Similar and yet different: oxygen sensing in animals and plants

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Abstract (50 words)

The ability to perceive oxygen levels and adapt metabolism on the basis of its availability is essential for most eukaryotic cells. Here, we retrace the essential steps that led to the identification of oxygen sensing mechanisms in animals and plants, and compare the essential features of the two strategies.

Main text (max 1200 words)

In 2019, the Nobel Assembly at the Karolinska Institute awarded the Nobel Prize in Physiology or Medicine jointly to William Kaelin, Peter Ratcliffe and Gregg Semenza '*for their discoveries of how* (animal) *cells sense and adapt to oxygen availability'*. This acknowledgement clearly reflects the relevance of the series of discoveries made by these three scientists to our understanding of animal physiology, including humans. Indeed, being oxygen essential for energy conversion in the mitochondria via oxidative phosphorylation, its cellular availability deeply affects cell and tissue functioning, maintenance and development. Thus, oxygen distribution and consumption require tight control and coordination to maintain its homeostasis. Nonetheless, plant and animal cells alike are frequently exposed to changes in oxygen availability, due to altered metabolic rates or alterations in oxygen collection and delivery, which often lead to a condition commonly defined as 'hypoxia'. Thus, evolution has enabled cells to adapt to such condition by the development of a set of processes that constitute the hypoxic response and include, but are not limited to, the production of new oxygen delivery avenues (angiogenesis and erythropoiesis in animals, aerenchyma in plants), and metabolic adaptations to decrease the demand for oxygen and optimize its usage.

The detailed picture of the molecular mechanisms that trigger the hypoxic response in animal cells, which is now available to us, is the product of hundreds of studies, among which the seminal ones reported by the three 2019 Nobel laureates and their teams in the last 25 years (Fig. 1). First, the isolation of the Hypoxia Responsive Element (HRE) enhancer in the erythropoietin gene allowed the identification of the Hypoxia Inducible transcription Factor (HIF) complex, consisting of two subunits: HIF-1α and HIF-1β [1]. Wang et al. [1] also reported that the HIF-1α subunit is ubiquitously and constantly produced in human cells, although degraded by the proteasome under normoxic conditions and stabilized by hypoxia. The identity of the E3 ubiquitin ligase complex responsible for HIF-1α proteolysis was revealed by two independent reports by Kaelin's and Ratcilffe's teams. Kaelin and co-workers, while studying the genetic determinant of the von Hippel-Lindau disease, noticed that inactivation of the tumour suppressor pVHL caused a cellular response that broadly overlapped with the hypoxic one [2]. Ratcliffe and co-workers provided experimental evidence for the role of pVHL in directly controlling HIF-1α abundance and activity [3]. Finally, Kaelin and Ratcliffe concomitantly showed that the oxygen-dependent hydroxylation of HIF-1α by Prolyl-hydroxylases (PHDs) enables binding by a pVHL-containing E3 ligase complex, and thus triggers its degradation [4,5]. In the following years, the field flourished with a number of studies that elicited further details of the post-translational regulation of HIF and added parallel mechanisms set into action in response to hypoxia in animal cells.

The discovery of oxygen sensing in the green lineage, instead, proceeded more slowly. After a surge of early investigations on the metabolic and anatomic adaptation of plants to flooding, a condition that restricts oxygen availability to the submerged organs, the scientific community rather focussed on plant's adaptive responses towards survival, aiming at reducing yield losses caused by this stress. In retrospect, the limited support to fundamental research in the field of plant hypoxia, likely due to the limited perception of its social or economic impact, might have contributed to delay its discovery by ten years as compared to the animal field (Fig. 1). The very idea that a cellular oxygen sensor might also exist in plants was debated, although the absence of obvious orthologs of the HIF/pVHL system favoured the hypothesis of indirect oxygen sensing, via oxidative stress, ethylene or cytosolic acidification.

In the early 2000s, the advent of molecular techniques allowed the identification of a core of genes specifically induced by hypoxia in several species. Among these, the group VII of Ethylene Response Factors caught the attention of different research groups, not only for their hypoxia inducibility, but also because some of them resulted to be the genetic determinants of increased submergence tolerance in rice (*Oryza sativa*) varieties [6]. Similar to HIF-1α, these transcription factors only exerted limited transcriptional activity when overexpressed under aerobic conditions. Moreover, their recognition as main drivers of the hypoxic response in plants was also hindered by their functional redundancy in plant genomes. As happened in the case of human hypoxia sensing,

two independent studies succeeded in shedding some light upon the matter. Originated by different observations, both studies [7,8] reached the same conclusion: oxygen sensing in higher plants relies on the recognition of a sulfinylated cysteine at the ERF-VII N-terminus by enzymes of the Arg/N-degron pathway that lead them to proteolysis under normoxia, while under hypoxia the N-terminal cysteine cannot be oxidized. Enzymatic control of this crucial step was demonstrated a few years later, with the identification of Plant Cysteine Oxidases (PCOs) as molecular switches for ERF-VII stability and activity and the confirmation of their involvement in the Cys-branch of the Arg/N-degron pathway in plant cells [9,10]. As observed for human *PHDs*, *PCO* genes are induced by hypoxia, revealing that oxygen sensors participate in a conserved negative feedback strategy in both animals and plants. Remarkably, PCO genes are among the markers of hypoxic treatments and their possible involvement as oxygen sensors was hypothesized some years before, in a speculative comparison with the HIF/pVHL/PHD system of animal cells. This model was finished by the identification of the DNA enhancer specifically recognized by the ERF-VII, which was named Hypoxia Responsive Promoter Element [11]. In the next years, details about the ancillary molecular mechanisms modulating the PCO/ERF-VII oxygen sensor emerged, also highlighting the role of nitric oxide, ethylene and low-ATP signaling [12,13].

The similarity between the oxygen sensing systems of plants and animals is remarkable: although exploiting completely different proteins, both consist in the oxygen-dependent proteolysis of transcriptional regulators that are constitutively expressed (Fig. 2). It is worth mentioning that the recognition of PCO functions led up to to the very recent discovery that the Cys-branch of the Arg/N-degron pathway, already proposed in animal cells as additional oxygen sensing mechanism to HIF/pVHL/PHD, is controlled by enzymatic oxidation of N-terminal cysteine in humans as well (Fig. 2) [14]. Thus, future research efforts should be aimed at resolving the evolution, hierarchy and differentiation of oxygen perception in Eukaryotes. While at first sight continuous aerobic turnover of regulatory proteins might seem an unreasonable expense of energy, the convergent development of such a strategy speaks up for its efficiency in ensuring prompt response to varying oxygen levels. Remarkably, these two systems are unparalleled among living organisms and therefore possibly in tight association with the high degree of cellular complexity and organization achieved by animals and plants. In support of this perspective, specific oxygen levels are essential for the development of tissues and organisms in the animal and plant kingdoms, and their respective oxygen sensing mechanisms contribute to ‘regular’ developmental programmes in perfectly aerobic environments [15].

The discovery of these oxygen sensing pathways has set milestones in our understanding of cell physiology. Their relevance is clearly demonstrated by the ever-increasing number of studies that link them to metabolism, disease, stress response and development, but also by the translation of this information into successful pharmacological approaches and breeding strategies.

Moreover, these reports certainly paved the way towards the discovery of additional strategies that conjugate cell biology with this essential element.

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Figures

Fig. 1. Milestones in the history of oxygen sensing research in plants and animals. This figure was created using BioRender (https://biorender.com/).

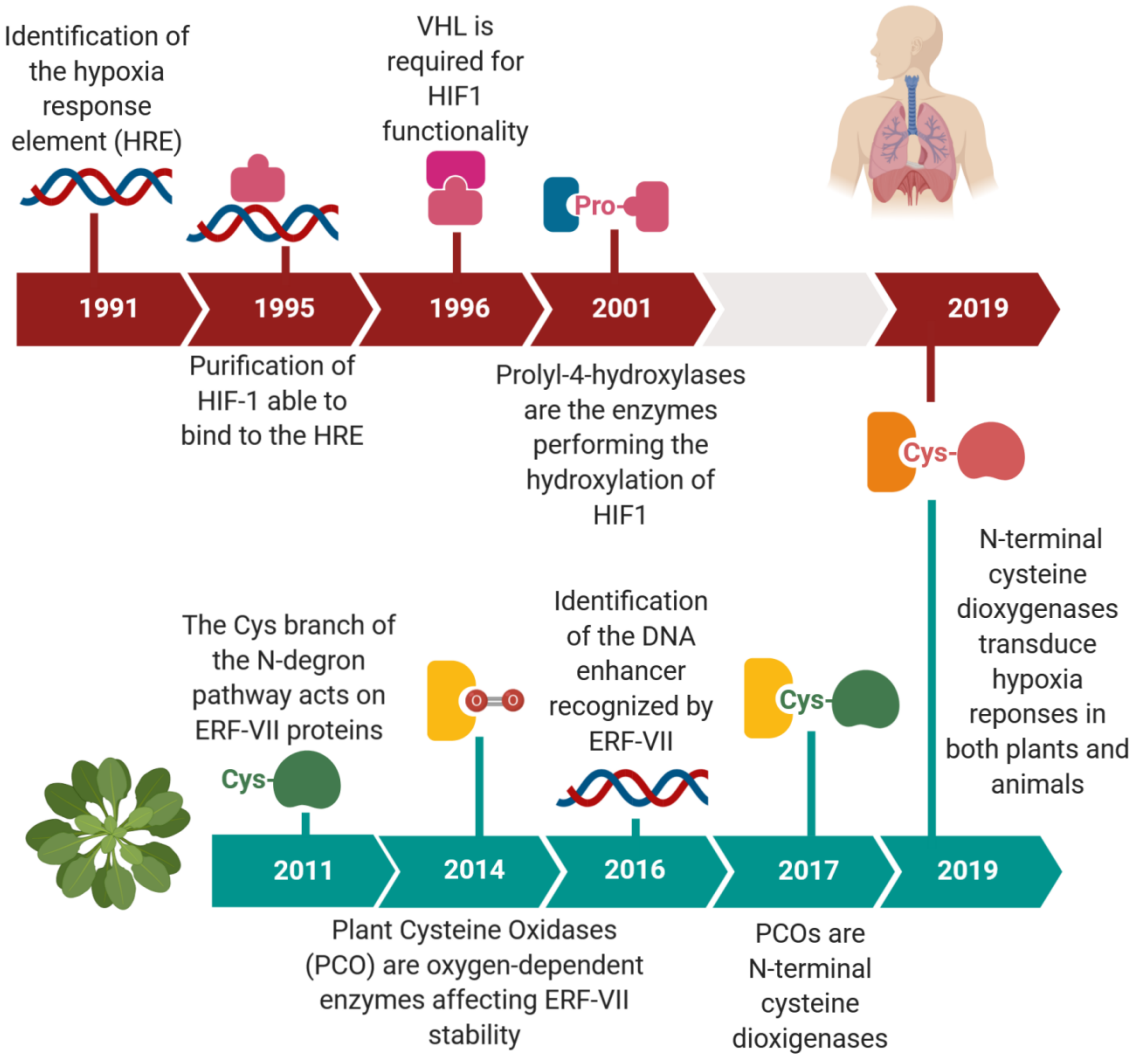

Fig. 2. Comparison of oxygen sensing mechanisms in plants and animals. In plants, the ERF-VII transcription factors are highly unstable in normoxia because their N-terminal Cys residue is oxidized by PCOs, leading to arginylation of the ERF-VII with subsequent proteasomal degradation. An oxygen sensing mechanism mirroring that of plants was recently discovered also in animal cells. Here, the RGS4/5 proteins also display a N-terminal Cys residue that is oxidised by cysteamine (2-aminoethanethiol) dioxygenase (ADO). Also in this case, oxidation of the Cys residue targets the protein for proteasomal degradation under normoxia. When oxygen is absent (hypoxia) neither ERF-VII nor RGS4/5 can be oxidised and are therefore stable and can perform their biological function. The canonical mechanism for oxygen sensing in animals instead relies on the oxidation of a Pro residue in HIF-1, catalysed by prolyl-4-hydroxylases (PHD) under normoxia, and followed by VHL-dependent proteasomal degradation. Also in this case, under hypoxia the protein (HIF-1) is stable and can activate the hypoxia responsive genes. This figure was created using BioRender (https://biorender.com/).