QnAs with Gregg Semenza

Leigh Cooper, *Science Writer*

In organisms that depend on oxygen, the element helps generate ATP, the cellular energy currency, and sustain cellular functions. But researchers have struggled to understand how organisms cope with shifts in oxygen availability. National Academy of Sciences member Gregg Semenza, a biochemist at the Johns Hopkins University School of Medicine, studies how cells sense and react to different oxygen levels. Semenza began his studies by identifying the transcription factor that regulates the expression of a gene that is turned on during low-oxygen conditions. Since those early findings, Semenza has explored the influence of the transcription factor on fetal development and cancer cells. Semenza shared the 2016 Lasker Basic Medical Research Award with biochemists William Kaelin, Jr. and Peter Ratcliffe. Semenza recently spoke with PNAS about his career.

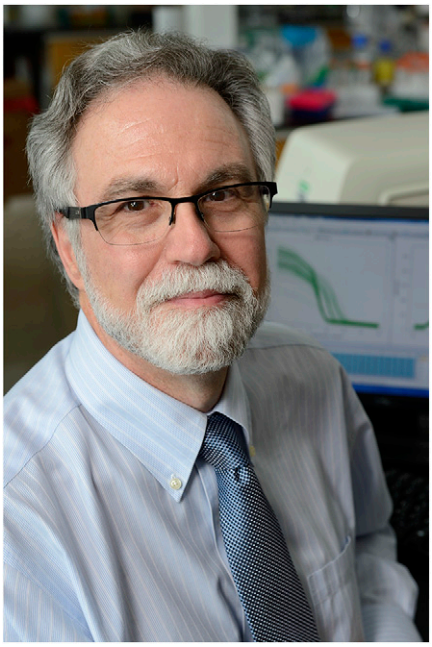
PNAS: What first drew you to the study of oxygen in cells?

Semenza: We started by studying the human erythropoietin (*EPO*) gene, which codes for the hormone that controls red blood cell production. We generated transgenic mice that expressed the human *EPO* gene and identified sequences flanking the gene that were important for its expression in the liver and kidney. Then, we turned our attention to regulation of the gene by oxygen, because when certain cell types are exposed to low oxygen, they will increase their production of EPO. We identified a short DNA sequence in the 3′ flanking region of the gene that was necessary for this response (1). We went on to identify the transcription factor that binds to the DNA sequence, and we called it hypoxia-inducible factor 1 or HIF-1 (2).

PNAS: What kinds of challenges did you face while identifying this gene sequence, called the hypoxia response element (HRE), and HIF-1?

Semenza: There were several major challenges during the course of that work. The first one was to identify the DNA binding activity. Our hypothesis was that a protein bound to the HRE sequence in cells that were hypoxic and did not bind in cells that were not hypoxic. We made nuclear extracts from hypoxic and nonhypoxic cells and incubated them with this short DNA sequence. We looked for increased binding of a protein in the hypoxic nuclear extracts. But the binding of any given protein to DNA is very idiosyncratic. Not all proteins will bind under the same conditions. We had to vary the conditions of the binding assay, hoping that we would identify some condition that would allow the binding of a hypoxia-induced protein, if such a protein existed. There was a lot of empirical trial and error. We were fortunate enough to finally perform the experiment under conditions that led to binding of HIF-1 that we could detect.

PNAS: You later discovered that HIF-1 is comprised of two molecules, HIF-1α and HIF-1β, during a series of challenging experiments. Can you elaborate?



Gregg Semenza. Image courtesy of Johns Hopkins University (Baltimore).

Semenza: Once we identified the binding activity, we wanted to determine the coding sequences for the protein. We started out using a bacteriophage library containing cDNAs prepared from the mRNA of hypoxic human cells. Each bacteriophage expressed a different human protein. We used the [HRE] binding sequence as a probe to see if it would bind to any of the proteins that were expressed by the bacteriophage. We got negative results for a long time and had to make a decision either to pursue this strategy further, to take a different approach to identify HIF-1, or just give up. We decided to take a biochemical approach and purify the protein based on its binding to the DNA sequence of the HRE (3).

PNAS: At one point, researchers believed EPO was produced only in the liver and kidney. But you found that HIF-1 binds and activates genes in mammalian cells that are not associated with EPO. What are the implications of the finding?

Semenza: It turns out that HIF-1 is present in almost all animals. The only animals that don't have HIF-1 are primitive sponges that have no cellular differentiation. The most primitive response controlled by HIF-1 is probably the mechanism that allows cells to use oxidative or glycolytic metabolism based on oxygen

availability. As larger and larger organisms evolved, it became necessary for specialized tissues to deliver oxygen to all of the cells in the body. For example, the body of the worm *C[aenorhabditis] elegans* consists of only 1,000 cells, and all of the cells receive oxygen by simple diffusion. However, a fruit fly is too large for oxygen to get to the interior of the organism by diffusion alone, so the fly has a series of tracheal tubes that conduct oxygen to the interior of the animal. The even larger mammals have specialized systems to capture oxygen: the lungs. To carry oxygen: the red blood cells. And to get those red cells to all parts of the body: the heart and blood vessels. HIF-1 controls all [of] those processes, both during development as well as in the adult organism.

PNAS: So HIF-1 comes into play during fetal development?

Semenza: After we isolated cDNA coding for HIF-1α, we performed homologous recombination in mouse embryonic stem cells to knock out the HIF-1α gene (4). We found that mouse embryos that did not produce any HIF-1α arrested around day 8.75 and died by day 10.5. They had failed development of the heart, decreased red cell production, and failed development of the blood vessels. All three components of the circulatory system do not form properly in the absence of HIF-1α. The embryos die when they grow so large that the circulatory system must function to deliver oxygen to all of the cells. HIF-1 controls both prenatal development of the circulatory system and postnatal physiological responses of the circulatory system that are necessary to maintain oxygen homeostasis, which is critical for everything else that goes on in the body.

PNAS: What are you working on now?

Semenza: We have been investigating the role of HIF-1 in cancer. Cancer cells must adapt to very low oxygen levels, and to do that they turn on expression of HIF-1 and HIF-2, which is a related protein that has similar functional attributes. The HIFs give cancer cells invasive and metastatic properties, which are critical components of the lethal cancer phenotype. Another really critical response to hypoxia is an increase in the number of cancer stem cells, and HIFs mediate this process. We have shown that chemotherapy also increases the number of breast cancer stem cells in a HIF-dependent manner, and this is particularly important for a subset of breast cancers called triple-negative breast cancers. In these cases, chemotherapy usually results in remission, but the patients generally relapse within a few months and have a high mortality rate. We have been exploring the mechanisms by which the HIFs promote metastasis and the cancer stem cell phenotype. One of our major interests is to develop drugs that will be effective in inhibiting HIF-1 and cancer progression.

PNAS: What motivates your research?

Semenza: We never know where our research is going to lead. We may design an experiment to test a hypothesis and find out the hypothesis is wrong. But it doesn't matter because if you designed the experiment properly, you still find out how nature has organized the response you were studying. I amuse myself with the number of hypotheses that turn out to be wrong but result in really interesting data. Those are the most interesting findings: the unexpected ones. The ones that go against whatever the dogma is.

1 Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE (1991) Hypoxia-inducible nuclear factors bind to an enhancer element located 3′ to the human erythropoietin gene. *Proc Natl Acad Sci USA* 88(13):5680–5684.

2 Semenza GL, Wang GL (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* 12(12):5447–5454.

3 Wang GL, Semenza GL (1995) Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* 270(3):1230–1237.

4 Iyer NV, et al. (1998) Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 12(2): 149–162.