Here comes the interesting part. There are some organisms that have both unicellular and multicellular forms: One form is capable of respiration, whereas the other is a fermenter. As Pasteur showed, the bread mold *Mucor* forms a network of threadlike filaments (a multicellular mycelium) that breaks down a food source in the presence of oxygen through aerobic respiration. In the absence of oxygen, however, it produces unicellular, yeastlike forms that are capable of fermenting the food. In other words, fermentation in *Mucor* is associated with the unicellular state, and respiration with the multicellular mycelium. The authors conclude that multicellularity arose precisely because the formation of multicellular organisms allowed cells to benefit from the efficiency of aerobic respiration in the presence of a slowly diffusing food source. The evolution of aerobic respiration allowed a kind of energetic luxury that in turn permitted the development of large genomes and cellular specialization. The Pfeiffer *et al.* work is interesting because it couples thermodynamics, biochemistry, and population biology to suggest a way in which multicellularity could have originated.

There is a different but somewhat parallel example in nature that fits in with this scheme. Two simple, and no doubt ancient organisms—the myxobacteria and the true slime molds or myxomycetes—feed and grow into a large mass before they form spore-bearing fruiting bodies that stick up into the air for effective dispersal of the spores. In the case of the myxobacteria, the individual bacterial cells group into swarms and, as Dworkin (*2*) pointed out some years ago, by doing so they are able to feed more effectively. Their strategy has a feature not found in the Pfeiffer *et al.* model. The myxobacteria obtain their energy from solid food, but to do so they must pump out extracellular enzymes that convert food into small organic molecules that can then be absorbed and converted into ATP by aerobic respiration. Dworkin appropriately called this phenomenon—the grouping of cells into large masses to enhance their ability to degrade food into small molecules—"wolf-pack feeding." So, there is a different but analogous advantage to the myxobacteria becoming multicellular.

This brief summary of the Pfeiffer *et al.* work does not do credit to all of the interesting details that the authors muster or to how their findings relate to the global problem of the evolution of multicellularity. As Pfeiffer *et al.* point out, their argument only applies to heterotrophs, and there are a number of photosynthetic organisms—nonheterotrophs or autotrophs—that independently invented multicellularity (*3*). They also raise the key point that the kind of advantage they propose for multicellularity means that it must have occurred after the atmosphere of Earth was invaded by oxygen and aerobic respiration was "invented." Bacteria were the first to acquire aerobic respiration. Later, eukaryotic cells evolved by the symbiotic incorporation of bacterial respirators, the forerunners of mitochondria. One wonders if Pfeiffer *et al.*'s argument about the advantage that multicellularity confers on eukaryotic respirators might also apply to prokaryotic respirators? We do not know of any existing multicellular prokaryotes that would fit that bill, but they might well have existed millions of years ago and become extinct (or perhaps they do exist and are waiting to be discovered).

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PERSPECTIVES: SIGNAL TRANSDUCTION

How Do Cells Sense Oxygen?

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In the early 20th century, the fledgling disciplines of physiology and biochemistry became interested in how animals and cells respond to changes in the amount of oxygen in their environment (oxygen homeostasis). Since then, much has been learned about how the cardiovascular and respiratory systems adjust to low oxygen tensions in tissues (hypoxia), how changes in tissue oxygen tension affect cellular metabolism and, within the last decade, how hypoxia affects programs of gene expression. But there is still much to learn about the ways in which cells sense reduced oxygen tensions and activate signal transduction pathways that lead to physiologically appropriate changes in gene expression. Papers by Ivan *et al.* on page 464 (*1*) and Jaakkola *et al.* on page 468 (*2*) in this week's issue add considerably to our understanding of this process by unraveling how a transcription complex, hypoxia inducible factor (HIF), controls gene expression in response to changes in oxygen tension.

When mammalian tissues are challenged by hypoxia, the expression of a number of physiologically important proteins is increased. For example, there is increased production of erythropoietin, a cytokine required for the formation of red blood cells; an increase in the number of erythrocytes enhances the delivery of oxygen to tissues. Vascular endothelial growth factor (VEGF) is a key regulator of blood vessel growth (angiogenesis). The induction of VEGF expression in hypoxic tissues results in enhanced blood flow, thereby providing protection against ischemic injury. VEGF is also important for tumor angiogenesis (*3*). Tyrosine hydroxylase is the rate-limiting enzyme in dopamine synthesis. The up-regulation of this enzyme in glomus cells of the carotid body in the neck enables the hypoxic animal to achieve a sustained increase in ventilation. Hypoxia also induces synthesis of certain glycolytic enzymes, enabling intracellular levels of the energy-rich molecule adenosine triphosphate to be maintained.

In hypoxic cells, the up-regulation of these and many other proteins depends on the activation of the HIF family of transcription factors (*3*). Heterodimers composed of HIFα and HIFβ subunits bind to pentanucleotide (5′-RCGTG-3′) response elements in genes encoding the proteins up-regulated in response to hypoxia. The HIF subunits are members of the PAS protein family, which includes not only transcription factors but also other proteins that sense perturbations in a cell's environment. For example, FixL in *Rhizobium* bacteria, a heuristic distant relative of PAS family members, is an oxygen-sensing fusion protein containing a heme binding domain and a protein kinase domain (*4*).

The HIF subunits are widely, perhaps universally, expressed in the cells and tissues of mammals, flies, worms and probably most other creatures. The β subunit, commonly called ARNT (arylhydrocarbon nuclear translocator), is a partner for the arylhydrocarbon receptor and is abundantly expressed independently of oxygen tension. In contrast, HIFα (*5*) cannot be detected unless cells are challenged by hypoxia. Above a critical intracellular oxygen ten-

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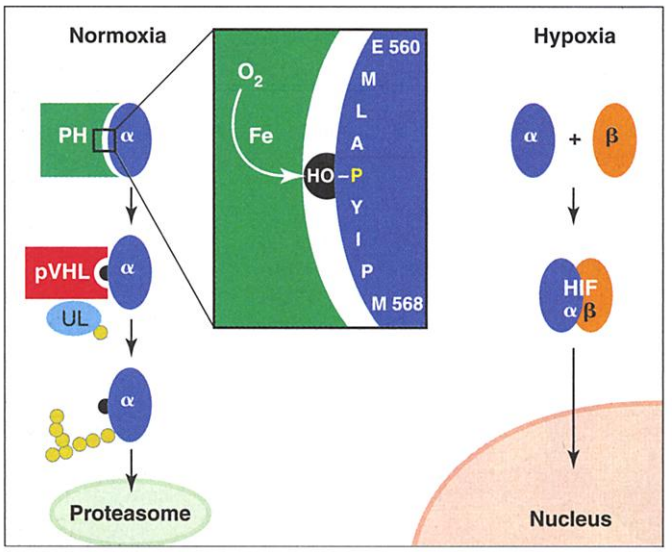
sion, HIFα is rapidly degraded in cellular organelles called proteasomes after its ubiquitination (a process in which ubiquitin molecules are added to proteins to tag them for degradation) (see the figure). HIFα contains an oxygen-dependent degradation domain (*6*) within which is a highly conserved region (*7*) containing a binding site for the tumor suppressor von Hippel–Lindau protein (pVHL) (*8–10*). The pVHL organizes the assembly of a complex that activates the ubiquitin E3 ligase, which then ubiquitinates HIFα targeting it for degradation. Interestingly, mutations in pVHL (encountered in certain tumors) prevent it from binding to HIFα, causing constitutive expression of this transcription factor and its target genes. Such mutations increase the potential for angiogenesis, probably through the continued production of VEGF (*11, 12*).

The nature of the oxygen sensor that regulates the activity of HIF remains elusive. There is circumstantial evidence implicating the participation of a heme protein (*13*) that generates reactive oxygen species (*14*). Besides hypoxia, HIF can also be activated by the transition metal cations Co^{2+}, Ni^{2+}, and Mn^{2+} and also by reagents that chelate iron. These observations hint that HIFα might be oxidatively modified by reactive oxygen species generated through a nonenzymatic oxygen- and iron-dependent process akin to that previously described for both bacterial and mammalian enzymes (*15*). However, if highly labile reactive oxygen species serve as messengers regulating HIFα activity, it is likely that short-range interactions are involved, requiring the participation of an enzyme. This concern is squarely addressed by Ivan *et al.* (*1*) and Jaakkola *et al.* (*2*). With a remarkable degree of accord, they provide convincing evidence that a prolyl hydroxylase enzyme encountered in a variety of mammalian cells is involved in sensing oxygen. In the presence of oxygen and iron, this enzyme targets a highly conserved residue in human HIF-1α, proline 564, and hydroxylates it (attaches an OH group). Hydroxylation of this proline appears to be both necessary and sufficient for the binding of pVHL to HIFα.

The immediate challenge is to isolate and further characterize the HIFα prolyl hydroxylase. Like the well-studied collagen prolyl 4-hydroxylases (*16*), the HIF-modifying enzyme depends on both oxygen and iron. Furthermore, the collagen and HIF enzymes respond to similar cofactors and inhibitors (*2*). However, collagen prolyl 4-hydroxylases are localized within the endoplasmic reticulum of the cell, whereas the HIF oxygen sensor is very likely to be cytosolic. Moreover, the two differ considerably in their interactions with oxygen. Hydroxylation of proline in collagen is insensitive to a wide range of oxygen concentrations, whereas the HIF oxygen sensor must have a considerably lower affinity for oxygen, enabling it to respond to subtle alterations in intracellular oxygen tension.

It will be important to learn whether there are other substrates for the HIF prolyl hydroxylase. Does the pVHL-dependent ubiquitination and degradation of other proteins depend on proline hydroxylation? Does the HIF prolyl hydroxylase control the hypoxia-dependent transcriptional activation of HIFα as well as of other transcription factors? These issues of specificity bear on whether it will be possible to develop drugs that target the oxygen sensing and signaling pathways that regulate HIF activity in response to hypoxia in clinical diseases. In particular, the controlled and local induction of VEGF by HIF could greatly enhance recovery from heart attacks or cerebral strokes. Conversely, tumor-specific suppression of VEGF would be expected to limit the growth and spread of malignancies.

The identification of HIF prolyl hydroxylase begs the question of whether other classes of proteins serve as oxygen sensors, and if so, whether they could be implicated in HIF activation. Candidates include flavoheme oxidoreductases (*14*), such as the recently identified cytochrome b5/b5 reductase fusion protein (*17*). An NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase containing $gp91^{phox}$ behaves as an oxygen sensor in pulmonary neuroepithelial bodies (*18*). These challenges appeal to a broad range of research disciplines and doubtless will be met in the near future.



Oxygen-dependent regulation of the HIF transcription factor. When the intracellular oxygen tension reaches a critical threshold, newly synthesized HIFα subunits (α) are oxidatively modified by a prolyl hydroxylase (PH) enzyme. This iron-dependent process results in the hydroxylation of a specific proline residue within a highly conserved region of the HIFα's internal oxygen-dependent degradation domain. This structural modification is necessary and sufficient for binding of HIFα to pVHL, which mediates the assembly of a complex (UL) that activates the ubiquitin-E3 ligase. Ubiquitination of HIFα is necessary for this transcription factor to be degraded by the proteasome. When cells are hypoxic (that is, the intracellular oxygen tension decreases below a critical threshold), the proline is not hydroxylated and so HIFα escapes degradation. HIFα then forms a stable heterodimer with HIFβ (ARNT). The HIFαβ heterodimer translocates to the nucleus, where it binds to hypoxia-response elements in genes that are switched on by hypoxia.

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