Cell Metabolism
Review

Oxygen Sensors at the Crossroad of Metabolism

Julián Aragonés,[1,2,4,5] Peter Fraisl,[1,2,5] Myriam Baes,[3] and Peter Carmeliet[1,2,*]
[1]Vesalius Research Center, K.U. Leuven, Leuven, B-3000, Belgium
[2]Vesalius Research Center, VIB, Campus Gasthuisberg, Herestraat 49, Leuven, B-3000, Belgium
[3]Laboratory of Cell Metabolism, K.U. Leuven, Leuven, Belgium
[4]Present address: Servicio de Inmunología, Hospital de la Princesa—Universidad Autónoma de Madrid, Diego de León 62, 28006 Madrid, Spain
[5]These authors contributed equally to this work
*Correspondence: peter.carmeliet@med.kuleuven.be
DOI 10.1016/j.cmet.2008.10.001

Aerobic organisms developed mechanisms to protect themselves against a shortage of oxygen (O_2). Recent studies reveal that O_2 sensors, belonging to the novel class of 2-oxoglutarate dependent iron(ii)-dioxygenases, have more important roles in metabolism than anticipated. Here, we provide a "metabolo-centric" overview of the role of the PHD/FIH members of this family in metabolism, in particular on how they regulate O_2 supply and consumption, energy compensation and conservation, O_2 conformance and hypoxia tolerance, redox and pH homeostasis, and other vital metabolic processes with implications in health and disease. These insights may offer novel opportunities for the treatment of ischemic diseases.

The appearance of oxygen (O_2) into the biosphere was one of the defining moments in evolution, as it offered organisms the advantage of generating energy more efficiently. According to the "endo-symbiosis" theory (Greek for "living together"), the ancestors of mitochondria were bacteria, capable of respiring O_2, that survived endocytosis by other species. The ability of symbiont bacteria to conduct cellular respiration in host cells, relying otherwise on anaerobic fermentation, would have provided a considerable evolutionary advantage for these hosts. This imposed a strong selection pressure for organisms to develop novel pathways of aerobic metabolism.

Because of the high energy potential of O_2, aerobic organisms have become dependent on this gas for their performance and survival. Apart from its role in energy metabolism, O_2 is also utilized as a substrate for the production of cellular constituents, including signaling mediators. Hence, aerobic species developed mechanisms to sense O_2 levels and regulate O_2 consumption, in order to cope with conditions of insufficient O_2 supply. Since C. Scheele's discovery, now more than 200 years ago, of O_2 as a gas "that made his chest feel peculiarly light," recent studies provide novel molecular insight as to how cells sense O_2, and how these O_2 sensors regulate metabolism. In this Review, we will discuss how a new class of O_2 sensors has key roles in reprogramming metabolism in response to changes in O_2 tension, and discuss consequences for health and disease.

The Challenge of Fluctuating O_2 Levels

Ischemic diseases, characterized by an imbalance in O_2 demand and supply, are leading causes of mortality worldwide. But even in healthy conditions, cells are exposed to greatly varying levels of O_2. Not all cells in our body are exposed to the same level of O_2, with substantial differences in the partial pressure of O_2 (PO_2) in distinct anatomical sites and physiological conditions. For instance, in most tissues, the PO_2 varies between 20 to 45 mmHg (corresponding to ~2.5–5.8 kPa) (Braun et al., 2001; Erecinska and Silver, 2001; Ward, 2008), but in some tissue compartments, such as the kidney medulla, bone marrow or intrauterine fetal compartment, the PO_2 is even lower, ranging between 10 to 25 mmHg (~1.3–3.3 kPa) (Chow et al., 2001; Leichtweiss et al., 1969; Singer and Muhlfeld, 2007). In solid tumors, certain regions are even exposed to extreme hypoxia, almost approximating anoxia (<0.1 mmHg; 0.01 kPa) (Helmlinger et al., 1997).

Another challenge is that O_2 levels are not constant, but fluctuate dynamically. It is therefore of vital importance that cells are equipped with mechanisms that sense subtle changes in O_2 tension to adjust metabolism to O_2 supply. During evolution, various mechanisms of O_2 sensing have evolved (Ward, 2008). In typical O_2 sensing tissues, such as the carotid body, pulmonary artery, and adrenal chromaffin cells, mitochondria likely act as O_2 sensors, possibly by using reactive oxygen species (ROS), energy state, or cytosolic redox state as intermediate signals (Ward, 2008); we will not discuss these mechanisms here. Instead, we will focus on a novel class of O_2-dependent enzymes, that has been recently discovered as O_2 sensors: in mammals, this family consists of four distinct "prolyl hydroxylase domain" (PHD) proteins, and a single asparaginyl hydroxylase called "factor inhibiting HIF" (FIH) (Bruick and McKnight, 2001; Epstein et al., 2001; Koivunen et al., 2007; Lando et al., 2002). We will now describe their molecular properties.

PHDs/FIH and HIF: The Molecular Players

The hypoxia-inducible transcription factors (HIF-1α, -2α, -3α) are key mediators of the cellular transcriptional response to hypoxia. Under hypoxic conditions, protein levels of the HIFα subunits rapidly increase; after translocation to the nucleus, they heterodimerize with their HIFβ subunit partner and enhance transcription of a large number of genes (Pouyssegur et al., 2006; Semenza, 2007). This program includes genes, involved in metabolic adaptation to low O_2 tension, including energy compensation, oxygen conformance, hypoxia tolerance, redox and pH homeostasis, O_2 supply, as well as many others processes (Semenza, 2003; Simon and Keith, 2008; Weidemann and Johnson, 2008) (Figure 1). An extensive list of genes, identified as targets

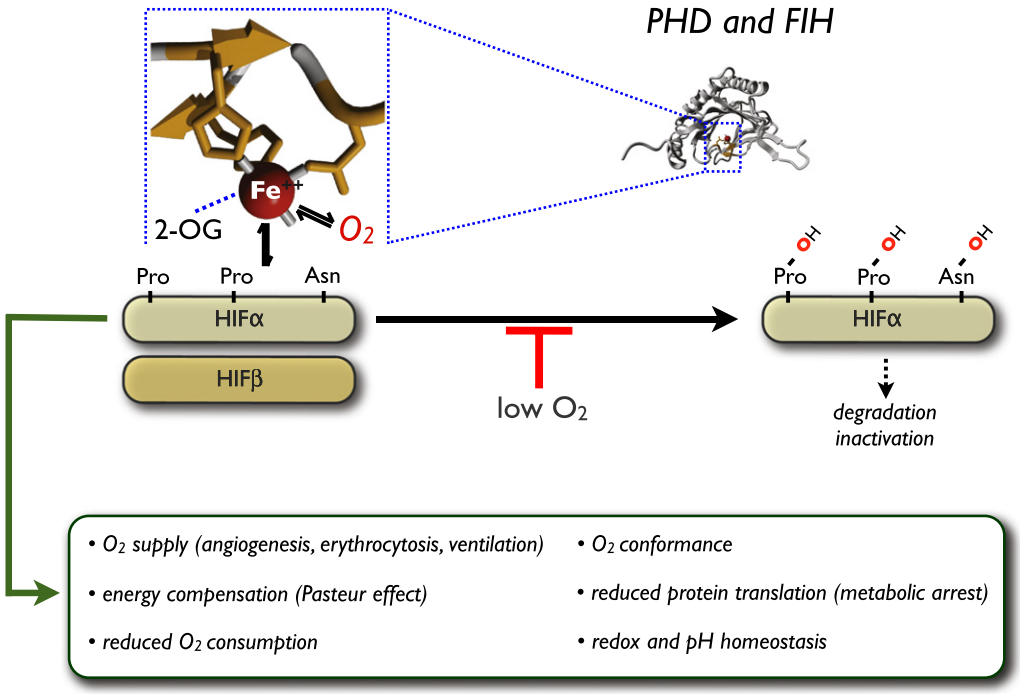

Figure 1. Molecular and Biological Properties of PHD/FIH

As an example for the PHDs/FIH, the three-dimenstional structure of murine PHD2 (amino acids 188–404) is shown, with its catalytic site (inset, left panel) containing the His-X-Asp/Glu-X_n-His triad required for docking of Fe^{2+}, O_2, 2-oxoglutarate (2-OG) and target HIFα amino acid residues, i.e., proline (Pro) for PHDs and asparagine (Asn) for FIH. In their hydroxylated form, HIFs are targeted for proteasomal degradation (in the case of Pro hydroxylation by PHDs) or loose transcriptional activity (Asn-hydroxylation by FIH); in contrast, upon inactivation of PHDs/FIH under low O_2 conditions, nonhydroxylated HIFα subunits stimulate transcription of a genetic program, leading to alterations oxygen supply and metabolism (other biological processes activated by HIFs are not listed).

of HIFs and involved in cellular adaptation to hypoxia, has been previously reviewed (Semenza, 2003).

HIFα subunits do, however, not sense O_2 directly; this activity is performed by PHDs and FIH. PHDs and FIH are 2-oxoglutarate dependent iron(ii)-dioxygenases, which use one of the atoms of O_2 to hydroxylate prolyl or asparaginyl residues, respectively (Kaelin and Ratcliffe, 2008; Lando et al., 2003; Schofield and Ratcliffe, 2004) (Figure 1). The second atom of O_2 is used to convert 2-oxoglutarate to carbon dioxide (CO_2) and succinate. Iron (Fe^{2+}), maintained in a reduced state by ascorbate, is a necessary cofactor. PHDs hydroxylate N- and C-terminal prolyl residues in HIFα subunits (Schofield and Ratcliffe, 2004). When the prolyl residues are hydroxylated in conditions of sufficient oxygen supply, HIFα subunits are recognized by the von Hippel-Lindau (vHL) protein in the multiprotein E3 ubiquitin ligase complex; through ubiquitination, this complex marks HIF proteins for subsequent degradation by the proteasome machinery (Safran and Kaelin, 2003; Schofield and Ratcliffe, 2004). FIH hydroxylates a C-terminal asparagyl residue in HIF-1α, which impairs interaction with the coactivator p300, that is essential for the transcriptional activity of HIF-1α (Lando et al., 2003) (Figure 1). When the O_2 supply drops, PHDs and FIH become progressively inactive, resulting in stabilized (elevated) levels of transcriptionally active HIF complexes (Figure 1).

Despite their relatively low affinity for O_2, with a K_M (100–250 μM for PHDs; 90 μM for FIH) (Ehrismann et al., 2007; Koivunen et al., 2004) that is much higher than tissue PO_2 levels (Gnaiger et al., 1998; Ward, 2008), PHDs/FIH have a sufficient dynamic response over the physiological range of PO_2 levels (Ward, 2008). Also, in cellulo, their overall activity is not only determined by the PO_2, but also by the availability of 2-oxoglutarate (cosubstrate), Fe^{2+} (cofactor), and other substituents such as mitochondrial ROS and ascorbate (Kaelin and Ratcliffe, 2008). These enzymes differ in their affinity for O_2. With FIH having a higher affinity for O_2 than PHDs, FIH remains active at reduced O_2 tensions, when PHDs have already lost their activity. This allows cells to activate different sets of genes in response to various degrees of hypoxia and thereby fine-tune the hypoxia response (Dayan et al., 2006; Koivunen et al., 2004).

Here, we will focus our discussion on how PHDs/FIH regulate metabolism and how, through such metabolic effects, they influence biological processes in health and disease. For further information about the molecular properties and regulation (for instance, by nitric oxide and ROS), expression pattern, and other targets of PHDs/FIH, the reader is referred to previous overviews (Berra et al., 2006; Cash et al., 2007; Kaelin and Ratcliffe, 2008).

Role of PHDs in Oxygen Supply

For cells to generate energy aerobically, they must receive O_2. Hence, when challenged with hypoxia, mechanisms come into action to restore oxygenation. Divergent taxa developed distinct mechanisms to improve O_2 carrying capacities in conditions of O_2 starvation. For instance, the water flea *Daphnia* produces more hemoglobin, while the avascular fruitfly *Drosophila*, which carries O_2 to distant cells via airways, expands its airway network (Gorr et al., 2006). Vertebrates, which use hemoglobin-rich

erythrocytes to transport O_2 via an elaborate network of blood vessels, stimulate angiogenesis, vasodilation, erythropoiesis, and ventilation. Screening of subjects with idiopathic erythrocytosis revealed two heterozygous mutations in the *PHD2* gene (P317R, R371H) that decrease enzyme activity (Percy et al., 2006, 2007). Consistent herewith, mice with targeted disruption of *PHD2* after birth have reduced PHD2 activity in various adult tissues (including kidney), resulting in the upregulation of renal erythropoietin levels and the development of polycythemia (Minamishima et al., 2008; Takeda et al., 2008). The other PHD isoforms are also involved, as combined loss of PHD1 and PHD3 in mice induces mild erythrocytosis through enhanced erythropoiesis in the liver, though, intriguingly, the pattern of activation of erythropoietin differs from that in PHD2 deficient mice (Takeda et al., 2008).

PHD2 has also been implicated in angiogenesis, though only to a limited extent so far. Indeed, postnatal disruption of *PHD2* in the heart, lung, kidney, and liver increases the number and size of blood vessels in these tissues (Takeda et al., 2007). Enhanced angiogenesis was also observed in the brain of these animals, despite inefficient disruption of the *PHD2* allele in this tissue, suggesting that the increase in angiogenesis in these mice might be mediated by circulating rather than locally produced angiogenic factors (Takeda et al., 2007). These mice die, however, prematurely because of circulatory congestion and dilated cardiomyopathy, indicating the need for tight regulation of the cardiovascular system by PHD2 (Minamishima et al., 2008). Silencing of PHD2 in vitro stimulates the proliferation of endothelial cells, at least of immortalized cells (Takeda and Fong, 2007). Recent studies indicate that partial loss of PHD2 in endothelial cells results in normalization of the endothelial layer in tumor vessels (M. Mazzone, D. Dettori, R. Leite de Oliveira, S. Loges, T. Schmidt, B Jonckx, Y.M. Tian, A.A. Lanahan, P. Pollard, C. Ruiz de Almodovar, F. De Smet, S. Vinckier, J.A., A. Luttun, S. Wyns, B. Jordan, A. Pisacane, B. Gallez, M.G. Lampugnani, E. Dejana, M. Simons, P. Ratcliffe, P. Maxwell, and P.C., unpublished data). None of these alterations was observed in mice lacking PHD1 or PHD3 (Takeda et al., 2007). Numerous studies also document a key role of HIFs in angiogenesis in health and disease (Carmeliet and Jain, 2000; Harris, 2002). By switching on a genetic program of angiogenic and arteriogenic factors, HIFs induce the formation of a mature, stable, and functional neovasculature (Carmeliet and Jain, 2000). Preclinical studies further reveal that *HIF-1α* gene transfer stimulates revascularization and improves functional recovery of ischemic tissues (Vincent et al., 2000). Therefore, given the involvement of HIFs in angiogenesis, the vascular changes in *PHD2* mutant mice are likely due to activation of HIFs. It remains, however, to be determined whether HIF-1α or HIF-2α are downstream of PHD2, since each of these HIFs seems to mediate distinct angiogenic processes (Ohneda et al., 2007; Tang et al., 2004).

PHDs/FIH Safeguard Cellular Metabolism via Energy Compensation

PHDs/FIH act as safeguards of cellular metabolism. Because of their relatively low affinity for O_2, they already initiate a defense before O_2 levels drop too low, which would otherwise lead to cellular demise (Koivunen et al., 2004). Hence, PHDs/FIH start to progressively lose activity when tissue PO_2 levels drop over a physiological range (Bracken et al., 2006; Wiesener et al., 1998), while mitochondrial respiration will only arrest when O_2 tension drops to very low levels, i.e., below 1 mmHg (Gnaiger et al., 1998). This safeguard activity of the O_2 sensors allows the cell to adapt to hypoxia, without becoming completely deprived of energy and challenged with life-threatening exhaustion of vital ATP.

A metabolic adaptation to hypoxia involves that cells switch from aerobic to anaerobic metabolism ("Pasteur effect") (Figure 2). With this energy compensation, the cell can continue to generate ATP and, despite the hypoxia, try to meet the metabolic demands and maintain energy expenditure of normoxic conditions. The O_2 sensors, in conjunction with HIFs, regulate various aspects of this metabolic adaptation. Upon O_2 deprivation, HIF-1α stimulates the glycolytic flux through upregulation of key glycolytic genes, including the glucose transporters *GLUT1* and *GLUT3*, the hexokinases *HK1* and *HK2*, and lactate dehydrogenase (*LDH*) (Iyer et al., 1998). In cancer cells, HIF-1α also cooperates with c-Myc to promote aerobic glycolysis by induction of hexokinase *HK2* and the pyruvate dehydrogenase kinases *PDK1* (Dang et al., 2008). Through phosphorylation, PDKs inhibit the activity of the pyruvate dehydrogenase complex (PDC), a key enzyme regulating the entry of pyruvate in the TCA cycle through conversion to acetyl CoA (Kim et al., 2006; Papandreou et al., 2006). A recent study also documents the role of the PDK3 isoform in this metabolic adaptation in cancer cells (Lu et al., 2008).

To prevent intracellular acidification during glycolysis, HIFs also drive expression of monocarboxylic transporters (MCT) and Na^+/H^+ exchanger-1 (NHE-1), that mediate lactate and proton efflux (Pouyssegur et al., 2006; Shimoda et al., 2006; Ullah et al., 2006) (Figure 2). Another key regulator of pH homeostasis, whose expression is induced in hypoxic conditions, includes carbonic anhydrase 9 (CAIX) (Pouyssegur et al., 2006; Wykoff et al., 2000). This extracellular enzyme catalyzes the conversion of CO_2 and H_2O to HCO_3^- (bicarbonate) and H^+ (protons); HCO_3^- exchangers then import extracellular bicarbonate into the cytosol, thereby further preventing intracellular acidification.

PHDs promote the shift to anaerobic metabolism also by sensing alterations in the levels of glycolytic and TCA cycle metabolites. For instance, glycolytic intermediates, such as pyruvate and lactate, but also TCA intermediates, such as oxaloacetate and malate, inhibit PHDs/FIH, possibly by acting as competitive antagonists for 2-oxoglutarate binding or by uncoupling enzyme reactions that lock PHDs into inactive states; this stabilizes HIFs, and reinforces the glycolytic flux (Lu et al., 2005). Moreover, accumulation of fumarate and succinate also reduces the activity of the PHDs, resulting in enhanced HIF signaling, as has been documented in certain cancer cells with loss-of-function mutations of fumarate hydratase (*FH*) or succinate dehydrogenase (*SDH*)-*B* (Isaacs et al., 2005; Selak et al., 2005).

Genetic studies in mice established the in vivo role of PHD1 in the above described mechanisms. Indeed, in the absence of PHD1, glycolytic flux is enhanced in ischemic muscle fibers; yet, these cells do not suffer lifethreatening acidosis in situ upon ligation of their blood supply (Aragones et al., 2008) (Figure 3). Molecular studies indicate that these metabolic and pH changes are attributable to several mechanisms (Aragones et al., 2008). Expression analysis reveals that loss of PHD1

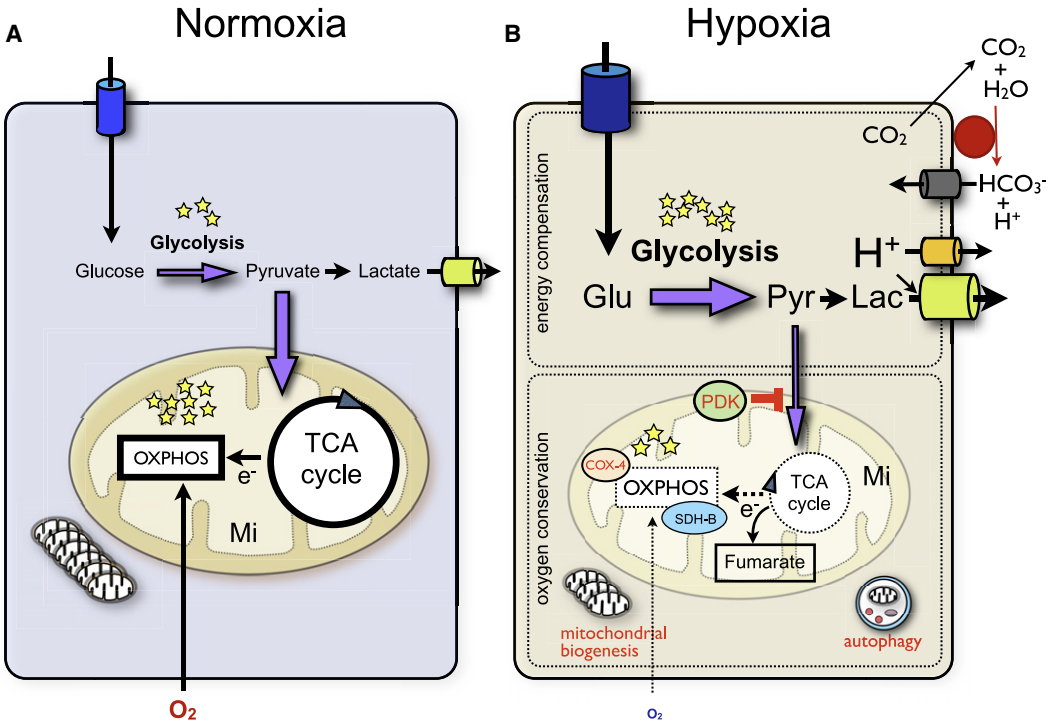

Figure 2. PHDs/HIFs Regulate Energy Compensation in Hypoxic Conditions

(A) In normoxia, glucose, taken up by its transporters (blue cylinder), is metabolized to pyruvate through glycolysis, yielding modest amounts of ATP (yellow stars). Pyruvate is then either converted to lactate, which exits the cell through monocarboxylate transporters (green cylinder), or enters the tricarboxylic acid (TCA) cycle in mitochondria (Mi), yielding electrons (e^-), that are used to generate high amounts of ATP in oxidative phosphorylation (OXPHOS). Mitochondrial biogenesis (indicated by the set of small mitochondria, lower left corner) promotes oxidative metabolism.

(B) In hypoxia, activation of HIF signaling by inactivation of PHDs initiates the illustrated changes in energy compensation and conservation. Oxygen conservation (lower box): O_2 consumption is reduced at the expense of mitochondrial energy production, resulting from (1) blocking entry of pyruvate (Pyr) into the TCA cycle via induction of pyruvate dehydrogenase kinase (PDK), (2) reducing expression of succinate dehydrogenase subunit B (SDH-B), (3) lowering the amount of mitochondria by attenuating mitochondrial biogenesis or increasing autophagy, or (4) switching cytochrome c oxidase subunits (COX-4) to optimize efficiency of respiration. Using fumarate as an alternative terminal electron acceptor can bypass the lack of O_2. Energy compensation (upper box): to compensate for the loss of mitochondrial ATP production, anaerobic glycolysis is switched on. The glycolytic end products lactate and protons (H^+), causing intracellular acidification, are exported by MCTs (green cylinder) and NHE-1 (yellow cylinder). CAIX (red circle) generates extracelular $HCO3^-$ that is imported by $HCO3^-$ exchangers (gray cylinder), thereby further preventing intracellular acidification. Increased glucose (Glu) uptake and conversion of pyruvate (Pyr) to lactate (Lac) by LDH, generating the necessary cofactor NAD^+, fuels glycolysis.

in myofibers upregulates the levels of the pyruvate dehydrogenase kinase isoforms PDK1 and PDK4, which reduces the entry of pyruvate in the TCA cycle. Second, LDH levels are upregulated in PHD1-deficient myofibers, resulting in a greater conversion of pyruvate to lactate. An advantage hereof is that NAD^+ is continuously being regenerated from NADH, thereby replenishing the supply of this essential cofactor for glycolysis and maintaining a high glycolytic flux. Since PHD1 deficiency also elevates the expression of the lactate transporter MCT4, lactate efflux is facilitated, preventing severe cellular acidification. Hence, despite an increased glycolytic flux in baseline conditions, the [lactate/pyruvate] ratio in ischemic conditions remains relatively low in ischemic PHD1-deficient compared to wild-type cells. Notably, PHD1-deficient myofibers enhance glycolytic flux without an apparent upregulation of various HIF-1α-dependent genes, such as the glucose transporter *GLUT-1*, the muscle-specific form of phosphofructokinase (*PFK-M*), phosphoglycerate kinase (*PGK*), M2-pyruvate kinase (*PKM2*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), possibly because HIF-2α is a more important mediator of the PHD1 deficient metabolic phenotype (see below).

Another condition, in which loss of function of PHDs (either resulting from somatic mutations in *PHD2* or epigenetic silencing of *PHD1*) has been associated with similar metabolic changes, is the malignant cancer cell (Calvisi et al., 2007; Kato et al., 2006). Indeed, by modulating the levels of HIFs, PHDs/FIH can regulate "aerobic glycolysis" (Warburg effect) in tumor cells at various steps, i.e., through an enhancement of glycolytic flux, restriction of TCA cycle-mediated respiration, and pH homeostasis (all discussed above). Another metabolic adaptation of tumors is that HIFs promote the production of lactate by tumor cells (through upregulation of LDH); released lactate is then taken up by stromal cells (through upregulation of MCTs), which then regenerate pyruvate (through increased levels of LDH) as internal fuel for glucose oxidation, or as external fuel for the cancer cell, as part of a micro-ecosystem (Gatenby and Gillies, 2008). It has been postulated that these metabolic changes offer tumor cells the advantage to generate more ATP and survive in conditions of fluctuating O_2 levels that would otherwise kill normal cells; tumor cells may also benefit from an acidic microenvironment (resulting from glycolytic generation of lactate) that provides a growth advantage; also, by shifting to aerobic glycolysis and reducing

mitochondrial respiration, malignant cells may suffer diminished oxidative stress (DeBerardinis et al., 2008; Gatenby and Gillies, 2008; Kroemer and Pouyssegur, 2008). Alternative metabolic adaptations of tumor cells include their hypoxia tolerance and oxygen conformance (see below).

PHDs/FIH Regulate Oxygen Consumption through Glucose Oxidation

Mitochondrial respiration relies on the availability of O_2; hence, mechanisms developed to slow down respiration in conditions of hypoxia. PHDs/FIH regulate this process, at least in part, through HIFs (Figure 2). Indeed, when PHDs/FIH are completely inhibited, O_2 consumption is reduced to ~15% of baseline rates in neonatal cardiomyocytes (Sridharan et al., 2008). HIFs reduce mitochondrial O_2 consumption by attenuating glucose oxidation. At the molecular level, HIFs upregulate the expression of PDKs, and thereby restrict the entry of the glycolytic intermediate pyruvate in the TCA cycle (Kim et al., 2006; Papandreou et al., 2006). HIFs also regulate the expression of the respiratory enzyme cytochrome c oxidase (COX). By inducing a switch from isoform COX4-1 to COX4-2, HIF-1α and HIF-2α optimize the efficiency of respiration at low O_2 tension (Fukuda et al., 2007). Further, HIF-1α lowers the expression of the mitochondrial SDH-B subunit (Dahia et al., 2005). It also inhibits mitochondrial biogenesis, by counteracting c-Myc, which enhances mitochondrial biogenesis via induction of PGC-1β (Zhang et al., 2007). Once cells can no longer generate sufficient ATP for survival, they turn to a form of "self cannibalism" termed autophagy; by inducing this program through upregulation of BNIP-3, HIF-1α reduces mitochondrial content and thereby lowers oxidative metabolism (Zhang et al., 2008). Moreover, HIF-1α upregulates the expression of type3 deiodinase (*DIO3*), which inactivates 3,5,3′-triiodothyronine (T3) and decreases thyroid hormone-induced O_2 consumption in cultured cell lines (Simonides et al., 2008).

From the above, it is clear that PHDs/FIH are key regulators of O_2 consumption. However, cells can recruit another mechanism of metabolic adaptation when challenged by severe O_2 deprivation, which has been observed in muscle in diving mammals, or in anoxic myocardium. Indeed, when PHDs/FIH are completely inhibited, some cells can switch to fumarate as an alternative terminal electron acceptor at complex II in the electron transport chain, allowing their mitochondria to maintain membrane polarization (Sridharan et al., 2008) (Figure 2). When PHDs are completely inhibited, certain cells are capable of hybrid respiration, utilizing O_2 for respiration when available, but switching to fumarate when oxidative respiration is paralyzed.

Genetic studies in mice reveal that PHD1 indeed controls O_2 consumption (Aragones et al., 2008) (Figure 3). Indirect calorimetry measurements show that whole body O_2 consumption is reduced in PHD1-deficient mice. As skeletal muscle constitutes 40%–50% of the body mass and is responsible for 40%–50% of the O_2 consumption in resting conditions, these data suggest that O_2 consumption in PHD1-deficient muscle is reduced. Indeed, high-resolution respirometry of permeabilized muscle fibers reveals that mitochondrial respiration through complex I is reduced in PHD1-deficient myofibers. The reduced O_2 consumption is not attributable to differences in mitochondrial biogenesis, O_2 delivery, erythropoiesis, vascularization, or perfusion of muscles. Instead, in vivo ^{13}C-NMR measurements of [^{13}C] glutamate resonances in muscle extracts after infusion of [U-$^{13}C_6$] glucose indicate that glucose oxidation is 35% lower in PHD1-deficient mice, consistent with the increased expression of PDK1 and PDK4 in muscle of these mice (see above). Ex vivo analysis using isolated muscles confirms that oxidation of [U-^{14}C] glucose is reduced in the absence of PHD1. Moreover, HIFs are downstream effectors of PHD1 in this metabolic regulation, since heterozygous deficiency of *HIF-2α* (and, to a lesser extent, of *HIF-1α*) nullifies the PHD1-deficient reprogramming of metabolism (Aragones et al., 2008).

Do PHDs/FIH Regulate Fatty Acid Oxidation?

Another important determinant of O_2 consumption is fatty acid oxidation; it remains, however, outstanding whether and how PHDs/FIH regulate this process as well. ^{13}C-NMR measurements of [^{13}C] glutamate resonances in muscle extracts after whole animal infusion of [U-^{13}C] fatty acids show that fatty acid oxidation is not altered in the absence of PHD1 (Aragones et al., 2008). This is somewhat surprising, since loss of PHD1 upregulates levels of PDK4 through PPARα activation (Aragones et al., 2008), a master regulator of metabolism that normally stimulates fatty acid oxidation at the expense of glucose oxidation (Lefebvre et al., 2006). The minimal role of PPARα in fatty acid oxidation in PHD1-deficient muscle may be explained by several reasons. First, PPARα levels are elevated in PHD1-deficient muscle (Aragones et al., 2008), but perhaps, this upregulation suffices to induce a shift in oxidative metabolism of glucose but not of fatty acids. Indeed, even supraphysiological levels of PPARα in skeletal muscle increase palmitate oxidation only by 1.5-fold (Finck et al., 2005). Second, in contrast to other organs, such as heart and liver, fatty acid oxidation in skeletal muscle is mainly controlled by PPARδ (Schuler et al., 2006), the expression of which is not altered in PHD1-deficient myofibers (Aragones et al., 2008). In addition, in other cell types, hypoxia downregulates the expression of PPARα or RXR, the obligate binding partner of PPARα, or reduces the DNA binding activity of the PPARα/RXR complex; these changes lower the levels of carnitine palmitoyltransferase I (*CPT-I*), the rate-limiting step in mitochondrial import of fatty acids for β oxidation; some of these effects are mediated by HIF-1α (Huss et al., 2001; Narravula and Colgan, 2001). Whether HIF-1α (by inhibiting PPARα signaling) counteracts the HIF-2α-driven upregulation of PPARα in PHD1-deficient mice, remains to be further explored.

Another intriguing question is whether PHDs/FIH are involved in hibernation. Indeed, similar to the PHD1-deficient state, hibernation relies, in part, on the induction of PDK4 and PPARα, and is also associated with attenuated glucose oxidation (Carey et al., 2003). Different from the PHD1-deficient mouse model, however, certain hibernating species combust their fat stores (Figure 4). In these species, induction of PPARα promotes lipid oxidation (Carey et al., 2003), presumably because levels of essential fatty acids, natural PPARα ligands, are elevated in hibernating species (Carey et al., 2003); this does not occur in PHD1 deficient mice, as their circulating fatty acids remain normal in fed conditions (Aragones et al., 2008).

Control of Oxygen Conformance by PHDs/FIH

Oxygen conformance refers to the ability of cells, upon exposure to insufficient O_2, to reduce energy expenditure, which allows

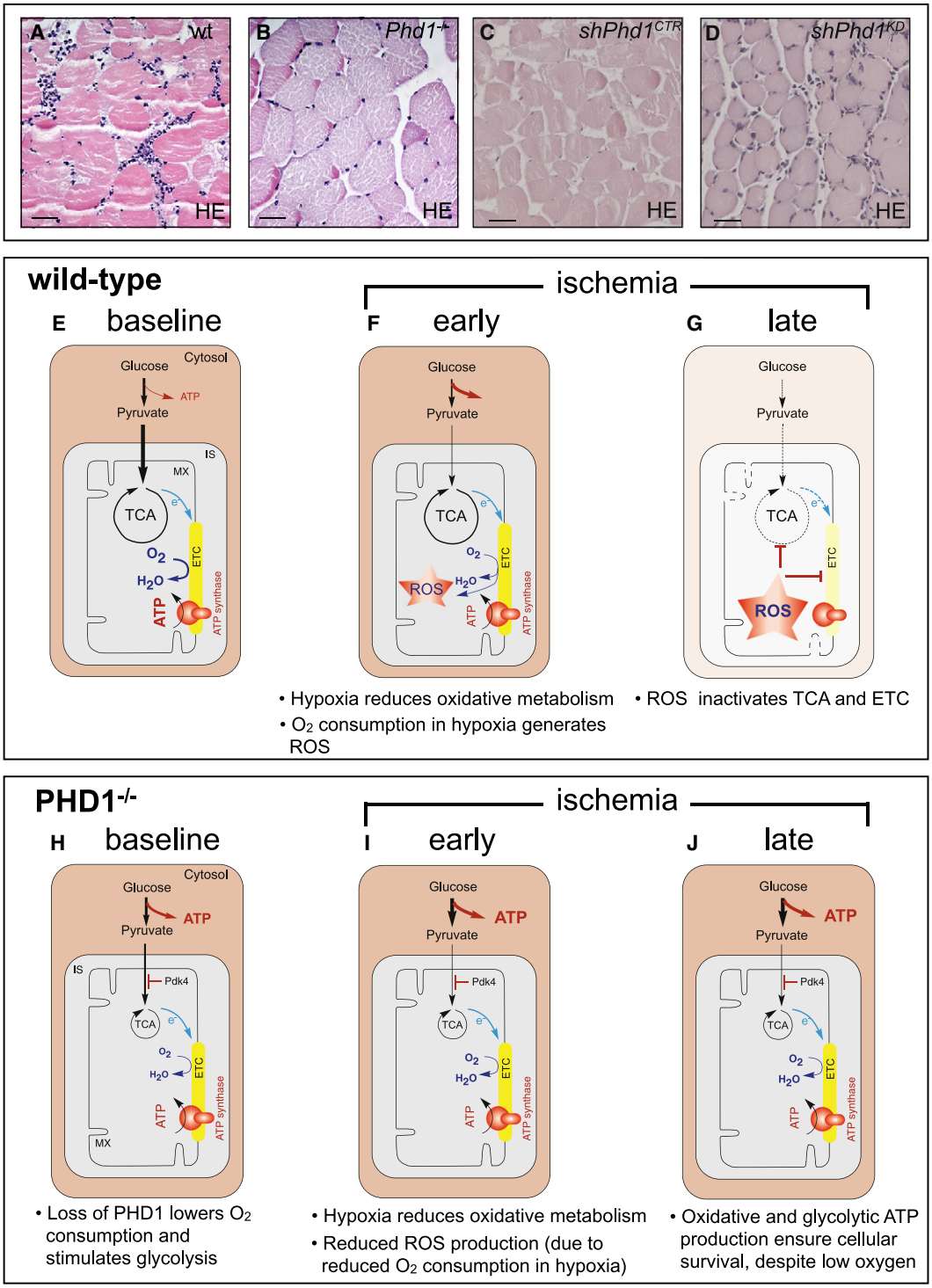

Figure 3. Loss of Phd1 Induces Hypoxia Tolerance

(A and B) Histopathology of gastrocnemius muscle 2 days after induction of hindlimb ischemia. Hematoxylin-eosin (HE) staining shows extensive coagulation necrosis and inflammatory cell infiltrates in wild-type muscle (A) but entirely healthy myofibers in ischemic PHD1 deficient muscle (B).

(C and D) Hematoxylin-eosin staining of heterozygous PHD1-deficient limb muscles (at 48 hr after ischemia) after shRNA-mediated knockdown of PHD1 (D) or electroporation with a mismatched shRNA-control construct (C) reveals protection against muscle necrosis upon knockdown of PHD1, but not in control-injected heterozygous *PHD1*-deficient muscle.

(E) Baseline energy metabolism in wild-type myofibers: ATP is generated by anaerobic glycolysis (cytosol) and by glucose oxidation in the TCA cycle (mitochondrial matrix; MX), where it provides electrons (e^-) for the mitochondrial electron-transport chain (ETC; yellow) that consequently drives the synthesis of ATP by the ATP synthase (red).

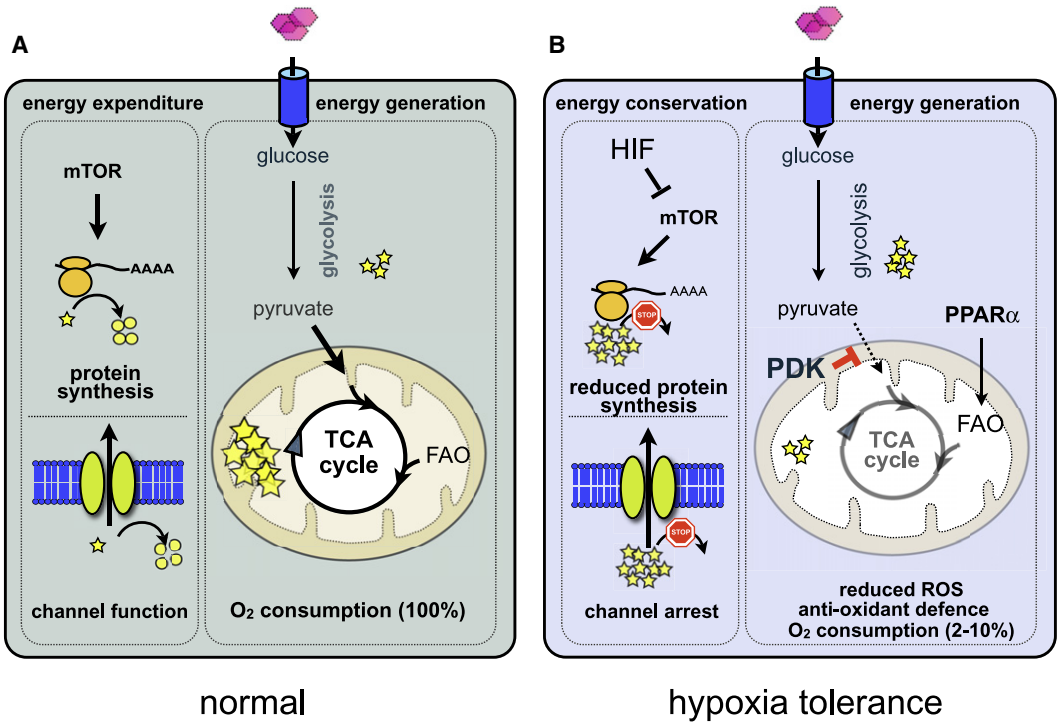

Figure 4. Role of PHDs and HIFs in Oxygen Conformance

(A) In normal metabolic conditions, a high level of energy expenditure is matched by a high level of energy generation. Depending on the cell type, ATP (yellow star) is generated glycolytically or oxidatively (an example of an oxidative cell type is shown for illustration; right box); ATP is consumed to ADP (yellow sphere) for protein synthesis, channel function, and other ATP-demanding processes (left box).

(B) During oxygen conformance, energy expenditure is reduced, in part because hypoxic activation of HIF inhibits overall protein synthesis by inhibiting mTOR signaling, thereby sparing ATP (left box). Cells also reduce O_2 consumption by lowering entry of glycolytic intermediates (via PDK) and reducing TCA flux and respiration (see Figure 2 for more explanation; right box). Depending on the species, glycolysis is compensatorily increased (Pasteur effect, as in PHD1-deficient mice or anoxia tolerant turtles) or remains unchanged (reverse Pasteur effect, as in *Mytilus* mussels), while in other species, PPARα-driven fatty acid oxidation (FAO) generates residual ATP in states of oxygen conformance (for instance, in hibernating grizzly bears). The reduced O_2 consumption also lowers production of ROS, while oxidative stress is further counteracted by increased antioxidant defense. Symbols and abbreviations: glucose (purple hexagons), ATP (yellow stars), ADP (yellow spheres), mTOR, mammalian target of rapamycin; Pyr, pyruvate; PDK, pyruvate dehydrogenase kinase; PPARα, peroxisome proliferator-activated receptor α; TCA cycle, tricarboxylic acid cycle; HIF, hypoxia inducible factor; ROS, reactive oxygen species.

them to lower O_2 consumption in advance of an energetic crisis. Such energy conservation enhances survival at low O_2 tension, and induces a state of hypoxia tolerance. Several species, such as fossorial or diving animals, estivating reptiles, hibernating mammals, and dormant *C. elegans* dauer larvae, or various invertebrates survive conditions of limiting O_2 supply, in part because of such metabolic depression (Ramirez et al., 2007). An extreme example is the crustacean *Daphnia*, which remains fully viable throughout a 24 hr anoxic challenge. But humans also show signs of oxygen conformance. For instance, hypoxia tolerance in highlanders or in the fetus, both exposed to low O_2 tension, results from reductions in O_2 demand. It has been postulated that fetal hypoxia tolerance is an adaptation of the neonate brain to resist the threats of low O_2 tension during the birth process.

In many cases, this hypometabolic state results from reduced glucose oxidation with a compensatory increase in anaerobic glycolysis. For instance, imaging studies reveal lower cerebral glucose utilization in Andean highlanders than lowlanders. Fetal hypoxia tolerance is also characterized by reduced glucose metabolism, with an associated increase in glucose transport, glycolytic enzymes, and glycogen stores (Singer and Muhlfeld, 2007). In some hypoxia tolerant invertebrates (such as intertidal *Mytilus* mussels or *Cardium* cockles), energy expenditure in hypoxic conditions drops so low that there is even no need for a compensatory increase in anaerobic glycolysis to generate

(F and G) Lethal response to hypoxia in ischemic wild-type myofibers. As a result of the reduced O_2 supply, oxidative phosphorylation is reduced. Nonetheless, ischemic wild-type myofibers continue to consume O_2, thereby generating excess ROS within the ETC (F). This increase in oxidative stress inactivates TCA and ETC enzymes (red line) during prolonged ischemia (G), ultimately causing irreversible mitochondrial damage (cristolysis, indicated by dotted line) and complete shutdown of oxidative energy metabolism. Anaerobic glycolysis cannot compensate for the loss of oxidative ATP production, leading to cellular energy exhaustion and demise.

(H) Baseline metabolic reprogramming in PHD1-deficient myofibers. Enhanced expression of PDK4 reduces glucose oxidation and O_2 consumption in PHD1 deficient myofibers. Coincidently, loss of PHD1 stimulates glycolytic production of ATP.

(I and J) Hypoxia tolerance in PHD1 deficient myofibers. Reduced O_2 consumption leads to attenuated ROS formation in ischemic PHD1 deficient myofibers (I). As a consequence, enzymes in the TCA and ETC are not (or are less) inactivated (J), thereby enabling residual oxidative phosphorylation (albeit lower than in baseline conditions because of the reduced O_2 supply). In addition, increased glycolysis together with the residual respiration provides sufficient ATP for survival. WT, wild-type. Adapted from Aragones et al. (2008).

ATP for survival ("reverse Pasteur effect"). As a result of the hypometabolic state, O_2 consumption is accordingly reduced, often more than 10-fold in hypoxia tolerant species (Andrews, 2002; Ramirez et al., 2007) (Figure 4).

Recent evidence implicates O_2 sensors as regulators of O_2 conformance. Indeed, pharmacological inhibition of PHDs/FIH or genetic loss of *PHD1* lowers O_2 consumption in cardiomyocytes and skeletal muscle cells, respectively, in vitro and in vivo (Aragones et al., 2008; Sridharan et al., 2007; Sridharan et al., 2008). This adaptation is caused, at least in part, by a reprogramming of basal metabolism, in particular by a reduction of oxidative glucose metabolism due to an upregulation of PDK1 and PDK4, which restrict the entry of glycolytic intermediates in the TCA cycle (see above) (Aragones et al., 2008) (Figures 3 and 4). Concomittant with the reduced O_2 consumption, oxidative muscle performance in PHD1-deficient mice is impaired, suggesting that not only mechanisms to reduce energy generation, but also to reduce energy expenditure are operational in *PHD1*$^{-/-}$ myofibers (Aragones et al., 2008). Indeed, they fatigue more rapidly when forced to run uphill, an exercise endurance test that recruits primarily oxidative muscle fibers and relies on oxidative metabolism. Loss of PHD1 does, however, not cause an extreme O_2 conformance program (as seen in water fleas and mussels), since it compensatorily upregulates glycolytic flux (Aragones et al., 2008). HIF-2α (and, to a lesser extent, HIF-1α) is a downstream effector of PHD1 in the hypometabolic adaptation (Aragones et al., 2008).

Another example of O_2 conformance, in which HIF/PHD signaling has been implicated, is the hibernating myocardium: to save energy and match the reduced blood supply, the chronic ischemic myocardium depresses oxidative metabolism, contractility, and pump function (Heusch et al., 2005). In a mouse model of myocardial hibernation, increased HIF levels drive a cardioprotective genetic program, that enhances glycolysis, through upregulation of *GLUT-1* and key glycolytic enzymes, including triphosphate isomerase (*TPI-1*), pyruvate kinase (*PK*), and *GAPDH* (May et al., 2008). Furthermore, the expression of motor proteins (*myosin 1B and tropomodulin 4*), ion pumps, and channels (*KCNIP2, KCNJ3, KCNJ8,* and *ATP1A2*) is downregulated, while *KCNE4*, an inhibitory subunit of potassium currents, is increased (May et al., 2008). Also, hibernating cardiomyocytes elevate levels of natriuretic peptides and adrenomedullin, cardioprotective factors that reduce cardiac load by promoting vasodilation and diuresis. Moreover, levels of *BNIP-3*, a HIF-inducible gene, are upregulated, resulting in autophagy of hibernating cardiomyocytes. *PHD3* expression is also upregulated, presumably in a hypoxia-driven feedback to keep HIF levels in check (May et al., 2008).

PHDs and HIFs not only control the switch to reduced energy generation, but they may also regulate the metabolic adaptation to reduced energy expenditure. To save energy in hypoxic conditions, cells downregulate nonessential anabolic or energy-consuming processes such as protein synthesis, folding, and degradation ("metabolic arrest"), ion pump activity ("channel arrest"), and other processes (Carey et al., 2003) (Figure 4). Initiation of translation is carried out by eukaryotic initiation factors (eIF), of which eIF2 and eIF4E are critical regulators of the preinitiation and cap-binding complex, respectively. Only inactivation of the eIF4F complex is regulated by HIF-1α in hypoxia (Liu and Simon, 2004). This results from the sequestration of its subunit eIF4E by 4E-BP1, when the latter is hypophosphorylated. HIF-1α controls this process by upregulating *Redd1*, an inhibitor of mTOR, which normally phosphorylates 4E-BP1 and thereby inactivates eIF4F (Brugarolas et al., 2004). Hypoxic tumor and other cells use this mechanism of oxygen conformance to survive in low O_2 conditions. To what extent and which energy conservation mechanisms are operational in PHD1-deficient muscle remains to be further studied.

Role of PHDs in Hypoxia Tolerance: Consequences for Ischemic Diseases

The role of PHDs in O_2 conformance has implications for hypoxia tolerance in ischemic conditions. This is exemplified by treatment of healthy mice with PHD/FIH inhibitors, which increases viability in (sub)lethal hypoxia, in part via upregulation of HIF-1α (Kasiganesan et al., 2007). Moreover, genetic studies in mice reveal that, upon permanent or transient ligation of supply arteries, or strenuous (ischemic) muscle exercise, muscle fibers, especially the ones relying on oxidative metabolism, in PHD1-deficient mice are almost completely protected against ischemic demise (Aragones et al., 2008) (Figure 3). Remarkably, this hypoxia tolerance is not attributable to an increase in O_2 supply through enhanced angiogenesis, vasodilation, or erythropoiesis, or a shift in muscle fiber from oxidative to glycolytic type, but, as explained below, to a depression of metabolism, which protects ischemic cells from lethal cellular oxidative damage.

It is well known that O_2 is of vital importance, yet also carries an inherent risk of toxicity. One theory even postulates that mitochondria became symbiont in hosts to escape possible deleterious effects of increasing O_2 tension in the earth's atmosphere, while the low intrauterine O_2 tension might be an adaptation to protect the immature fetus against O_2 toxicity (Singer and Muhlfeld, 2007). Also, Joseph Priestly, another scientist credited with the discovery of O_2, was mindful of the toxicity of O_2, writing "For as a candle burns much faster in dephlogisticated (O_2 enriched) than in common air, so we might live out too fast, and the animal powers be too soon exhausted in this pure kind of air." This toxicity of O_2 relates to the generation of ROS. The latter are generated as a result of electron leakage from the mitochondrial electron transfer chain during normal respiration (Kulkarni et al., 2007). A long-existing, though debated, model postulates that mitochondria are able to generate increased amounts of ROS under hypoxic conditions (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005). When excessive, formation of ROS in ischemic and ischemia-reperfusion events causes irreversible cellular damage and death. Notably, loss of PHD1 protects ischemic muscle fibers against such O_2 toxicity (Aragones et al., 2008), as explained in the next paragraph.

Upon ligation of the supply artery in wild-type mice, staining of myofibers for the hypoxia marker pimonidazole reveals that myofibers, despite being deprived of their O_2 supply by 70%, continue to consume O_2 (Aragones et al., 2008). Also, measurements of the reduced versus oxidized fraction of the antioxidant glutathione, of the muscle area that stains for 8-hydroxy-2-deoxy guanosine, a marker of deoxyguanosine oxidation, and of the level of protein carbonylation reveal that ischemic myofibers in wild-type mice generate large amounts of toxic ROS, that

irreversibly damage cellular organelles. Indeed, aconitase, a TCA enzyme that is highly sensitive to oxidative stress, becomes inactive in ischemic myofibers, which also exhibit severe ultrastructural signs of mitochondrial damage.

In contrast, ischemic PHD1-deficient myofibers do not become pimonidazole positive, consistent with findings that these cells consume less O_2 in baseline conditions (see above). Also, when using the above-mentioned assays, no or only minimal signs of oxidative stress can be detected in ischemic PHD1 mutant muscle. Hence, these findings suggest that, because of their reduced O_2 consumption, PHD1-deficient mitochondria exhibit a reduced electron transport and therefore generate less oxidative stress in ischemic conditions; this protects cell organelles against destruction. Indeed, ischemic PHD1-deficient myofibers have normal levels of active aconitase, and also do not exhibit ultrastructural signs of mitochondrial degeneration. Furthermore, in vivo ^{13}C-NMR measurements of [^{13}C] glutamate resonances in muscle extracts after infusion of [U-$^{13}C_6$] glucose indicate that, compared to ischemic wild-type myofibers, ischemic PHD1-deficient myofibers better preserve mitochondrial respiration (glucose oxidation), and are capable of regenerating ATP already within minutes after acute ischemia (Aragones et al., 2008). Hence, by switching from aerobic to anaerobic metabolism, and by slowing down mitochondrial respiration, PHD1-deficient cells protect themselves against deleterious effects of oxidative damage in hypoxic conditions (Aragones et al., 2008; Kim et al., 2006) (Figure 3).

Notably, even transient silencing of PHD1 rapidly induces hypoxia tolerance and protection against ischemic damage, warranting further evaluation of PHD1 inhibitors for therapy of ischemic disease (Aragones et al., 2008) (Figure 3). Pharmacological inhibition of PHDs/FIH also exhibits cytoprotection against ischemia/reperfusion injury (Hill et al., 2008; Ockaili et al., 2005). An outstanding question is whether PHDs/FIH may provide ischemic protection via other mechanisms, for instance, by regulating mechanisms of ischemic preconditioning. Some recent studies indeed suggest a role of the PHD/HIF pathway in this process. For instance, myocardial ischemic preconditioning is lost upon silencing of HIF-1α versus enhanced upon silencing of PHD2 (Eckle et al., 2008). Moreover, pharmacological inhibition of PHDs/FIH induces myocardial preconditioning in vivo (Philipp et al., 2006).

Notably, silencing of PPARα abrogates the protection of PHD1-deficient myofibers against ischemic damage after femoral artery ligation, indicating that PPARα is essential for ischemia tolerance (Aragones et al., 2008). Conversely, pharmacological activation of PPARα, by agonists such as fenofibrate, protects skeletal muscle in wild-type mice against ischemic injury (Aragones et al., 2008). This beneficial effect is also found in other organs, such as the heart and liver, where PPARα has a key role in ischemia tolerance (Okaya and Lentsch, 2004; Yue et al., 2003). The mechanisms underlying the role of PPARα in ischemia tolerance are not completely understood, and may differ form one organ to another. As explained above, in PHD1-deficient skeletal muscle, PPARα-dependent upregulation of PDK4 seems to contribute to hypoxia tolerance by attenuation of glucose oxidation, O_2 consumption, and mitochondrial ROS formation (Aragones et al., 2008). The role of PPARα in ischemia tolerance needs, however, further study, as transgenic animals with supraphysiological levels of PPARα in the heart are, in fact, more susceptible to ischemia/reperfusion injury (Sambandam et al., 2006); whether this suggests gene dosage-dependent activities of PPARα in this process remains to be explored.

Metabolic Regulation of Aging by PHDs/FIH

Senescence, a state of irreversible growth arrest, has been linked to oxidative metabolism and stress (including oxidative damage of mitochondrial DNA (Druzhyna et al., 2008), and is counteracted by hypoxia, likely through mechanisms mediating hypoxia tolerance. Of note, hypoxia tolerance is reduced in elderly (Mariani et al., 2000) and associated with impaired hypoxia-inducible gene expression (Di Giulio et al., 2005). Hypoxia increases the expression of a telomerase in a HIF-1α-dependent manner, thereby opposing age-related telomere shortening (Yatabe et al., 2004). The role of PHDs in oxidative metabolism and oxygen conformance may therefore explain why PHD2 induces cell senescence through repression of HIF activity (Kato et al., 2006). Expression of PHD3 is also upregulated in aging rodents; this induction is counteracted by caloric restriction (Rohrbach et al., 2008) that promotes longevity. The protection against oxidative stress in the absence of PHD1 (Aragones et al., 2008) versus the aggravated oxidative stress in mice lacking HIF-2α (Scortegagna et al., 2003) further warrant analysis of O_2 sensing pathways in senescence. Also, the age-associated increase of *PHD3* expression inversely correlates with the expression of macrophage migration inhibitory factor, a HIF target that inhibits cellular senescence (Rohrbach et al., 2008; Welford et al., 2006).

Perspectives

It is evident from the above discussion that PHDs/FIH are important multitasking regulators of metabolism, with far reaching impact on processes such as metabolic fuel selection, oxidative performance, oxidative stress, hypoxia tolerance, hibernation, and others. Considering the involvement of these processes in numerous medical disorders, ranging from ischemic heart disease, stroke, inflammatory disorders, cancer, and various metabolic disorders, further study of the distinct role of these O_2 sensors is warranted. A better understanding of their (patho)-physiological role will also aid in the preclinical development of therapeutic strategies, based on the use of selective PHD/FIH inhibitors.

ACKNOWLEDGMENTS

The authors would like to apologize for not being able to cite numerous other important contributions because of space limitations. P.C. declares to be named as inventor on patent application WO2007/082899, claiming subject matter that is partially based on the results described in this paper. Should the aforementioned patent application or any resulting patent be licensed, this may result in a royalty payment to P.C. This work is supported, in part, by grant GOA2006/11/KULeuven from the University of Leuven, Belgium, Methusalem Program, KU Leuven, grant IUAP06/30 from the Federal Government Belgium, grants FWO G.0265 and FWO G.0652 from the Flanders Research Foundation, Belgium. P.F. is supported by a postdoctoral fellowship from the Marie Curie Program of the European Commission.

REFERENCES

Andrews, R.M. (2002). Low oxygen: a constraint on the evolution of viviparity in reptiles. Physiol. Biochem. Zool. *75*, 145–154.

Aragones, J., Schneider, M., Van Geyte, K., Fraisl, P., Dresselaers, T., Mazzone, M., Dirkx, R., Zacchigna, S., Lemieux, H., Jeoung, N.H., et al. (2008). Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. Nat. Genet. *40*, 170–180.

Berra, E., Ginouves, A., and Pouyssegur, J. (2006). The hypoxia-inducible-factor hydroxylases bring fresh air into hypoxia signalling. EMBO Rep. *7*, 41–45.

Bracken, C.P., Fedele, A.O., Linke, S., Balrak, W., Lisy, K., Whitelaw, M.L., and Peet, D.J. (2006). Cell-specific regulation of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha stabilization and transactivation in a graded oxygen environment. J. Biol. Chem. *281*, 22575–22585.

Braun, R.D., Lanzen, J.L., Snyder, S.A., and Dewhirst, M.W. (2001). Comparison of tumor and normal tissue oxygen tension measurements using OxyLite or microelectrodes in rodents. Am. J. Physiol. Heart Circ. Physiol. *280*, H2533–H2544.

Brugarolas, J., Lei, K., Hurley, R.L., Manning, B.D., Reiling, J.H., Hafen, E., Witters, L.A., Ellisen, L.W., and Kaelin, W.G., Jr. (2004). Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Dev. *18*, 2893–2904.

Bruick, R.K., and McKnight, S.L. (2001). A conserved family of prolyl-4-hydroxylases that modify HIF. Science *294*, 1337–1340.

Brunelle, J.K., Bell, E.L., Quesada, N.M., Vercauteren, K., Tiranti, V., Zeviani, M., Scarpulla, R.C., and Chandel, N.S. (2005). Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metab. *1*, 409–414.

Calvisi, D.F., Ladu, S., Gorden, A., Farina, M., Lee, J.S., Conner, E.A., Schroeder, I., Factor, V.M., and Thorgeirsson, S.S. (2007). Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. J. Clin. Invest. *117*, 2713–2722.

Carey, H.V., Andrews, M.T., and Martin, S.L. (2003). Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. Physiol. Rev. *83*, 1153–1181.

Carmeliet, P., and Jain, R.K. (2000). Angiogenesis in cancer and other diseases. Nature *407*, 249–257.

Cash, T.P., Pan, Y., and Simon, M.C. (2007). Reactive oxygen species and cellular oxygen sensing. Free Radic. Biol. Med. *43*, 1219–1225.

Chow, D.C., Wenning, L.A., Miller, W.M., and Papoutsakis, E.T. (2001). Modeling pO(2) distributions in the bone marrow hematopoietic compartment. II. Modified Kroghian models. Biophys. J. *81*, 685–696.

Dahia, P.L., Ross, K.N., Wright, M.E., Hayashida, C.Y., Santagata, S., Barontini, M., Kung, A.L., Sanso, G., Powers, J.F., Tischler, A.S., et al. (2005). A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. PLoS Genet. *1*, 72–80.

Dang, C.V., Kim, J.W., Gao, P., and Yustein, J. (2008). The interplay between MYC and HIF in cancer. Nat. Rev. Cancer *8*, 51–56.

Dayan, F., Roux, D., Brahimi-Horn, M.C., Pouyssegur, J., and Mazure, N.M. (2006). The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. Cancer Res. *66*, 3688–3698.

DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G., and Thompson, C.B. (2008). The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. *7*, 11–20.

Di Giulio, C., Bianchi, G., Cacchio, M., Artese, L., Rapino, C., Macri, M.A., and Di Ilio, C. (2005). Oxygen and life span: chronic hypoxia as a model for studying HIF-1alpha, VEGF and NOS during aging. Respir. Physiolo. Neurobiol. *147*, 31–38.

Druzhyna, N.M., Wilson, G.L., and LeDoux, S.P. (2008). Mitochondrial DNA repair in aging and disease. Mech. Ageing Dev. *129*, 383–390.

Eckle, T., Kohler, D., Lehmann, R., El Kasmi, K., and Eltzschig, H.K. (2008). Hypoxia-inducible factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning. Circulation *118*, 166–175.

Ehrismann, D., Flashman, E., Genn, D.N., Mathioudakis, N., Hewitson, K.S., Ratcliffe, P.J., and Schofield, C.J. (2007). Studies on the activity of the hypoxia-inducible-factor hydroxylases using an oxygen consumption assay. Biochem. J. *401*, 227–234.

Epstein, A.C., Gleadle, J.M., McNeill, L.A., Hewitson, K.S., O'Rourke, J., Mole, D.R., Mukherji, M., Metzen, E., Wilson, M.I., Dhanda, A., et al. (2001). C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell *107*, 43–54.

Erecinska, M., and Silver, I.A. (2001). Tissue oxygen tension and brain sensitivity to hypoxia. Respir. Physiol. *128*, 263–276.

Finck, B.N., Bernal-Mizrachi, C., Han, D.H., Coleman, T., Sambandam, N., LaRiviere, L.L., Holloszy, J.O., Semenkovich, C.F., and Kelly, D.P. (2005). A potential link between muscle peroxisome proliferator- activated receptor-alpha signaling and obesity-related diabetes. Cell Metab. *1*, 133–144.

Fukuda, R., Zhang, H., Kim, J.W., Shimoda, L., Dang, C.V., and Semenza, G.L. (2007). HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell *129*, 111–122.

Gatenby, R.A., and Gillies, R.J. (2008). A microenvironmental model of carcinogenesis. Nat. Rev. Cancer *8*, 56–61.

Gnaiger, E., Lassnig, B., Kuznetsov, A., Rieger, G., and Margreiter, R. (1998). Mitochondrial oxygen affinity, respiratory flux control and excess capacity of cytochrome c oxidase. J. Exp. Biol. *201*, 1129–1139.

Gorr, T.A., Gassmann, M., and Wappner, P. (2006). Sensing and responding to hypoxia via HIF in model invertebrates. J. Insect Physiol. *52*, 349–364.

Guzy, R.D., Hoyos, B., Robin, E., Chen, H., Liu, L., Mansfield, K.D., Simon, M.C., Hammerling, U., and Schumacker, P.T. (2005). Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab. *1*, 401–408.

Harris, A.L. (2002). Hypoxia–a key regulatory factor in tumour growth. Nat. Rev. Cancer *2*, 38–47.

Helmlinger, G., Yuan, F., Dellian, M., and Jain, R.K. (1997). Interstitial pH and pO2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. Nat. Med. *3*, 177–182.

Heusch, G., Schulz, R., and Rahimtoola, S.H. (2005). Myocardial hibernation: a delicate balance. Am. J. Physiol. Heart Circ. Physiol. *288*, H984–H999.

Hill, P., Shukla, D., Tran, M.G., Aragones, J., Cook, H.T., Carmeliet, P., and Maxwell, P.H. (2008). Inhibition of hypoxia inducible factor hydroxylases protects against renal ischemia-reperfusion injury. J. Am. Soc. Nephrol. *19*, 39–46.

Huss, J.M., Levy, F.H., and Kelly, D.P. (2001). Hypoxia inhibits the peroxisome proliferator-activated receptor alpha/retinoid X receptor gene regulatory pathway in cardiac myocytes: a mechanism for O2-dependent modulation of mitochondrial fatty acid oxidation. J. Biol. Chem. *276*, 27605–27612.

Isaacs, J.S., Jung, Y.J., Mole, D.R., Lee, S., Torres-Cabala, C., Chung, Y.L., Merino, M., Trepel, J., Zbar, B., Toro, J., et al. (2005). HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. Cancer Cell *8*, 143–153.

Iyer, N.V., Kotch, L.E., Agani, F., Leung, S.W., Laughner, E., Wenger, R.H., Gassmann, M., Gearhart, J.D., Lawler, A.M., Yu, A.Y., and Semenza, G.L. (1998). Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. *12*, 149–162.

Kaelin, W.G., Jr., and Ratcliffe, P.J. (2008). Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol. Cell *30*, 393–402.

Kasiganesan, H., Sridharan, V., and Wright, G. (2007). Prolyl hydroxylase inhibitor treatment confers whole-animal hypoxia tolerance. Acta. Physiol. (Oxf) *190*, 163–169.

Kato, H., Inoue, T., Asanoma, K., Nishimura, C., Matsuda, T., and Wake, N. (2006). Induction of human endometrial cancer cell senescence through modulation of HIF-1alpha activity by EGLN1. Int. J. Cancer *118*, 1144–1153.

Kim, J.W., Tchernyshyov, I., Semenza, G.L., and Dang, C.V. (2006). HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. *3*, 177–185.

Koivunen, P., Hirsila, M., Gunzler, V., Kivirikko, K.I., and Myllyharju, J. (2004). Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases. J. Biol. Chem. *279*, 9899–9904.

Koivunen, P., Tiainen, P., Hyvarinen, J., Williams, K.E., Sormunen, R., Klaus, S.J., Kivirikko, K.I., and Myllyharju, J. (2007). An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor alpha. J. Biol. Chem. *282*, 30544–30552.

Kroemer, G., and Pouyssegur, J. (2008). Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell *13*, 472–482.

Kulkarni, A.C., Kuppusamy, P., and Parinandi, N. (2007). Oxygen, the lead actor in the pathophysiologic drama: enactment of the trinity of normoxia, hypoxia, and hyperoxia in disease and therapy. Antioxid. Redox Signal. *9*, 1717–1730.

Lando, D., Gorman, J.J., Whitelaw, M.L., and Peet, D.J. (2003). Oxygen-dependent regulation of hypoxia-inducible factors by prolyl and asparaginyl hydroxylation. Eur. J. Biochem. *270*, 781–790.

Lando, D., Peet, D.J., Gorman, J.J., Whelan, D.A., Whitelaw, M.L., and Bruick, R.K. (2002). FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev. *16*, 1466–1471.

Lefebvre, P., Chinetti, G., Fruchart, J.C., and Staels, B. (2006). Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. J. Clin. Invest. *116*, 571–580.

Leichtweiss, H.P., Lubbers, D.W., Weiss, C., Baumgartl, H., and Reschke, W. (1969). The oxygen supply of the rat kidney: measurements of intrarenal pO2. Pflugers Arch. *309*, 328–349.

Liu, L., and Simon, M.C. (2004). Regulation of transcription and translation by hypoxia. Cancer Biol. Ther. *3*, 492–497.

Lu, H., Dalgard, C.L., Mohyeldin, A., McFate, T., Tait, A.S., and Verma, A. (2005). Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. J. Biol. Chem. *280*, 41928–41939.

Lu, C.W., Lin, S.C., Chen, K.F., Lai, Y.Y., and Tsai, S.J. (2008). Induction of pyruvate dehydrogenase kinase 3 by HIF-1 promotes metabolic switch and drug resistance. J. Biol. Chem. 10.1074/jbc.M803508200.

Mansfield, K.D., Guzy, R.D., Pan, Y., Young, R.M., Cash, T.P., Schumacker, P.T., and Simon, M.C. (2005). Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation. Cell Metab. *1*, 393–399.

Mariani, J., Ou, R., Bailey, M., Rowland, M., Nagley, P., Rosenfeldt, F., and Pepe, S. (2000). Tolerance to ischemia and hypoxia is reduced in aged human myocardium. J. Thorac. Cardiovasc. Surg. *120*, 660–667.

May, D., Gilon, D., Djonov, V., Itin, A., Lazarus, A., Gordon, O., Rosenberger, C., and Keshet, E. (2008). Transgenic system for conditional induction and rescue of chronic myocardial hibernation provides insights into genomic programs of hibernation. Proc. Natl. Acad. Sci. USA *105*, 282–287.

Minamishima, Y.A., Moslehi, J., Bardeesy, N., Cullen, D., Bronson, R.T., and Kaelin, W.G., Jr. (2008). Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. Blood *111*, 3236–3244.

Narravula, S., and Colgan, S.P. (2001). Hypoxia-inducible factor 1-mediated inhibition of peroxisome proliferator-activated receptor alpha expression during hypoxia. J. Immunol. *166*, 7543–7548.

Ockaili, R., Natarajan, R., Salloum, F., Fisher, B.J., Jones, D., Fowler, A.A., 3rd, and Kukreja, R.C. (2005). HIF-1 activation attenuates postischemic myocardial injury: role for heme oxygenase-1 in modulating microvascular chemokine generation. Am. J. Physiol. Heart Circ. Physiol. *289*, H542–H548.

Ohneda, O., Nagano, M., and Fujii-Kuriyama, Y. (2007). Role of hypoxia-inducible factor-2alpha in endothelial development and hematopoiesis. Methods Enzymol. *435*, 199–218.

Okaya, T., and Lentsch, A.B. (2004). Peroxisome proliferator-activated receptor-alpha regulates postischemic liver injury. Am. J. Physiol. Gastrointest. Liver Physiol. *286*, G606–G612.

Papandreou, I., Cairns, R.A., Fontana, L., Lim, A.L., and Denko, N.C. (2006). HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab. *3*, 187–197.

Percy, M.J., Zhao, Q., Flores, A., Harrison, C., Lappin, T.R., Maxwell, P.H., McMullin, M.F., and Lee, F.S. (2006). A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. Proc. Natl. Acad. Sci. USA *103*, 654–659.

Percy, M.J., Furlow, P.W., Beer, P.A., Lappin, T.R., McMullin, M.F., and Lee, F.S. (2007). A novel erythrocytosis-associated PHD2 mutation suggests the location of a HIF binding groove. Blood *110*, 2193–2196.

Philipp, S., Cui, L., Ludolph, B., Kelm, M., Schulz, R., Cohen, M.V., and Downey, J.M. (2006). Desferoxamine and ethyl-3,4-dihydroxybenzoate protect myocardium by activating NOS and generating mitochondrial ROS. Am. J. Physiol. Heart Circ. Physiol. *290*, H450–H457.

Pouyssegur, J., Dayan, F., and Mazure, N.M. (2006). Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature *441*, 437–443.

Ramirez, J.M., Folkow, L.P., and Blix, A.S. (2007). Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. Annu. Rev. Physiol. *69*, 113–143.

Rohrbach, S., Teichert, S., Niemann, B., Franke, C., and Katschinski, D.M. (2008). Caloric restriction counteracts age-dependent changes in prolyl-4-hydroxylase domain (PHD) 3 expression. Biogerontology *9*, 169–176.

Safran, M., and Kaelin, W.G., Jr. (2003). HIF hydroxylation and the mammalian oxygen-sensing pathway. J. Clin. Invest. *111*, 779–783.

Sambandam, N., Morabito, D., Wagg, C., Finck, B.N., Kelly, D.P., and Lopaschuk, G.D. (2006). Chronic activation of PPARalpha is detrimental to cardiac recovery after ischemia. Am. J. Physiol. Heart Circ. Physiol. *290*, H87–H95.

Schofield, C.J., and Ratcliffe, P.J. (2004). Oxygen sensing by HIF hydroxylases. Nat. Rev. Mol. Cell Biol. *5*, 343–354.

Schuler, M., Ali, F., Chambon, C., Duteil, D., Bornert, J.M., Tardivel, A., Desvergne, B., Wahli, W., Chambon, P., and Metzger, D. (2006). PGC1alpha expression is controlled in skeletal muscles by PPARbeta, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. Cell Metab. *4*, 407–414.

Scortegagna, M., Ding, K., Oktay, Y., Gaur, A., Thurmond, F., Yan, L.J., Marck, B.T., Matsumoto, A.M., Shelton, J.M., Richardson, J.A., et al. (2003). Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1−/− mice. Nat. Genet. *35*, 331–340.

Selak, M.A., Armour, S.M., MacKenzie, E.D., Boulahbel, H., Watson, D.G., Mansfield, K.D., Pan, Y., Simon, M.C., Thompson, C.B., and Gottlieb, E. (2005). Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell *7*, 77–85.

Semenza, G.L. (2003). Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer *3*, 721–732.

Semenza, G.L. (2007). Life with oxygen. Science *318*, 62–64.

Shimoda, L.A., Fallon, M., Pisarcik, S., Wang, J., and Semenza, G.L. (2006). HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes. Am. J. Physiol. Lung Cell. Mol. Physiol. *291*, L941–L949.

Simon, M.C., and Keith, B. (2008). The role of oxygen availability in embryonic development and stem cell function. Nat. Rev. Mol. Cell Biol. *9*, 285–296.

Simonides, W.S., Mulcahey, M.A., Redout, E.M., Muller, A., Zuidwijk, M.J., Visser, T.J., Wassen, F.W., Crescenzi, A., da-Silva, W.S., Harney, J., et al. (2008). Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats. J. Clin. Invest. *118*, 975–983.

Singer, D., and Muhlfeld, C. (2007). Perinatal adaptation in mammals: the impact of metabolic rate. Comp. Biochem. Physiol. A Mol. Integr. Physiol. *148*, 780–784.

Sridharan, V., Guichard, J., Bailey, R.M., Kasiganesan, H., Beeson, C., and Wright, G.L. (2007). The prolyl hydroxylase oxygen-sensing pathway is cytoprotective and allows maintenance of mitochondrial membrane potential during metabolic inhibition. Am. J. Physiol. Cell Physiol. *292*, C719–C728.

Sridharan, V., Guichard, J., Li, C.Y., Muise-Helmericks, R., Beeson, C.C., and Wright, G.L. (2008). O(2)-sensing signal cascade: clamping of O(2) respiration, reduced ATP utilization, and inducible fumarate respiration. Am. J. Physiol. Cell Physiol. *295*, C29–C37.

Takeda, K., and Fong, G.H. (2007). Prolyl hydroxylase domain 2 protein suppresses hypoxia-induced endothelial cell proliferation. Hypertension *49*, 178–184.

Takeda, K., Cowan, A., and Fong, G.H. (2007). Essential role for prolyl hydroxylase domain protein 2 in oxygen homeostasis of the adult vascular system. Circulation *116*, 774–781.

Takeda, K., Aguila, H.L., Parikh, N.S., Li, X., Lamothe, K., Duan, L.J., Takeda, H., Lee, F.S., and Fong, G.H. (2008). Regulation of adult erythropoiesis by prolyl hydroxylase domain proteins. Blood *111*, 3229–3235.

Tang, N., Wang, L., Esko, J., Giordano, F.J., Huang, Y., Gerber, H.P., Ferrara, N., and Johnson, R.S. (2004). Loss of HIF-1alpha in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. Cancer Cell *6*, 485–495.

Ullah, M.S., Davies, A.J., and Halestrap, A.P. (2006). The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. J. Biol. Chem. *281*, 9030–9037.

Vincent, K.A., Shyu, K.G., Luo, Y., Magner, M., Tio, R.A., Jiang, C., Goldberg, M.A., Akita, G.Y., Gregory, R.J., and Isner, J.M. (2000). Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF-1alpha/VP16 hybrid transcription factor. Circulation *102*, 2255–2261.

Ward, J.P. (2008). Oxygen sensors in context. Biochim. Biophys. Acta *1777*, 1–14.

Weidemann, A., and Johnson, R.S. (2008). Biology of HIF-1alpha. Cell Death Differ. *15*, 621–627.

Welford, S.M., Bedogni, B., Gradin, K., Poellinger, L., Broome Powell, M., and Giaccia, A.J. (2006). HIF1alpha delays premature senescence through the activation of MIF. Genes Dev. *20*, 3366–3371.

Wiesener, M.S., Turley, H., Allen, W.E., Willam, C., Eckardt, K.U., Talks, K.L., Wood, S.M., Gatter, K.C., Harris, A.L., Pugh, C.W., et al. (1998). Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. Blood *92*, 2260–2268.

Wykoff, C.C., Beasley, N.J., Watson, P.H., Turner, K.J., Pastorek, J., Sibtain, A., Wilson, G.D., Turley, H., Talks, K.L., Maxwell, P.H., et al. (2000). Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res. *60*, 7075–7083.

Yatabe, N., Kyo, S., Maida, Y., Nishi, H., Nakamura, M., Kanaya, T., Tanaka, M., Isaka, K., Ogawa, S., and Inoue, M. (2004). HIF-1-mediated activation of telomerase in cervical cancer cells. Oncogene *23*, 3708–3715.

Yue, T.L., Bao, W., Jucker, B.M., Gu, J.L., Romanic, A.M., Brown, P.J., Cui, J., Thudium, D.T., Boyce, R., Burns-Kurtis, C.L., et al. (2003). Activation of peroxisome proliferator-activated receptor-alpha protects the heart from ischemia/reperfusion injury. Circulation *108*, 2393–2399.

Zhang, H., Gao, P., Fukuda, R., Kumar, G., Krishnamachary, B., Zeller, K.I., Dang, C.V., and Semenza, G.L. (2007). HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. Cancer Cell *11*, 407–420.

Zhang, H., Bosch-Marce, M., Shimoda, L.A., Tan, Y.S., Baek, J.H., Wesley, J.B., Gonzalez, F.J., and Semenza, G.L. (2008). Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J. Biol. Chem. *283*, 10892–10903.