypoxia response(s). Many of the signaling pathways that mediate the effects of hypoxia, food deprivation, and H2S signaling have also been implicated in the control of lifespan. Understanding how these pathways are coordinated therefore has the potential to reveal new cellular and organismal homeostatic mechanisms that contribute to longevity assurance in animals.

Keywords: hypoxia, anoxia, oxygen, hydrogen sulfide, suspended animation, diapause, dietary restriction, homeostasis

All organisms must maintain homeostasis to survive. Walter Cannon defined the modern concept of homeostasis as “the coordinated physiological reactions which maintain most of the steady states in the body…” (Cannon, 1929). At the cellular level, maintaining homeostasis requires the coordination of metabolic reactions and cellular processes with environmental conditions. Homeostatic mechanisms are also centrally important for regulating longevity assurance. One consequence of the physiological decline associated with aging is degeneration of the ability to maintain homeostasis which narrows the range of conditions that can be tolerated. At least partly as a result of this defect in homeostasis, the likelihood of death from injury, infection, and disease increases. Oxygen (O2) is an essential environmental resource for all metazoans, with only one known exception (Danovaro et al., 2010). The ability to sense and respond to changes in O2 likely arose early in evolution (O’Farrell, 2001). Nevertheless, even short exposure to decreased O2 availability (hypoxia) leads to irreversible cellular damage and death in most metazoans. Interestingly, responses to hypoxia have molecular and physiological similarities to the effects of food deprivation. Moreover, there is accumulating evidence that hydrogen sulfide (H2S) improves outcomes after ischemia, suggesting that H2S signaling can modulate effects of hypoxia in animals. In this article, we review physiological responses to hypoxia and consider similarities and interactions with adaptation to food deprivation and H2S signaling (Figure 1).
O2 at the tissue level is lower than ambient, varies between tissue types, and depends both on O2 delivery and tissue metabolic activity (Montgomery, 1957; Dyson and Singer, 2011). Fluctuations in ambient O2 supply or tissue metabolic demand stimulate compensatory responses to increase blood flow and O2 delivery, including vasodilation, increased respiratory rate, and production of red blood cells. This makes it difficult to experimentally control the hypoxic exposure of cells in an intact animal in order to investigate different cellular responses to hypoxia. It is important also to consider that it is experimentally difficult or impossible to separate damage that occurs in hypoxia or ischemia from effects that occur as a result of reoxygenation. In contrast, C. elegans does not have a circulatory system, relying instead on diffusion for O2 delivery to cells. This allows for precise experimental control of both genotype and cellular environment (Shen and Powell-Coffman, 2003; Fawcett et al., 2012). Because it is an attractive model for hypoxia research we have built a framework of hypoxia responses as a function of O2 tension using C. elegans, drawing connections with other systems when possible. There have been several excellent reviews recently about signaling pathways that coordinate cellular responses to hypoxia (Coore et al., 2006; Powell-Coffman, 2010; Hand et al., 2011; Padilla and Ladage, 2012). In this review we compare how strategies to respond to hypoxia vary with O2 concentration, and focus on how response mechanisms could integrate with other signaling pathways to influence organism physiology and lifespan.

ADAPTATIONS TO ANOXIA

In the laboratory, C. elegans, Drosophila melanogaster, and Danio rerio all survive without O2 (anoxia; operationally defined as <10 ppm O2) by entering into a state of suspended animation (Foe and Alberts, 1985; DiGregorio et al., 2001; Padilla and Roth, 2001; Padilla et al., 2002). In suspended animation, all microscopically observable activity reversibly arrests, including embryonic cell divisions, post-embryonic development, movement, and reproduction. Upon reoxygenation, developmental processes resume and animals grow to healthy, fertile adults. Suspended animation can be successfully maintained for several days in C. elegans, weeks in Drosophila embryos, and years in the brine shrimp Artemia franciscana (Foe and Alberts, 1985; Clegg, 1997; Padilla et al., 2002). Mechanisms that underlie the ability to survive severe hypometabolic and quiescent states may be widely conserved. Metabolism is dramatically reduced in dogs that survive for several hours after total exsanguination with cold saline flush, for example (Behringer et al., 2003).

One common feature of suspended animation is the reversible arrest of cell divisions. The point at which cell cycle arrest occurs differs between organisms. C. elegans embryonic blastomeres arrest in interphase, prophase, and metaphase, but the transition to anaphase will not occur in anoxia (Padilla et al., 2002; Nystul et al., 2003; Hajeri et al., 2005). The spindle assembly checkpoint is activated by anoxia, and stopping the cell cycle is important to prevent lethal chromosome segregation defects. Embryos that have been depleted of san-1, a component of the spindle assembly checkpoint, by RNAi die when exposed to anoxia and exhibit chromosome segregation defects (Nystul et al., 2003). In cells that arrest in interphase or prophase, the chromatin condenses and
Chromosomes align near the nuclear envelope, whereas metaphase blastomeres display reduced spindle and astral microtubule density. The prophase arrest is characterized by inactivation of cdk-1, and requires the npp-16 nucleoporin (Haeriz et al., 2003). These results indicate that there are at least two distinct cell cycle checkpoints activated to arrest embryonic cell divisions in anoxia-induced suspended animation in C. elegans. The spindle assembly checkpoint is not required for suspended animation in adults, possibly because somatic cells are all post-mitotic. However, germline stem cell divisions arrest in adults in suspended animation without any apparent decrease in full reproductive potential (Paddilla et al., 2002; our unpublished observation). Thus, there may be other mechanisms that contribute to anoxia-induced suspension of cell division post-embryonically. The mechanisms by which anoxia signaling integrates with the spindle checkpoint are not well understood, though the effect is conserved. Drosophila embryos exposed to anoxia also arrest during interphase, prophase, and metaphase, and the arrest is characterized by chromatin localization near the nuclear membrane (Foe and Alberts, 1985; Douglas et al., 2001). Similarly, Danio rerio embryos suspend cell division in anoxia, though arrest is exclusively during interphase (Paddilla and Roth, 2001).

In anoxia metabolic networks must be substantially rearranged, with important phenotypic consequences. O2 is essential for both mitochondrial respiration and fatty acid oxidation. A major consequence of O2 deprivation is that cellular energy metabolism is disrupted. The survival of both embryos and adult C. elegans in anoxia is correlated with available glycogen stores, which serve as a source for glycolytic energy production (Frazier and Roth, 2009; LaRue and Paddilla, 2011). Glycogen decreases progressively as embryos are exposed to anoxia (Frazier and Roth, 2009). Mutations in genes that have little in common, other than decreased glycogen content, all show an anoxia-sensitive phenotype during embryogenesis (Frazier and Roth, 2009). Similarly, hypoxia-inducible shock, an environmental perturbation that increases glycolysis at the expense of glycogen, reduces the viability of embryos in anoxia (Frazier and Roth, 2009). In contrast, in adults hypomorph loss-of-function mutations in the insulin/IGF receptor homolog daf-2 increase glycogen content and survival in anoxia (Scott et al., 2002; Mendenhall et al., 2006; Frazier and Roth, 2009; LaRue and Paddilla, 2011). Diet-induced increases in glycogen are also associated with increased survival in anoxia in Drosophila (Vigne et al., 2009). Depletion of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (gpd-2/3) by RNAi decreases survival of adult daf-2 mutant animals in anoxia (Mendenhall et al., 2006). The significance of this result is not clear, insofar as gpd-2/3/RNAi does not reduce survival of wild-type animals in anoxia (Mendenhall et al., 2006). One possibility is that the difference between wild-type and daf-2 mutant animals reflects a difference in metabolic state. Both gene expression, oxygen consumption measurements, and physiological studies suggest that the daf-2 mutant animals have a metabolic architecture that is very different from wild-type (Van Voorhis and Ward, 1999; Lee et al., 2003; Murphy et al., 2003; Hothersoff et al., 2005). Moreover, RNAi directed against other glycolytic enzymes does not alter survival in anoxia (Mendenhall et al., 2006). This may suggest that simply decreasing glycolysis does not explain the effect on anoxia survival. However, it is difficult to assess whether the RNAi treatment sufficiently decreased the activity of the glycolytic enzymes in these experiments, and no direct measurements of effects on glycogen were reported.

In anoxia, fatty acid oxidation is not possible. Instead, increased fatty acid synthesis may be important for anaerobic activity and to regenerate reducing equivalents for continued glycolytic activity. Fatty acid synthesis is a hallmark of hypoxic tumor cells (Romero-Garcia et al., 2011), and in C. elegans the SREBP homolog slp-1 is required for fatty acid accumulation after anoxia (Taghibiglou et al., 2009). This result suggests that changes in lipid metabolism are essential parts of the response to hypoxia. However, it is also possible that lipid signaling plays an important role during O2 deprivation. Consistent with this view, mutations that are predicted to disrupt ceramide synthesis modulate survival in anoxia. Survival was decreased by loss-of-function of hyl-2, whereas similar mutations in the related hyl-1 increase survival in anoxia (Menuez et al., 2009). In mammalian models, altered ceramide signaling has been associated with hypoxia-induced changes in tumors and may contribute to cell death in neurological disorders including cerebral ischemia (Jana et al., 2009; Yin et al., 2010).

hyl-2 and hyl-2 are functional homologs, of LAG1 (longevity assurance gene 1), which was reported to increase replicative lifespan in Saccharomyces cerevisiae (U’Mollo et al., 1994). However, RNAi knockdown of neither hyl-1 nor hyl-2 increase lifespan in C. elegans (Menuez et al., 2009). Lipid metabolism and signaling are increasingly recognized as playing an important role in the regulation of aging and lifespan (Lapierre and Hansen, 2012). Considering the important role that aberrant lipid signaling plays in the progression of cancer cells, elucidating the role that these processes play in adaptations to hypoxia is likely to be a productive direction for future research.

There is surprising overlap between genes and pathways that increase survival in anoxia and those that modulate lifespan, though the mechanistic basis of this correlation is not understood. In a screen for genes that increased survival in anoxia when depleted by RNAi, 11 of 198 hits (5.6%) had previously been identified to increase lifespan in C. elegans (Mabon et al., 2009). In contrast, the frequency of finding genes that increase lifespan from RNAi screens that use longevity as the primary phenotype ranged from 0.1 to 0.5% (Hamilton et al., 2005; Hansen et al., 2005). Thus, the genes identified by enhanced anoxia survival are enriched for longevity genes. In addition to a variety of metabolic genes identified in this screen, anoxia survival also requires autophagy, which may serve as an important source for catabolic energy production. Disruption of genes important for autophagy by RNAi or mutation reduce survival in anoxia (Samokhvalov et al., 2008). In mammalian systems, autophagy is regulated by hypoxia, particularly in cancer cells (Rosaschop and Wouters, 2009; Eskelinen, 2011). Moreover, autophagy is important for increased lifespan by both daf-2/1 loss-of-function mutations and dietary restriction (DR) in C. elegans (Meléndez et al., 2003; Hansen et al., 2008). Overexpression of autophagy gene LC3/Atg8 in the nervous system increases lifespan in Drosophila (Simonsen et al., 2008). The insulin/IGF signaling (IIS) pathway is another conserved pathway that is involved both in longevity assurance and the response to hypoxia. In C. elegans, the IIS receptor homolog daf-2 increases
lifespan as well as survival in anoxia (Kenyon et al., 1993; Scott et al., 2002; Mendenhall et al., 2016). Increased stress resistance is a well-known feature of daf-2(e1370) mutant animals, suggesting that increased survival in anoxia is a consequence of a correlation between increased stress resistance and lifespan (Lithgow et al., 1995; Honda and Honda, 1999; Mendenhall et al., 2006; Scott et al., 2002). However, five of six daf-2 regulated gene products depleted by RNAi increased resistance to anoxia but had no effect on lifespan (Mabon et al., 2009). Moreover, mutations that increase resistance to osmotic stress, including loss-of-function alleles of dpy-19 and oom-7, decrease survival in anoxia (Wheeler and Thomas, 2006; Frazier and Roth, 2009). Thus, a general increase in stress resistance does not explain the relationship between lifespan and anoxia resistance.

Protein metabolism is another central aspect of cellular physiology affected by hypoxia. Protein synthesis and the chaperones that help to maintain cellular proteins in the correctly folded state are energetically expensive. The coordination of protein synthesis, quality control, and degradation, referred to as proteostasis, is essential to maintain cellular function (Hartl et al., 2011; Taylor and Dillin, 2011). Reduced protein translation is associated with increased lifespan in C. elegans (Hansen et al., 2007; Pan et al., 2007). Many genes that increase survival in anoxia when depleted by RNAi are involved in protein translation. Protein translation is inhibited in low O2 (Blochchina et al., 1996; Teodoro and O'Farrell, 2007; Storey and Storey, 2004; Wouters et al., 2005; Liu et al., 2006), making it somewhat surprising that genetic manipulations that inhibit protein translation could increase energy stores available in anoxia. Another possibility is that reduced translation rates improve proteostasis networks and improve the capacity to deal with unfolded protein stress in anoxia. In the endoplasmic reticulum, the ERO1 enzyme uses O2 to catalyze oxidative protein folding (Ito and Weissman, 2002), which would be inhibited in anoxia. In C. elegans, the ER unfolded protein response (UPR) is activated in anoxia, and UPR genes dhp-1 and ire-1 are required for survival (Mao and Crowder, 2010). This suggests that anoxia increases the burden of misfolded proteins in the secretory path. Decreasing translation by knock-down of aminoacil tRNA synthase genes reduces expression of UPR mediators, and increases survival in anoxia (Anderson et al., 2009). UPR activity is increased by decreased O2 in pancreatic β-cells and liver (but not cardiomyocytes), suggesting that it plays a conserved role in the cellular response to hypoxia (Tagliavacca et al., 2012; Zheng et al., 2012). Understanding general mechanisms that integrate stress homeostasis pathways with the proteostasis network could reveal new strategies to manipulate proteostasis. This would have broad significance, particularly as defects in proteostasis have been associated with the aging process (Haigis and Yankner, 2010; Zheng et al., 2012).

Responses to Hypoxia When Some O2 Is Available

A common strategy to survive hypoxia is to avoid conditions with insufficient O2. Indeed, animals have evolved sophisticated behavioral strategies to avoid hypoxic conditions. In a gradient of O2 blue crabs, New Zealand snapper, and C. elegans will all avoid low O2 and show preference for an optimal O2 environment (Dusenbery, 1980; Bell et al., 2009; Gray et al., 2004; Cook and Herbert, 2012). Interestingly, other environmental conditions can modulate what is perceived as the optimal O2 concentration. Hypoxia avoidance in C. elegans decreases as animals are starved (Dusenbery, 1980). Both alligators and cold-submerged frogs prefer lower ambient temperature in hypoxia (Branco et al., 1993; Tattersall and Boutilier, 1997). This may reflect a physiological interaction between temperature and O2. Consistent with this idea, C. elegans survive much longer in anoxia at low temperature than at higher temperature (Padilla et al., 2002; Scott et al., 2002; Mendenhall et al., 2006). It is not clear if the mechanisms that regulate survival are identical in these conditions, though the insulin/IGF receptor ortholog daf-2 can increase survival at both temperatures (Scott et al., 2002; Mendenhall et al., 2006). The interaction between temperature and hypoxia may also have clinical relevance, as therapeutic hypothermia can reduce neurodevelopmental disability in infants surviving hypoxic ischemic encephalopathy from perinatal asphyxiation, and is used in adults clinically to improve outcome after pelvic surgery, cardiac arrest, and brain ischemia (Selway, 2010; Finley, 2011; Sunde and Sereide, 2011; Yenari and Han, 2012).

In moderate hypoxia (5,000–20,000 ppm O2) C. elegans embryos complete development and grow to gravid adults, albeit more slowly than in room air (Jiang et al., 2001; Nystul and Roth, 2004; Miller and Roth, 2009). This indicates that the response to these hypoxic conditions is physiologically distinct from anoxia, in which animals enter suspended animation. Consistent with this, embryos do not require san-1, the spindle assembly checkpoint protein essential for suspended animation (Nystul and Roth, 2004), to survive exposure to hypoxia. Instead, HIF-1, the single worm homolog of the hypoxia-inducible factor (HIF) is required for embryo survival in 5,000–20,000 ppm O2 (Jiang et al., 2001; Nystul and Roth, 2004). HIF is a highly conserved Mollusca and annelid domain transcription factor that helps maintain O2 homeostasis by coordinating the transcriptional response to hypoxia in metazoans. There are many excellent reviews of HIF function and its role in development and disease (e.g., Semenza, 2009, 2010, 2011, 2012; Majmundar et al., 2010; Powell-Coffman, 2010). HIF was first identified biochemically as the factor that bound the erythropoietin promoter in hypoxia (Wang and Semenza, 1993). HIF is directly regulated by O2 levels. HIF is hydroxylated at the conserved proline in the LxxLAP motif by a 2-oxoglutarate-dependent prolyl hydroxylase of the EGLN family, named after egl-9 in C. elegans (Epstein et al., 2001). Hydroxylated HIF is then recognized by an E3 ubiquitin ligase, the von Hippel–Lindau factor VHL-1, and degraded by the proteasome (Kaehl, 2008). In hypoxia the hydroxylase is inefficient and HIF accumulates, dimerizes with the aryl hydrocarbon nuclear translocator (ARNT, ahl-1), and induces expression of target genes that facilitate adaptation to hypoxia. In mammals, HIF is essential for early developmental events, and both HIF1α and HIF2α mutant mice die early in embryogenesis (Iyer et al., 1998; Compernolle et al., 2002). HIF homologs are also important for tracheal branching in Drosophila and neuronal patterning in C. elegans, highlighting the conserved role for HIF in development (Keith and Simon, 2007; Centanin et al., 2008).
Constitutive stabilization of HIF has been implicated in tumor progression and mutations in VHL, a negative regulator of HIF, are associated with Von Hippel-Lindau syndrome, which is characterized by renal clear cell carcinoma (Kim and Kaelin, 2004; Shen and Kaelin, 2012). Importantly, HIF-1 is not required for embryos to survive suspended animation in C. elegans, demonstrating that these two physiological responses to low O2 are genetically distinct. Although HIF has been the focus of most studies into transcriptional responses to hypoxia, there is also evidence that other factors are involved. HIF-independent transcriptional responses to hypoxia have been observed in C. elegans and mammals (Dong et al., 2001; Shen et al., 2005; Pier et al., 2006; Nishizato et al., 2010). The factors that mediate these effects are not well understood.

Despite the fact there are at least two separate adaptive responses to low O2—suspended animation in anoxia or continued development in moderate hypoxia—there are hypoxic conditions that are lethal during embryogenesis. Isolated embryos die when exposed to O2 concentrations between 100 and 1,000 ppm O2 (Nystul and Roth, 2004). In these conditions, continued developmental progress is associated with increased lethality. Embryos exposed to 1,000 ppm O2 undergo more cell divisions and experience a higher rate of lethality than those exposed to 100 ppm O2, for 24 h (Nystul and Roth, 2004). Although the cellular mechanisms that underlie these defects are not well understood, this has been demonstrated that inducing suspended animation in isolated embryos using carbon monoxide rescues embryo survival in hypoxia (Nystul and Roth, 2004). Anoxia-induced suspended animation also protects C. elegans embryos against otherwise lethal cold exposure (Chan et al., 2010). These results suggest that arresting cell division and development facilitates coordination between cellular events and prevents irreversible errors.

Although embryos cannot autonomously engage suspended animation in these hypoxic conditions, embryos exposed to 1,000 ppm O2 in utero arrest development and survive (Miller and Roth, 2009). Embryo survival in utero requires san-1, suggesting that the embryos are in a state genetically related to anoxia-induced suspended animation (Miller and Roth, 2009). We refer to this as a hypoxia-induced diapause, because it is reminiscent of mammalian embryonic diapause, in which the adults remain active but arrest development of embryos in utero (Benttree and Shaw, 2000). This embryonic diapause is coordinated by as-yet uncharacterized maternal factors that alter the uterine environment to impinge on embryonic development. Many facets of suspended animation and the mechanisms by which suspended animation can be non-autonomously controlled in the presence of O2 remain a mystery and are likely to be a fruitful area of future research.

Developmental context also influences the response to hypoxia, with greater flexibility after embryogenesis. Newly hatched larvae survive in hypoxic conditions that are lethal to embryos (1,000 ppm O2), and survival is associated with a reversible arrest of postembryonic development (Miller and Roth, 2009). This suggests that there are mechanisms that can arrest cell division in 1,000 ppm O2, but that embryos cannot enact this response. The arrest of postembryonic cell division is genetically distinct from suspended animation, in that san-1 is not required to arrest cell division of germline stem cells (Miller and Roth, 2009). One caveat to this interpretation is that it has not been demonstrated that san-1 is required for successful suspension of germline stem cell divisions in adults exposed to anoxia, and it is possible that suspended animation in adults employs different strategies to arrest cell division. Further delineation of the mechanisms used to arrest cell division in these conditions is required to evaluate this possibility. In addition to this developmental arrest, adults exposed to 1,000 ppm O2 enter a reproductive diapause (Miller and Roth, 2009). Gravid adults cease laying eggs, arrest the development and fertilization of oocytes, and halt embryonic development in utero. The arrest of progeny production ensures that embryos are not produced into conditions where they cannot survive. Moreover, energy shunted away from reproductive activity can be used instead for locomotion to search for a new environment. Therefore, by delaying progeny production animals can find a time and place more suited to successful reproduction. In this way, hypoxia-induced reproductive diapause is similar to diapause in insects and mammals that ensures progeny production is synchronized with seasonal and nutritional conditions that maximize fitness (Renfree and Shaw, 2000; Tatar et al., 2011; Allen, 2007; Guidetti et al., 2008; Tachibana and Watanabe, 2008).

HIF-1 is not required for hypoxia-induced diapause, as animals with a null allele of hif-1 arrest post-embryonic development and reproduction in 1,000 ppm O2 as efficiently wild-type animals (Miller and Roth, 2009). Unlike the situation in embryos, hif-1(−/) mutant larvae and adults exposed to 5,000 ppm O2 survive 24 h with >90% viability to adult upon reoxygenation (Nystul and Roth, 2004; Miller and Roth, 2009). Nevertheless, HIF-1 is necessary for the normal response to 5,000 ppm O2. Whereas wild-type animals continue development in these conditions, hif-1(−/) mutant animals precociously enter into hypoxia-induced developmental and reproductive diapause (Miller and Roth, 2009). This observation supports the idea that responses to hypoxia are specific to the concentration of O2 that is available, and that HIF-1 does not play a major role in the response to 1,000 ppm O2. In fact, even constitutive activation of HIF-1, by loss-of-function mutations in negative regulator vhl-1 or egl-8, does not prevent diapause in 1,000 ppm O2. This result further suggests that HIF-1 promotes continued developmental activity in both larva and embryos, though it may have different targets in each developmental context. Although hif-1 is expressed in most, if not all, cells (Jiang et al., 2001), expression only in neurons is sufficient to regulate hypoxia-induced diapause in 5,000 ppm O2 (Miller and Roth, 2009). This suggests that there are neuroendocrine signaling pathways that coordinate development with the response to hypoxia.

In contrast, early stage hif-1 mutant embryos die in 5,000 ppm O2, suggesting that HIF-1 acts autonomously during embryogenesis, when the nervous system is not fully developed, to protect against hypoxia (Jiang et al., 2001; Nystul and Roth, 2004). The neuronal circuits and neuroendocrine factors that coordinate the systemic response to hypoxia have not been delineated, though it has been shown that hypoxia-induced diapause does not require the same neurons that mediate hypoxia avoidance behavior (Gray et al., 2004; Miller and Roth, 2009).

The AMP-activated protein kinase (AMPK) is also involved in regulating hypoxia-induced diapause in 5,000 ppm O2. AMPK
AMPK directly phosphorylates many components of the cell cycle at physiological levels (Laderoute et al., 2006; Liu et al., 2006; Papandreou et al., 2003). CAMKKα cells, activation of AMPK by hypoxia is abrogated by depletion hypoxia contexts has not been investigated. In mammalian recent proteomic studies have revealed that machinery (Banko et al., 2011). These studies suggest a preliminary model in which HIF-1 acts upstream or in parallel to AMPK, which regulates cell division in hypoxia. Working out the mechanistic details that govern this effect is likely to provide unique insight into how AMPK coordinates cellular activities in response to metabolic stress.

**RELATIONSHIP BETWEEN HYPOXIA AND FOOD DEPRIVATION**

Hypoxia and food deprivation are similar stresses in that they both affect central aspects of cellular metabolism. The absence of either food or O₂ disrupts energy-generating pathways, and there are similarities in physiological responses and molecular genetic pathways that are activated in these two situations. The integration of these pathways is highlighted by the interactions between hypoxia and nutrient availability. Rats that are subject to alternate-day feeding have reduced neuronal damage and improved behavioral outcomes after focal cerebral ischemia (Yu and Mattson, 1989). Similarly, mice that are fasted for only 3 days are resistant to surgically induced renal and hepatic ischemia/reperfusion (I/R) injury (Mitchell et al., 2010; Verweij et al., 2011). In contrast, both severe and moderate food restriction decrease survival after gut I/R from occlusion of the superior mesenteric artery (Ueno et al., 2005). Given the therapeutic potential, there is much interest in understanding the mechanistic basis of the interaction between fasting and hypoxia and I/R.

In both C. elegans and Droshophila exposure to hypoxia increases lifespan, though the relationship is not linear and different hypoxic conditions increase lifespan in these species (Honda et al., 1993; Mehta et al., 2009; Rascón and Harrison, 2010). Decreased food intake, DR, also increases lifespan in these and other species (Koubbova and Guarente, 2003; Bishop and Guarente, 2007; Fontana et al., 2010), though there are some genetic backgrounds and species in which DR does not increase lifespan (Mockett et al., 2006; Swindell, 2012). Many genetic pathways that are involved in mediating the effects of DR on lifespan also have roles in the response to hypoxia, and vice versa. This suggests that responses to DR and hypoxia may physiologically interact as well. Indeed, dietary conditions that maximize lifespan are different for Drosophila in hypoxia and normoxia. Flies chronically adapted to 50,000 ppm O₂ live longer at lower yeast (protein) levels than normoxic cohorts (Vigne and Frelin, 2007). In C. elegans the IIS pathway downstream ofdaf-2, AMPK, and the target of rapamycin (TOR) kinaselet-363 have all been shown to be important for increased lifespan in DR (Greer and Brunet, 2009). As noted above, bothdaf-2 and AMPK are important in mediating responses to decreased O₂. Although a role ofTOR/let-363 in C. elegans hypoxia response has not been demonstrated, TOR is negatively regulated by AMPK, and TOR mediates the translational arrest observed in mammalian cells exposed to hypoxia (Liu et al., 2006; Lee et al., 2008). This suggests the possibility that these factors mediate increased lifespan in response to both decreased food and hypoxia.

HIF-1 has recently been shown to modulate lifespan in C. elegans. Curiously, bothhif-1(−) andvha-1(−)mutant animals, which have constitutively stabilized HIF-1, exhibit increased lifespan (Chen et al., 2009; Mehta et al., 2009; Zhang et al., 2009). It may be that different environmental contexts underlie this effect. Thehif-1 mutant was subsequently shown to be long-lived at low temperature but not at high temperature (Lesier et al., 2011). HIF-1 is required for C. elegans to adapt to changes in temperature (Trenin et al., 2003), and HIF is stabilized in both crucian carp and mice exposed to high temperature (Katschinski et al., 2002; Riisanen et al., 2006). Thus, HIF may have an important role in responding to thermal stress as well as hypoxia. Notably, thehif-1(−)mutant animal does not have increased lifespan in DR. While C. elegans that overexpress HIF-1 due to a mutation inegf-9 show modest increases in lifespan under DR, the effect is...
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EMERGING EVIDENCE SUGGESTS THAT H2S SIGNALING CAN MODULATE THE PHYSIOLOGICAL EFFECTS OF HYPOXIA IN MAMMALS. H2S IS NATURALLY PRODUCED IN ANIMAL CELLS AS A PRODUCT OF AMINO ACID METABOLISM THROUGH THE TRANSULFURATION PATHWAY (DOMINYY AND STIPANUK, 2004; STIPANUK, 2004). ENDGENOUSLY PRODUCED H2S HAS MANY IMPORTANT ROLES IN CELLULAR SIGNALING, NEUROMODULATION, AND REGULATION OF VASCULAR TONE (KIMURA, 2011; VANDIVER AND SNYDER, 2012; WANG, 2012). AT LOW CONCENTRATIONS EXOGENOUS H2S HAS Dramatic Physiological Effects That Improve Survival in Changing Conditions. MICE Exposed to 80 ppm H2S, in Otherwise Normal Room Air, Enter into a Suspended-animation-like State in Which Basal Metabolic Rate Is Depressed and Core Body Temperature Is Maintained Only Slightly Above Ambient (Blackstone et al., 2003; Volpato et al., 2008). MICE Exposed to Low H2S Survive in Otherwise Lethal Hypoxia (Blackstone and Roth, 2007), and H2S Improves Outcome in a Variety of Mammalian Models of I/R Spanning Multiple Organ Systems, Including Myocardial Infarct, Hepatic I/R, and Lung Injury from Smoke Inhalation (Szabo, 2007; Nicholson and Calvert, 2010; King and Lefer, 2011).

THE MECHANISMS BY WHICH H2S SIGNALING INTEGRATES WITH HYPOXIA ARE NOT WELL UNDERSTOOD. PHARMACOLOGICAL INHIBITORS OF KATP CHANNELS AND PROTEIN KINASE C (PKC) ABROGATE THE PROTECTIVE EFFECT OF NAHS, THE IONIZED FORM OF H2S, IN A NEURAL CELL CULTURE MODEL OF HYPOXIC INJURY (TAY et al., 2010). SIMILARLY, THE VASODILATORY EFFECTS OF NAHS DEPEND PARTIALLY ON PLASMA MEMBRANE KATP SUBUNIT SUR2 (LIANG et al., 2011). THE ABILITY FOR H2S TO STIMULATE RAT KATP CHANNELS Heterologously Expressed IN HEK293 CELLS REQUIRES SPECIFIC CYSTEINE RESIDUES (LIANG et al., 2010), SUGGESTING THAT H2S DIRECTLY SULFHYDRATES THE KATP CHANNEL TO MODULATE ITS ACTIVITY. HOWEVER, ENDENGENOUSLY PRODUCED H2S POST-TRANSLATIONALLY MODIFIES UP TO 80% OF CELLULAR PROTEINS (MASTON et al., 2009), AND ELUCIDATING THE FUNCTIONALLY RELEVANT TARGETS OF H2S IN DIFFERENT CONTEXTS IS A MAJOR CHALLENGE. IN ADDITION TO
KATP channels, H2S has been proposed to directly activate mitochondrial energy production in smooth muscle of mice (Fu et al., 2012). Similar activity has been reported for ciliated mussel gills (Doellier et al., 1999) and isolated chicken liver mitochondria (Yong and Seary, 2001), suggested that the ability to stimulate cellular energy production may be a conserved features of H2S (Theissen and Searcy, 2001), suggested that the ability to stimulate cellular energy production may be a conserved features of H2S (Theissen and Searcy, 2001), suggested that the ability to stimulate cellular energy production may be a conserved features of H2S (Theissen and Searcy, 2001). Cardioprotective effects of H2S administration in murine models of myocardial ischemia require the Nrf2 transcriptional factor (Calvert et al., 2009, 2010). C. elegans require the Nrf2 homolog skn-1 to survive H2S, and some early transcriptional changes in H2S depend on skn-1 (Miller et al., 2011). SKN-1 is important for the response to various oxidative stresses, though the gene products that are regulated can vary depending on context (An and Blackwell, 2003; Olivoza et al., 2009; Li et al., 2011). SKN-1 is required for increased stress resistance and lifespan resulting from inhibiting either TOR or IIS (Tullet et al., 2008; Robbels-Stubbbs et al., 2012), and it is also required in the two ASI neurons for increased lifespan by DR (Bishop and Guarente, 2007).

The transcriptional response to H2S requires hif-1 in C. elegans, suggesting a potential mechanistic link between the response to hypoxia and H2S. HIF-1 is stabilized and accumulates in the nucleus upon exposure to H2S in C. elegans (Budde and Roth, 2010). Similarly, NaHS induces expression and accumulation of HIF in rat endothelial cells (Liu et al., 2010). Increased expression of hif-1 target genes and survival in H2S requires CYSL-1, which binds to EGL-9 and is proposed to inhibit its ability to hydroxylate HIF-1 (Budde and Roth, 2010; Ma et al., 2012). CYSL-1 is member of the cystathionine β-synthase/cysteine synthase family of pyridoxal-5'-phosphate (PLP)-dependent enzymes that has O-acetylsertines a thioldehydroacetyl activity in vitro (Ma et al., 2012). All of the transcripts that accumulate after 1 h exposure to H2S require hif-1 (Miller et al., 2011). However, it is not yet clear how H2S affects on HIF contribute to protection in hypoxia. The hif-1-mediated response is essential for animals to survive exposure to H2S (Budde and Roth, 2010), whereas hif-1(ad4) mutant animals can survive 24 h exposure to hypoxia (Miller and Roth, 2009). Moreover, there is curiously little overlap between gene products that require hif-1 to accumulate in response to hypoxia and H2S (Miller et al., 2011). The source of this variation has not been determined, but could reflect different fissue response, context- dependent transcription factors, or context- dependent effects on HIF-1 activity deriving from other signaling events.

H2S increases lifespan and thermotolerance in C. elegans (Miller and Roth, 2007), and overexpression of dCBS, a H2S-producing enzyme in the transsulfuration pathway, modestly increases lifespan in Drosophila (Kabir et al., 2011). Pharmacological inhibition of dCBS and RNAi-mediated knockdown of dCBS abrogates increased lifespan by DR (Kabir et al., 2011). These experiments suggest the possibility that H2S signaling also integrates with nutrient sensing pathways. In C. elegans the effects of H2S on lifespan require the conserved sirtuin, sir-2.1 (Miller and Roth, 2007). Sirtuin activity is intrinsically linked with metabolic adaptations to stress, as its activity can be modulated by changes in redox state and metabolic status (Yang et al., 2006; Schwer and Verdin, 2008; Weyrich et al., 2008; Longo, 2009; Yu and Ausw, 2009; Donmez and Guarente, 2010; Haigis and Yankner, 2010). In mammals, the SIRT1 sirtuin deacetylates and activates HIF (Liu et al., 2010). This suggests the possibility that sir-2.1 activates hif-1, leading to physiological responses to H2S that increase lifespan.

CONCLUSION

Signaling pathways that mediate responses to decreased O2, food deprivation, and H2S are integrated with fundamental aspects of cellular physiology and metabolism. As a result, these (and other) stress responses depend on the initial state of the organism. Anything that changes the physiological state – such as aging or previous stress exposure – will necessarily change response(s) to subsequent stresses. In this way, stress responses can be considered to be path dependent: the initial conditions determine the magnitude and trajectory of the response. A greater understanding of the systems biology of stress responses will provide insight into how physiological systems change with age, and may suggest new strategies to delay age-associated disruptions in homeostasis.

Many important questions remain to be answered that will advance our understanding of mechanisms that underlie how context-dependent stress responses are coordinated. For example, we understand little about how conserved factors such as AMPK and HIF have different effects in different conditions. The physiological basis for H2S signaling effects physiological functions, including lifespan and stress response are relatively unexplored. Similarly, the mechanisms by which proteostasis networks are integrated with conditional stress responses are not well understood. In order to address questions requires that both genetic and environmental conditions can be precisely controlled experimentally. The power of genetically tractable model organism systems provides great promise in this regard, as do unbiased approaches that have the potential to reveal novel regulators in these responses. Moreover, these studies will reveal neuroendocrine signaling factors that coordinate the organism-wide response to changing conditions.

Insufficient or inappropriate responses to hypoxia contribute to the progression of many human diseases, suggesting that it may be possible to exploit context-dependent physiological responses for clinical benefit. For example, the observation that the fasting response protects normal cells from chemotherapeutic agents more than cancerous cells led to the simple idea of using fasting to improve the efficacy of chemotherapeutics (Raffaghello et al., 2008; Powell-Coffman, 2010; Lee et al., 2012). This promising study demonstrates the importance of understanding how diverse stress responses are coordinated with each other and an excellent example of the promise of this emerging research area.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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