How to catch a HIF—the work of Gregg Semenza's lab on hypoxia-inducible factor 1

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Molecular oxygen is essential for the viability and function of every cell of the animal body. Because skin, tissues, and organs impede oxygen diffusion directly from the air, molecular oxygen concentrations inside the body often are less than 5% (1), much lower than the 21% in Earth's atmosphere.

As multicellular organisms evolved, this steep oxygen gradient necessitated the development of structures such as the circulatory system and of biochemical mechanisms that monitor and control oxygen levels in the body.

A major player in sensing potentially harmful drops in cellular oxygen concentrations (hypoxia) is the transcription factor hypoxia-inducible factor 1 (HIF-1).[2] HIF-1, and its close relative HIF-2, regulate many genes, including the *erythropoietin* (*EPO*) gene, which encodes a hormone that stimulates production of red blood cells (2, 3).

One important milestone in uncovering HIF-1's pivotal role in oxygen sensing was its purification and biochemical characterization in the mid-1990s by the lab of Gregg Semenza (Fig. 1), a geneticist at Johns Hopkins University School of Medicine. This work was reported in two JBC papers recognized as Classics here (4, 5).

"JBC was the first choice," says Semenza, referring to the publication of the first Classics article. "To me, that was a classic JBC paper."

A few years before this work, Semenza's team had found that hypoxia induces the binding of a nuclear protein to a 50-nucleotide-long enhancer region located in the 3′-flanking region of the *EPO* gene (2, 3). Although the researchers could delineate the DNA region that is bound by the hypoxia-induced protein, the protein's identity was unknown, prompting Semenza to look for it.

Semenza's team first had to cross some unexpectedly rough waters. "We used an expression cloning strategy," he says. The researchers expressed human proteins from cDNAs in bacteriophages and then used the HIF-binding oligonucleotide as a probe to find proteins binding to it. "We screened millions and millions of clones and got nothing."

What went wrong? "We had initially performed experiments that suggested that there was a single subunit that could bind to the DNA fragment," says Semenza. As the later results of the two Classics papers revealed, HIF-1 is a heterodimeric protein (4, 5), a fact that probably hobbled the bacteriophage-based approach, since each phage clone typically contains only a single gene.

Semenza and his team were undeterred. "We could give up and let someone else do it—but that did not sound like a good idea," says Semenza.

The team changed tack, embarking on a biochemical purification. They grew HeLa cells in large-scale (>100-liter) cultures, exposed them to hypoxia and cobalt chloride (which also induces HIF activity), prepared nuclear extracts from them, and purified HIF-1 via DNA-affinity chromatography with an oligonucleotide that contained the HIF-binding site.

After isolating the HIF-1 proteins in a preparative gel-shift assay along with glycerol sedimentation, the authors could show that HIF-1 is composed of two subunits: a larger one of 120 kDa, called HIF-1α, and a smaller one of 91–94 kDa, called HIF-1β (4).

The purification yielded enough protein of the two HIF-1 subunits for amino acid microsequencing that gave short protein sequences. The researchers used that sequence information in a cloning approach to identify and sequence full-length cDNAs of the HIF-1 subunits (6).

The cDNA sequences showed that both HIF-1 subunits belong to a group of transcription factors containing a basic helix-loop-helix (bHLH) motif required for dimerization and DNA binding and a Per-Arnt-Sim (PAS) domain for protein–protein interactions.

The HIF-1β sequence was identical to that of a previously identified protein, aryl hydrocarbon receptor nuclear translocator (ARNT), a subunit of the dioxin receptor, which responds to dioxins and other toxic chemicals. However, the HIF-1α sequence was previously unknown and only partially similar to single-minded homolog (Sim), a transcription factor in *Drosophila*.

The second Classics paper (5) helped define the regions in HIF-1α and HIF-1β required for dimerization of the two subunits, DNA binding, and activation of transcription of hypoxia-induced genes.

What might now look like a series of straightforward steps of purifying and sequencing a protein was more an exercise in continual improvisation.

"My lab was a molecular genetics laboratory," says Semenza. "We did not even own a fraction collector," required for protein purification. Luckily for Semenza, the lab of another Johns Hopkins researcher, Thomas Kelly, was just across the street.

"Tom was one of the first people to purify a protein based on its binding to DNA," notes Semenza, giving his project a vital boost. "We could not have [isolated HIF-1] without the help from Tom Kelly's laboratory."

JBC Associate Editor Ruma Banerjee at the University of Michigan nominated these papers as Classics.

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[2] The abbreviations used are: HIF, hypoxia-inducible factor; PHD, prolyl-4-hydroxylase domain.

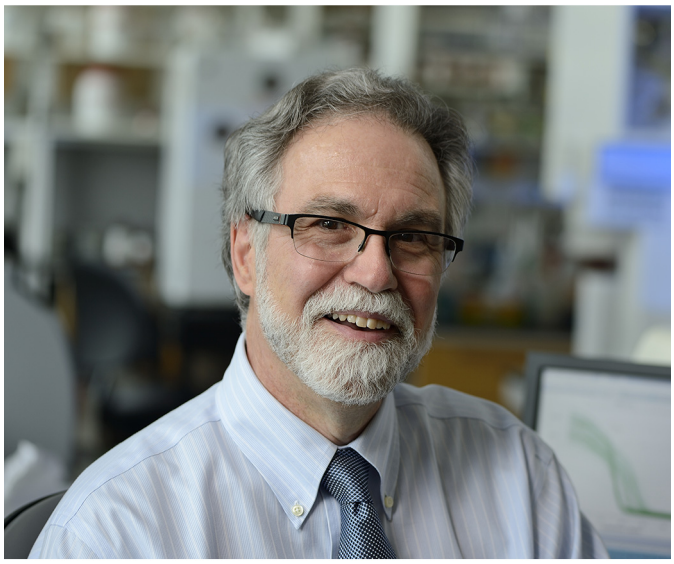

Figure 1. Gregg Semenza and colleagues isolated and biochemically characterized the HIF-1 protein. Photo courtesy of Johns Hopkins Medicine.

The biochemical characterization of HIF-1 by Semenza and colleagues threw open the doors to many additional studies from Semenza's group and also other labs.

What emerged from these investigations is that the specific HIF-1–binding site is ubiquitous in the human genome. It's been found at many locations, including the 5′-flanking regions and introns of many genes, says Semenza. This hinted at HIF-1's role as the central hub of metabolic regulation in response to oxygen.

"These genes are not induced by hypoxia in every cell type under every condition," Semenza notes. "They're induced by hypoxia in some cell types under some conditions—the plasticity of the [hypoxic] response is remarkable."

The HIF-1–regulated genes encode proteins in many metabolic processes, including glycolysis, angiogenesis, and wound healing. They are also expressed when people move from lower to higher altitudes, and they are hyperactive in some diseases, most notably cancer (7, 8). Consequently, HIF-mediated signaling is now a therapeutic target to combat cancer and manage disorders such as altitude sickness.

A key feature of HIF-1 activity is its regulation by specific prolyl-4-hydroxylase domain (PHD) proteins. In the presence of normal oxygen concentration, the PHDs hydroxylate one or both of two conserved proline residues in HIF-1α, leading to binding by the von Hippel-Lindau protein (VHL), which targets HIF-1α for proteasomal degradation (9).

"So [cells] make HIF1α, but it's being degraded when oxygen is available," explains Semenza. When oxygen levels become limiting, the PHD proteins are inhibited, increasing the fraction of HIF-1α that's not hydroxylated. This stabilizes the HIF-1 protein, causing its rapid accumulation and leading to the activation of its target genes.

"It is a really beautiful system," says Semenza.

For his work on the role of HIF-1 in oxygen sensing, Semenza was awarded the Nobel Prize in Physiology or Medicine in 2019 (shared with William G. Kaelin, Jr., and Peter J. Ratcliffe for their work on related oxygen-sensing pathways) and the Albert Lasker Award for Basic Medical Research in 2016.

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