NOBEL PRIZE FOR PHYSIOLOGY AND MEDICINE 2019

Most life forms need oxygen for energy production but the fundamental oxygen sensing mechanisms that translate changes into action inside cells has been elusive.

During the last two decades **William G. Kaelin, Sir Peter J. Ratcliffe and Gregg L. Semenza** have worked towards understanding this and have now been awarded the 2019 Nobel Prize of Physiology or Medicine for the seminal discoveries of molecular machinery regulating the activity of genes in response to varying levels of oxygen. Their work revealed how cells respond to changes in oxygen levels. These findings are important in understanding and treating conditions and diseases like anemia, heart attacks, strokes and cancer.

BACKGROUND

Oxygen is essential for life. It is an electron acceptor and used by mitochondria to provide energy for the cells in an enzymatic process. To ensure adequate supply of oxygen to tissues and cells, specific mechanisms have developed during evolution. The foundation for understanding these mechanisms, laid by Otto Warburg, the recipient of the 1931 Nobel Prize in Physiology or Medicine, revealed that this conversion is an enzymatic process and a 'mode of actions of a respiratory enzyme'. In 1938, this Nobel was awarded to Corneille Heymans for the discovery of the role of sinus and aortic mechanisms in respiration regulation. He discovered how the carotid body controls respiratory rate via blood oxygen sensing and that the carotid body communicates directly with the brain.

THE BEGINNING – ERYHTROPOIETIN (EPO)

A few decades later, first evidence for oxygen sensing mechanism in animals came from the work describing erythropoietin (EPO) (1). EPO is a hormone produced by the kidney and stimulates red blood cell production in response to low blood oxygen levels. EPO was first purified in 1977 and the gene encoding it was cloned in 1985 (2) but how varied oxygen levels regulate EPO expression remained a mystery.

In the early 1990s Gregg Semenza had studied the EPO gene and concluded that it must work like other genes, i.e. that a nearby stretch of DNA must activate it. He was interested in finding out how this worked and how varying oxygen levels can affect this.

By using gene-modified mice, specific DNA segments located next to the EPO gene were shown to mediate the response to hypoxia. Eventually he was able to show that a region now called 'hypoxia-responsive element' or HRE, at the other end of the EPO gene was able to bind nuclear factors and be induced by variations in oxygen levels (3).

At the same time Ratcliffe and his research group were studying O_2-dependent regulation of the EPO gene. They found out that this HRE element described by Semenza was present in cells that had nothing

to do with EPO production. This lead Ratcliffe to understand that oxygen sensing mechanisms might in fact affect expression of other genes as well (4) and quite quickly they found HRE elements in the genes encoding glycolytic enzymes (5). These findings pointed out that changes in oxygen levels, and especially hypoxia, could confer adaptations at cellular level by regulating glycolysis for oxygen-independent energy production. Finally, as it was discovered that hypoxia also induced the expression of angiogenic growth factors, it became clear that there indeed was a wide oxygen sensing network in cells that governed expression of a number of fundamental genes in response to oxygen availability (6) and that the oxygen sensing process operated in mammalian cells.

BAKER RUSKINN RELEVANCE: In studying this they faced some problems, for instance that not all cells responded well at low (1% O_2). As it is now understood, this was because different cell types differ greatly in oxygen consumption and it was (and still is) easy to miss responses without directly measuring oxygen (7). At this time Ratcliffe and his colleagues were using oxygen-controlled incubators (Baker Ruskinn chambers were not available yet) where low oxygen conditions were lost at each door opening. At this time, however, this was not an issue because 1) it was not understood how rapid the changes oxygen caused were 2) they were using stable reporter gene constructs and not HIF (as you see below, HIF had not been discovered yet).

DISCOVERY OF THE HIF SYSTEM

As it was now known that there were oxygen regulated nuclear factors that bound the EPO gene, it needed to be figured out what they were. Hypoxia-inducible factor 1 or HIF-1 was purified in 1995 by Semenza and colleagues (8). HIF-1 is essential for the formation of the vascular system, as well as red blood cell production (8). It is composed of two subunits, namely HIF-1alpha and HIF-1beta. They were both expressed in both ambient and low oxygen conditions but only HIF-1beta seemed to be stably expressed; HIF-1alpha was constantly degraded in ambient oxygen conditions. This was a clear indication that somehow HIF-1alpha -levels were regulated in the presence of oxygen.

William Kaelin had read about the identification of a gene for a hereditary cancer called von Hippel-Lindau (VHL) disease. Patients with this cancer often develop tumors in the kidneys, adrenal glands, or pancreas. They also often have tumors in the central nervous system that look like nests of blood vessels. Kaelin thought that tumors produce EPO (and thus stimulate red blood cell productions) and saw that this greatly resembles the behavior of a tissue that is short of oxygen. Thus he wondered whether the VHL disease itself, and especially the gene behind the condition (VHL), involved an abnormal response to ambient oxygen levels.

Knowing that in VHL disease VHL gene is often deleted or mutated, Kaelin and his team were able to show that this VHL loss could lead to the stabilization of genes that increased the formation of vasculature; one such gene being vascular endothelial growth factor (VEGF) (10). As it was already shown by Ratcliffe that hypoxia also induced the expression of angiogenic growth factors, the likes of VEGF, Kaelin soon discovered that loss of VHL leads to upregulation of VEGF even in ambient oxygen conditions (11).

Ratcliffe and his research group then made a key discovery: demonstrating that VHL can physically interact with HIF-1α and is required for its degradation at normal oxygen levels (10,12). This conclusively linked VHL to HIF-1α. The only thing left that was not known, was how oxygen levels regulated the interaction between HIF and VHL.

BAKER RUSKINN RELEVANCE: In the 1999 Nature paper they in fact made a slight mistake. They initially reported that the association between VHL and HIF was sensitive to DFO and cobalt (DFO is an iron-chelator and stabilizes HIF-1alpha; cobalt induces HIF-1alpha in normoxia by preventing HIF binding to VHL) but not oxygen levels. They made this mistake because oxygen was getting into the buffers used in their cell culture, and because the cells were rapidly re-oxygenated when they were taken out of the tri-gas incubator. Only after collaborating with Andrew Skinn, and getting an InvivO$_2$ hypoxia workstation from him, they were able to carry out the essential experiments that showed that the interaction between HIF and VHL was indeed oxygen sensitive. This is because they were able to produce stable hypoxic conditions without exposure to oxygen during vital experimentation.

In a similar way Baker Ruskinn Workstations have enabled a whole range of other techniques in the Ratcliffe laboratory. Techniques such as chromatin IP (ChIP) absolutely require that the oxygen atmosphere is preserved as assay material is being prepared.

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A lot of information gained during the research, focused on the part of HIF-1alpha protein that was known to be important for VHL-dependent degradation. It was likely that the oxygen sensitive section would reside in this area of the protein and indeed in 2001 they published the seminal findings that under ambient oxygen hydroxyl groups are added to HIF-1alpha (a protein modification called prolyl hydroxylation), leading to its recognition by VHL protein and its subsequent degradation (13,14,15,16,17).

Together Kaelin, Ratcliffe and Semenza were able to compose the oxygen sensing system in cells. Working independently they were able to build, from different angles (Kaelin being an oncologist, Ratcliffe kidney specialist and Semenza a medical geneticist), this great contribution to medical science that now awarded them with 2019 Nobel Prize for Physiology or Medicine.

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