ROS: Really involved in Oxygen Sensing

The role of reactive oxygen species (ROS) in the cellular response to cellular oxygen sensing has been controversial. Three papers in this issue of *Cell Metabolism* (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005) used genetic tools to establish that ROS produced by mitochondria are required for the normal induction of HIF (hypoxia-inducible factor), which is a master regulator of oxygen-sensitive gene expression, by low oxygen.

Understanding how cells sense and adapt to changes in oxygen availability is important, in part because the leading causes of death in the developed world are diseases such as myocardial infarction, cancer, and stroke, which are characterized by impaired tissue oxygenation. Over the years, there have been contradictory reports as to the importance of mitochondria, as well as reactive oxygen species (ROS), with respect to oxygen sensing. Three papers in this issue of *Cell Metabolism* strengthen the earlier contention that ROS generated by mitochondria under low-oxygen (hypoxic) conditions play an important role in this process (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005).

Deconvoluting complex biological processes usually requires both genetic and biochemical approaches to establish causality and mechanism. Several components of the pathway used by metazoans to respond to changes in oxygen have now been genetically validated and biochemically characterized. These include transcription factor HIF (hypoxia-inducible factor), the von Hippel-Lindau protein (pVHL), and prolyl hydroxylase EglN (also called HPH or PHD).

HIF consists of a labile α subunit (such as HIF-1α or HIF-2α) and a stable β subunit (such as HIF-1β, which is also called ARNT). Under hypoxic conditions, HIF-α is stabilized, binds to HIF-β, and transcriptionally activates a cadre of genes involved in adaptation to low oxygen. But how is HIF-α stability coupled to changes in oxygen? A clue came from the study of von Hippel-Lindau disease, a human hereditary cancer syndrome caused by inactivation of the pVHL tumor-suppressor protein. In cells lacking pVHL, HIF-α is relatively stable irrespective of changes in ambient oxygen, leading to the constitutive activation of hypoxia-inducible genes. Later, it was shown that pVHL is the substrate adaptor for a ubiquitin ligase complex that targets HIF-α for destruction when oxygen is present. The interaction between pVHL and HIF-α is oxygen sensitive because HIF-α must be hydroxylated on one of two prolyl residues in order to be recognized by pVHL (Kaelin, 2004). This reaction is intrinsically oxygen dependent (the oxygen atom of the hydroxyl group is derived from molecular oxygen) and is carried out by EglN (Epstein et al., 2001; Bruick and McKnight, 2001; Ivan et al., 2002; Figure 1). In human cells, there are three EglN family members (EglN1, EglN2, and EglN3), although EglN1 (also called PHD2 or HPH2) appears to be the primary HIF prolyl hydroxylase under normal conditions (Berra et al., 2003).

Elimination of the single EglN family member in *C. elegans* and *D. melanogaster* leads to constitutive HIF stabilization, as does elimination of EglN1 from mammalian cells (Berra et al., 2003; Bruick and McKnight, 2001; Epstein et al., 2001). In addition, EglN hydroxylase activity is very sensitive to changes in oxygen availability in vitro because of its relatively high Km for oxygen (Epstein et al., 2001; Hirsila et al., 2003). Therefore, EglN1 is an excellent candidate to be an oxygen sensor. However, EglN did not evolve in a test tube but rather evolved to function in the complex environment of a cell. It is conceivable that a number of inputs impinge upon EglN activity and contribute to the hypoxic response. In this regard, these enzymes require 2-oxoglutarate (which is converted to succinate during the hydroxylation reaction), ascorbate, and Fe^{2+} in addition to oxygen. There is already experimental evidence supporting the prediction that EglN1 activity is sensitive to changes in intracellular succinate levels, through feedback inhibition, and to changes in redox status (Gerald et al., 2004; Selak et al., 2005).

In 1998, the senior authors of the three papers in this issue, then working together, reported that mitochondria produced a burst of ROS in response to hypoxia and that this burst was both necessary and sufficient to stabilize HIF

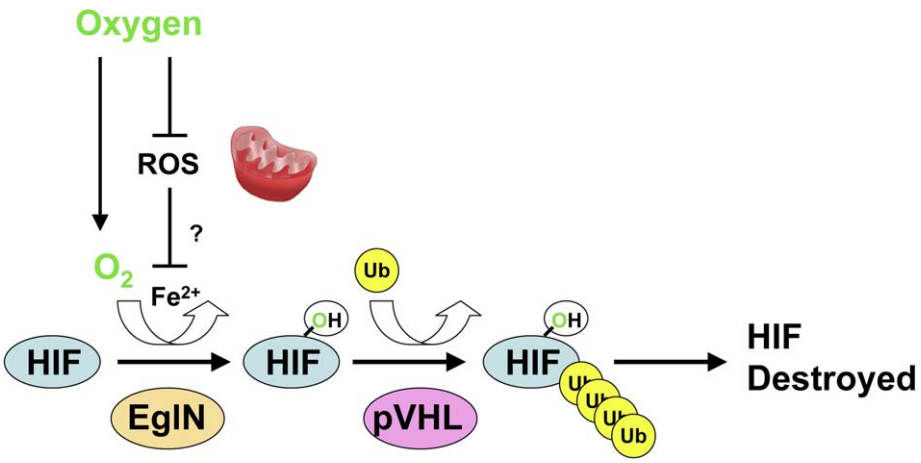

Figure 1. Regulation of HIF stability by reactive oxygen species

In the presence of oxygen, the α subunit of the heterodimeric transcription factor HIF is hydroxylated on one of two conserved prolyl residues by EglN. This creates a binding site for a ubiquitin ligase that contains pVHL (for simplicity, the other components of the pVHL complex are not shown). Polyubiquitination targets HIF-α subunits for destruction. EglN requires oxygen, Fe^{2+}, 2-oxoglutarate, and ascorbate. Low-oxygen conditions inhibit prolyl hydroxylation by limiting the amount of oxygen available for the hydroxylation reaction and through increased ROS, which might oxidize EglN bound iron.

(Chandel et al., 1998). This work was initially met with some skepticism, in part because it relied heavily on pharmacological tools and because others reported that cells lacking mitochondria still stabilized HIF in response to hypoxia (Kaelin, 2004). The current papers use a variety of approaches to substantiate their earlier claims.

First, Guzy et al. (2005) used a novel FRET probe containing a redox-sensitive linker to confirm the earlier conclusion, reached with redox-sensitive dyes, that hypoxia leads to increased ROS production. Along with Brunelle et al. (2005), they then used siRNA to inactivate the Rieske iron-sulfur protein of mitochondrial complex III to abrogate this burst; Mansfield et al. (2005) exploited cells in which the cytochrome c locus was disrupted to accomplish this end. Blocking ROS production, genetically or pharmacologically, led to impaired HIF induction by hypoxia. Conversely, treating cells with agents that produce ROS induced HIF. Importantly, HIF could still be induced by profound hypoxia (anoxia) in cells in which ROS production was experimentally suppressed, arguing that ROS produced by the mitochondria alter the shape of the oxygen/prolyl hydroxylation dose-response curve. This might partially explain the conflicting reports regarding the necessity of mitochondria for oxygen sensing. It is likely that ROS produced in response to hypoxia affect the oxidation status of EglN bound iron, although this remains to be proven (Figure 1).

One caveat, highlighted by a recent study of the effects of nitric oxide on HIF, relates to the possibility that perturbing mitochondrial function could decrease oxygen consumption (Hagen et al., 2003). In theory, this could lead to increased intracellular oxygen concentrations and thus suppress HIF induction at a given ambient oxygen concentration. The authors of the present studies attempted to control for this by showing that cells defective in oxidative phosphorylation or treated with various mitochondrial poisons that decrease oxygen consumption without generating ROS induced HIF normally under hypoxic conditions. A decrease in oxygen consumption would also not readily explain the impairment of HIF induction observed with various peptidic and organic ROS scavengers. Nonetheless, it will be important to determine whether any of the experimental manipulations carried out in the current papers indirectly affect intracellular oxygen, succinate, or ascorbate concentrations. Likewise, it will be important to gain a clearer understanding of how decreased oxygen availability leads to increased ROS production by mitochondrial complex III.

As recently as five years ago, knowledge of oxygen sensing by metazoans was a bewildering morass. In this context, control of HIF stability by an oxygen-dependent enzyme provided a satisfying and surprisingly simple explanation for how changes in oxygen are translated into changes in gene expression. The current papers suggest that additional layers of complexity are about to be added to this picture, no doubt reflecting the importance of oxygen in cellular homeostasis.

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