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## Researchers Identify How Cells Respond to Oxygen Starvation

Two research groups, working independently, have discovered a key step in a chemical reaction that protects cells from oxygen starvation. The finding gives scientists a glimpse of proteins that may one day be manipulated to enhance oxygenation of tissues in stroke or heart attack victims or to cut off a tumor's oxygen supply.

In articles published in the April 20, 2001, issue of the journal *Science*, groups led by Howard Hughes Medical Institute investigator [William G. Kaelin, Jr.](#), and University of Oxford researcher Peter J. Ratcliffe identified a key chemical reaction affecting a transcription factor known as hypoxia-inducible factor (HIF).

When oxygen is present in tissues at normal levels, HIF is continually degraded and prevented from switching on genes that enhance oxygen delivery by increasing red blood cell formation and blood vessel growth. In addition, HIF switches on genes that alter metabolism to allow cells to adapt to low oxygen. When there is not enough oxygen present in tissues, HIF is stabilized and is allowed to activate gene expression.

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Scientists had previously found that HIF degradation occurs when a protein called von Hippel-Lindau protein (pVHL) binds to HIF. "That was very satisfying," said Kaelin, "because now we had a fairly direct link between the function of the von Hippel-Lindau protein and the regulation of HIF. But then the major question was how in the absence of oxygen does the pVHL protein know that it should leave HIF alone?"

The scientists began to search for the source of the chemical alteration that occurs in HIF that enables pVHL to bind to HIF and mark it for destruction.

Kaelin's and Ratcliffe's groups showed that the critical chemical reaction is the addition of a single hydroxyl group to a specific amino acid, proline, in the HIF protein. Since this reaction requires oxygen, the scientists believe it may play a key role in oxygen-sensing in the cell.

Commenting on the work in a *Perspective* article also published in *Science*, Harvard Medical School scientists Hao Zhu and H. Franklin Bunn write that the finding "adds considerably to our understanding of this process by unraveling how a transcription complex, hypoxia inducible factor, controls gene expression in response to changes in oxygen tension."

Since oxygen is fundamental to life, there has been intense interest in how cells sense changes in oxygen and adapt to those changes, said Kaelin, who is at the Dana-Farber Cancer Institute and Brigham and Women's Hospital. "While we have come to understand the changes that occur at the tissue and organismal level, we haven't really understood the changes that occur at the cellular level," he said.

The research by Kaelin and colleagues builds on earlier work, including studies by Ratcliffe's team and their own, which showed that binding of pVHL to HIF is crucial for HIF degradation. Kaelin's team found that alterations to HIF that occurred after the protein is translated were necessary in order for pVHL to bind to HIF.

Following up on those clues, Mircea Ivan, in Kaelin's lab, decided to search for the critical target region of interaction between pVHL and HIF, so as to narrow the list of possible modifications. Ivan finally narrowed down the binding region to a short protein segment, or peptide.

By studying how alterations in this target peptide affected pVHL binding -- and exploring the scientific literature on such effects -- they surmised that hydroxylation of a single proline was likely the key step in rendering HIF vulnerable to pVHL attachment. This was confirmed by analytic means, including mass spectrometry, as well as by studying the behavior of HIF-derived peptides synthesized to contain proline or hydroxyproline. They also showed that cell extracts can hydroxylate HIF in vitro. Enzymes called prolyl hydroxylases carry out proline hydroxylation.

"These were also very satisfying results, because it's known that the classical prolyl hydroxylases require both oxygen and iron to function," said Kaelin. "Thus, one can now understand how HIF becomes stabilized in the absence of oxygen or in the presence of 'hypoxia-mimetics' such as iron chelators."

One of the next steps, said Kaelin, is to identify which prolyl hydroxylase modifies HIF. Kaelin noted that the HIF prolyl hydroxylase is likely to be novel since the known prolyl hydroxylases do not typically reside in the region of the cell where HIF is found. Identifying the enzyme could lead to clinical applications, he said.

“I think we’ve identified an important piece of the puzzle in terms of the molecular control of hypoxia adaptation, including angiogenesis. So, for example, if you can block the interaction between pVHL and HIF that could conceivably promote angiogenesis in cases of heart disease or stroke. However, it remains an open question whether one can direct this pro-angiogenic effect to specific tissues.

“Conversely, it’s clear that HIF is overproduced in a variety of tumors, so we may be able to come up with strategies to either down-regulate HIF in tumors or to turn high levels of HIF into an Achilles’ heel.”