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Into Thin Air: How We Sense and Respond to Hypoxia

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This year's Lasker Basic Medical Research Award is shared by William Kaelin, Peter Ratcliffe, and Gregg Semenza for discovery of the pathway by which cells sense and adapt to changes in oxygen availability, which plays an essential role in human adaptation to a wide variety of physiologic and pathologic conditions.

One thing we all learned in grade school is that oxygen is really important. Each time we breathe, oxygen is taken up through our lungs into the bloodstream and carbon dioxide is expelled. The oxygen consumed is essential for producing a robust energy supply with which to maintain the viability and function of the 40 trillion cells within our bodies. This is because our cells have two major mechanisms for producing ATP from glucose: glycolysis, which can take place anaerobically but is highly inefficient at generating ATP, and oxidative phosphorylation, which maximizes ATP yield but requires oxygen. Cells can also use oxidative phosphorylation to produce ATP from fatty acid and amino acid degradation, further increasing the amount of energy that can be extracted from nutrients. Since the metabolic demands of a complex nervous system or muscle-based motility are impossible without efficient ATP production, the harnessing of environmental oxygen for energy production was essential for the development of multicellular animal life as we know it.

As a result, how cells catabolize nutrients and oxygen to produce the ATP required to maintain cellular bioenergetics has been the subject of numerous scientific prizes. Moreover, when it became clear that organs such as the brain, heart, and muscle are uniquely dependent on efficient production of ATP through oxidative phosphorylation, another generation of prizes was awarded for the discovery and characterization of proteins, such as hemoglobin and myoglobin, that can either carry oxygen to or buffer oxygen reserves in these vital tissues. Despite these extraordinary accomplishments, until recently, one aspect of oxygen metabolism remained unresolved—namely, how animals sense and adapt to changing levels of oxygen availability, a function that is essential for adaptation to high altitudes and is dysregulated in disease states such as cardiopulmonary failure or anemia. This year's Lasker Basic Research Award is being awarded to three scientists for their discovery of the central oxygen-sensing system that allows animals to sense and adapt to physiological variations in oxygen.

Finding the Factor

Although William Kaelin, Peter Ratcliffe, and Gregg Semenza worked independently, their work collectively uncovered the molecular mechanism by which organisms sense and adapt to low oxygen levels, or hypoxia. The solution to the problem began with erythropoietin, a hematopoietic growth factor produced by the liver and kidney. Erythropoietin promotes generation of red blood cells in the bone marrow, thereby raising the blood's oxygen carrying capacity.

In an elegant series of experiments that started with his interest in learning more about gene regulation mechanisms, Semenza made the key initial discoveries that broke open the field. Using transgenic mice, he uncovered sequence-specific binding of a transcription factor to the hypoxia-response element (HRE) in the 3′ enhancer region of erythropoietin when liver cells were subjected to hypoxia (Semenza and Wang, 1992), and then biochemically purified the factor (Wang et al., 1995), a heterodimer that he dubbed HIF-1 (hypoxia inducible factor 1). Both components of this heterodimer were members of the bHLH-Pas family of transcription factors, and while the HIF-1β component (also known as ARNT) was constitutively expressed, expression of HIF-1α varied in an oxygen-dependent fashion.

Betting that HIF-1 did more than regulate erythropoietin expression, Semenza started digging deeper. A critical advance came when he discovered that HIF-1α also induced vascular endothelial growth factor (VEGF), which plays a key role in angiogenesis (Forsythe et al., 1996). This suggested that HIF-1α was at the center of an oxygen-sensing mechanism important for controlling both the generation of the vascular system and the production of red cells to carry O_2 within the bloodstream. To confirm the essential role of HIF-1 in these organismal processes, Semenza then made HIF-1α mutant mice. In the absence of HIF-1α, he indeed found that vascular development and oxygen-dependent gene expression were severely impaired, leading to embryonic lethality (Iyer et al., 1998).

Over this time period, Semenza's work was complemented and extended by the work of Peter Ratcliffe. Oxygen sensing was long thought to be a property restricted to a specialized set of cell types, but Ratcliffe showed that HRE DNA binding activity was regulated in an oxygen-dependent fashion in all cell types he tested (Maxwell et al., 1993). This suggested that HIF-1 was a part of a universal cellular response mechanism to changes in oxygen availability. Ratcliffe confirmed this by demonstrating that the genes encoding glycolytic enzymes were also regulated by hypoxia response elements in their enhancers (Firth et al., 1994). Thus, HIF-1 was not only responsible for long-term adaptation to hypoxia at the organismal level, but also could dynamically regulate the rate of glycolysis, the oxygen-independent mechanism of ATP generation, at the cellular level.

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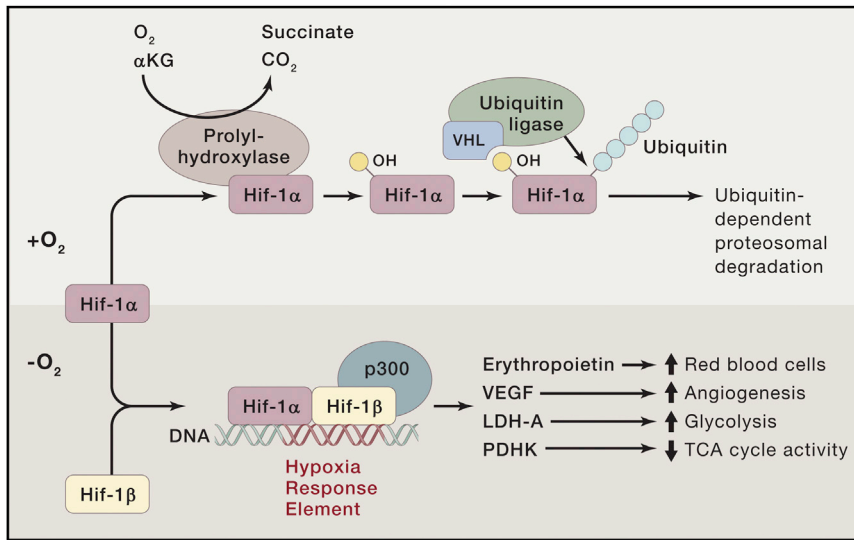
Figure 1. Oxygen-Dependent Regulation of the HIF-1 Transcription Factor by Prolyl Hydroxylases

Under normoxic conditions, the HIF-1α component of the heterodimeric transcription factor, HIF-1, undergoes hydroxylation through the enzymatic activity of prolyl hydroxylases. The resulting prolyl hydroxylation of HIF-1α is recognized by the tumor suppressor V(H)L, leading to V(H)L-dependent recruitment of a ubiquitin ligase complex and HIF-1α ubiquitination and proteosomal degradation. In the reaction carried out by prolyl hydroxylases, the concentration of oxygen is rate limiting. This allows the hydroxylation reaction to regulate the accumulation of HIF-1α in direct relation to oxygen availability. Under hypoxic conditions, the accumulating HIF-1α dimerizes with HIF-1β, leading to nuclear translocation and binding to hypoxia-response elements (HRE) in genes required for hypoxic adaptation. Sequence-specific DNA binding of HIF-1 leads to the recruitment of transcriptional co-activators such as p300 and the transcription of target genes that stimulate increased vascularization (VEGF), enhance the oxygen carrying capacity of blood (erythropoietin), stimulate oxygen-independent production of ATP through glycolysis (LDH-A), and suppress TCA cycle activity and oxidative phosphorylation (PDHK).

Clues from the Clinic

Unlike Semenza and Ratcliffe, Kaelin came upon the oxygen adaptation pathway through his efforts to make sense of observations in the cancer biology field. From his clinical training, he knew that certain kidney tumors had increased vasculature and lost the tumor suppressor V(H)L, first identified in the von Hippel-Lindau syndrome. However, the cloning of V(H)L had not provided any insights into its potential molecular function. In 1995, Kaelin's group was one of several to show that V(H)L formed a complex with Elongin B, Elongin C, and CUL2 (Kibel et al., 1995). Since Elongin C and CUL2 showed homology to the yeast proteins SKP1 and Cdc53, the V(H)L/Elongin/CUL2 complex might function as a ubiquitin ligase and therefore control protein stability. But how did this lead to increased vasculature of kidney tumors? Kaelin hypothesized that the absence of V(H)L might lead cells to produce the angiogenic factor VEGF. Indeed, he found that V(H)L

deficiency led to constitutive upregulation of HIF-dependent targets defined by Ratcliffe and Semenza, including VEGF, even in the presence of oxygen (Iliopoulos et al., 1996).

Making the Oxygen Link

Based on these discoveries, both Ratcliffe and Kaelin went on to uncover a simple and elegant post-translational oxygen-sensing mechanism. They first showed that V(H)L acted as an E3 ubiquitin ligase for HIF-1α and that ubiquitination of HIF-1α led to its degradation. In back-to-back papers in 2001, Ratcliffe and Kaelin reported that HIF-1α underwent proline hydroxylation in the presence of oxygen and that V(H)L specifically bound hydroxylated HIF-1α to target it for degradation in an oxygen-dependent fashion (Ivan et al., 2001; Jaakkola et al., 2001). This of course raised a question: what was controlling the oxygen-dependent hydroxylation of HIF-1α? Ratcliffe, Kaelin, and Steve McKnight indepen-

dently reported the discovery of a family of prolyl-4-hydroxylases with this function (Bruick and McKnight, 2001; Ivan et al., 2002; Epstein et al., 2001). A second oxygen-dependent mechanism of HIF-1α hydroxylation, this time on asparagine residues, was subsequently found to block the ability of HIF-1α to recruit transcriptional coactivators (Lando et al., 2002). This provided cells with a back-up system to block any residual HIF-1 that had escaped degradation from activating transcription of target genes.

Sensing More Than Just Oxygen

The discovery of HIF-1α-modifying hydroxylases led to a broader interest in the role of these enzymes as metabolite sensors. In addition to their originally described function as oxygen sensors, subsequent studies have shown that they can also be regulated by other reactants, as well as the products of the reactions they carry out. Each of the enzymes contains a catalytic Fe^{2+} in the active site to hydroxylate targets using oxygen and alpha-ketoglutarate (αKG) as reactants, producing succinate and carbon dioxide as byproducts (Figure 1). Sustained inhibition of HIF hydroxylation in hypoxia is also accomplished in part through lactate dehydrogenase (LDH-A)-dependent conversion of αKG to L-2-hydroxyglutarate (L-2HG). The resulting L-2HG acts as a competitive inhibitor of αKG binding to the HIF hydroxylases (Harris, 2015).

The broader relevance of this metabolic sensor pathway in cancer became evident in studies involving two other tumor suppressor syndromes with a hypervascular microenvironment similar to that observed in V(H)L mutant renal cell carcinomas. In these tumor syndromes, loss-of-function mutations in either succinate dehydrogenase or fumarase led to high levels of HIF-1 in the presence of oxygen. Without these enzymes, the TCA cycle intermediates succinate and fumarate accumulate, leading to end product inhibition of the HIF hydroxylases. Thus, accumulation of succinate and/or fumarate can prevent hydroxylation of HIF even in an oxygen-rich environment.

The HIF-1 prolyl hydroxylases have also been shown to be redox sensors. Both the catalytic Fe^{2+} and cysteines required for HIF-1's three-dimensional structure are highly susceptible to inactivation by

oxidation under conditions where the mitochondrial electron transport chain activity exceeds cellular ATP consumption. The redox sensitivity component of the proline hydroxylases thus allows cells to adapt not only to hypoxia but also conditions when glucose metabolism exceeds cellular ATP demand. Under these conditions, HIF-1-induced expression of pyruvate dehydrogenase kinase (PDHK) suppresses TCA cycle activity, and HIF-1 induction of LDH-A redirects glycolytic pyruvate into lactate for excretion from the cell. Thus, HIF-1 can protect cells not only from hypoxia, but also from excess nutrient catabolism, by directing cell metabolism from the highly efficient oxidative phosphorylation to the less efficient glycolysis, thus preventing redox stress.

Beyond ATP Production

The discovery that the activity of HIF prolyl hydroxylases are dynamically regulated within the physiological range of oxygen concentration has led to a renewed interest in the role of oxygen in controlling other cellular activities. As a result, a number of additional oxygen-dependent enzymes have emerged as potential regulators of cellular adaptation to hypoxia. For example, both the TET family of DNA methyl cytosine hydroxylases and the Jumonji family of histone demethylases carry out similar molecular reactions to the proline hydroxylases. These enzymes hydroxylate methyl groups on DNA and proteins, respectively. Like the HIF-1-prolyl and asparaginyl hydroxylases, they also use αKG and oxygen as reactants to hydroxylate substrates and decarboxylate αKG. Since these enzymes have been implicated in the regulation of chromatin accessibility during cellular differentiation, their oxygen dependence has been proposed to provide a potential molecular explanation for why in most adult mammalian tissues, stem cell maintenance is carried out in relatively hypoxic niches.

Impairment of the αKG-dependent dioxygenases involved in histone and DNA demethylation has been shown to result from cancer-causing mutations in either isocitrate dehydrogenase (IDH) 1 or 2. These IDH mutations result in a neomorphic enzymatic activity that produces D-2-hydroxyglutarate, which competes with αKG for binding to this family of dioxygenases, thereby impairing DNA and histone demethylation (Harris, 2015). These results suggest that the chemistry involved in αKG-dependent dioxygenase function may be used reiteratively in metazoan biology to drive not only adaptive metabolic responses to hypoxia, but to also regulate embryonic development/differentiation under suboptimal nutrient conditions, such as oxygen depletion and TCA cycle dysfunction.

New Appreciation for Oxygen

It took just under a decade for Kaelin, Ratcliffe, and Semenza to unravel this elegant oxygen-sensing system. However, it is only in the last 15 years that scientists have begun to fully appreciate the broad implications of their discovery. For example, HIF-1 plays an essential role in organismal adaptation to high altitudes, congestive heart failure, chronic obstructive pulmonary disease, and other forms of pulmonary distress and contributes to stroke, coronary artery disease, and cancer. It also plays a critical role in cellular bioenergetic adaptation to prevent redox stress and to preserve macromolecular synthesis under both normoxic and hypoxic conditions.

The role of αKG-dependent dioxygenases in cell processes beyond HIF-1α destabilization has led to a broader appreciation of the role of oxygen in mammalian cell biology well beyond its use in oxidative phosphorylation. It is now appreciated that a wide variety of other cellular processes are controlled by oxygen-dependent enzymes whose dynamic range of activity occurs within the physiologic variation in oxygen levels of viable tissues. Oxygen is required not just for the maintenance of ATP production, it is increasingly recognized as a critical component of the regulation of a wide variety of host events including cellular differentiation, immune defense, and tissue repair.

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