conditions. The ethylene-stabilized ERF-VIIs may localize at the plasma membrane through an interaction with ACBPs [4]. A drop in ATP content, which is typical of hypoxic conditions, is necessary to reduce the activity of LONG-CHAIN ACYL-COA SYNTHETASE (LACS), which eventually affects the composition of the acyl-CoA pool [12]. Increased hypoxia-dependent levels of oleoyl-CoA trigger the release of RAP2.12 from ACBP and consequently activate HRG transcription [12]. Alternatively, assuming that the ERF-VII proteins are stabilized and translocated to the nucleus, post-translational modifications could be essential to activate the proteins as transcriptional activators of HRGs. These questions await an answer in the rapidly expanding field of plant hypoxia. In the context of the increasingly important need to produce plants tolerant to submergence, manipulation of the ethylene responsiveness of *PGB1* genes could be used to develop plants in which passive ethylene entrapment upon flooding rapidly preadapts crops to later-occurring hypoxia.

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Forum

Similar and Yet Different: Oxygen Sensing in Animals and Plants

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

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 acknowledgment clearly reflects the relevance of the series of discoveries made by these three scientists to our understanding of animal physiology, including humans. Indeed, as oxygen is 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 variable 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 a 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 now detailed picture of the molecular mechanisms that trigger the hypoxic response in animal cells is the product of hundreds of studies, notably the seminal works that were reported by the three 2019 Nobel laureates and their teams over the past 25 years (Figure 1). First, the isolation of the hypoxia responsive element 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

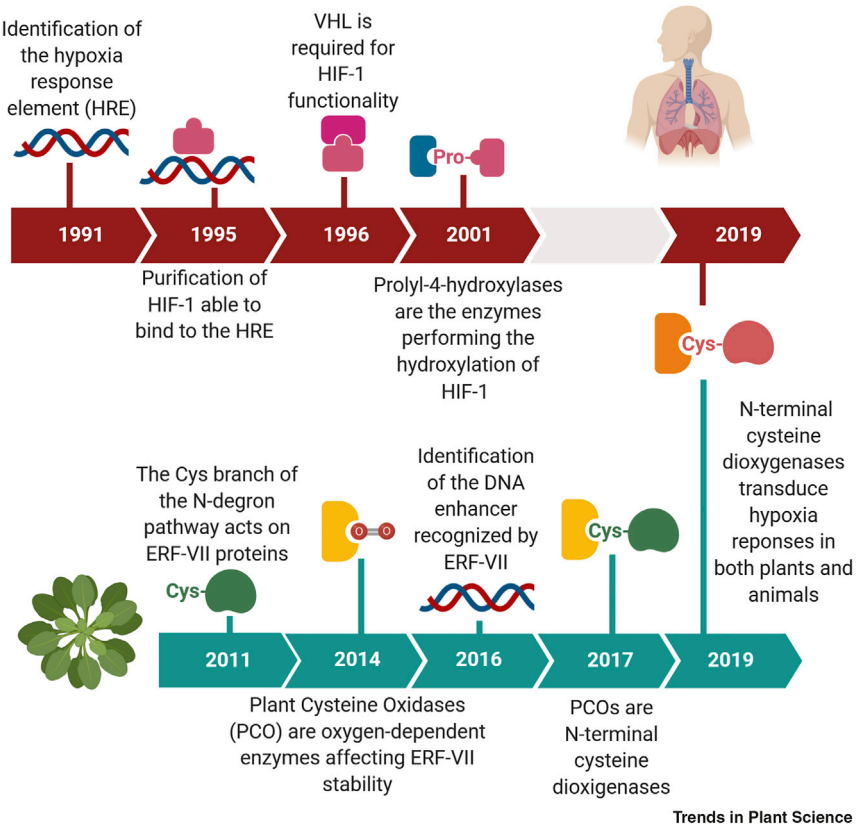

Figure 1. Milestones in the History of Oxygen-Sensing Research in Plants and Animals.
Abbreviations: ERF-VII, Group VII of ethylene response factors; HIF, hypoxia inducible transcription factor; HRE, hypoxia response element; PCO, plant cysteine oxidase. This figure was created using BioRender (https://biorender.com/).

ubiquitin ligase complex responsible for HIF-1α proteolysis was revealed by two independent reports by Kaelin's and Ratcliffe's teams. Kaelin and coworkers, while studying the genetic determinant of the von Hippel-Lindau disease, noticed that inactivation of the tumor suppressor pVHL caused a cellular response that broadly overlapped with the hypoxic one [2]. Ratcliffe and coworkers 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, thus triggering 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 focused 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 10 years as compared with the animal field (Figure 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 favored 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 genes, the group VII of ethylene response factors (ERF-VII) 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, whereas, 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

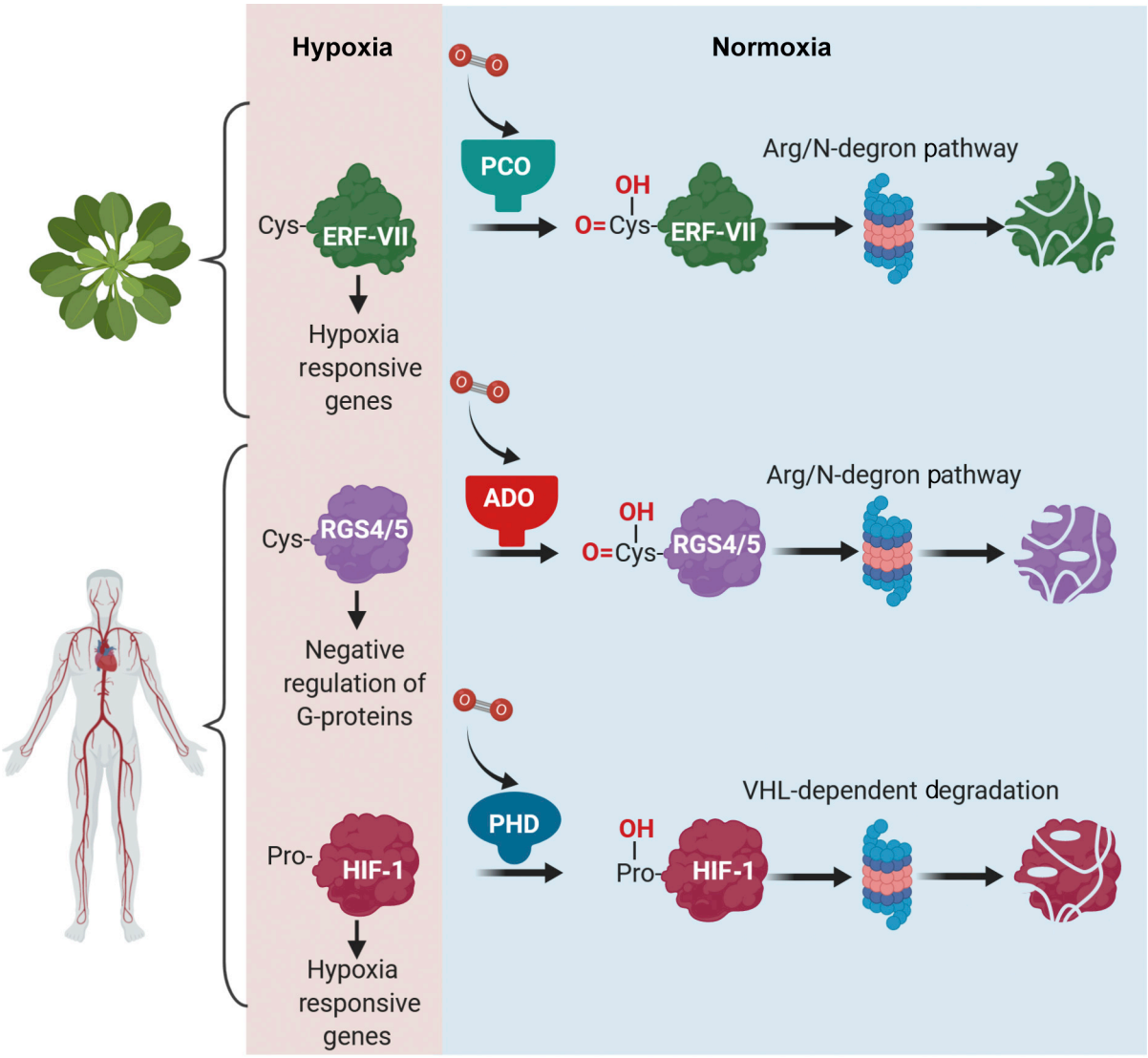

Figure 2. Comparison of Proteolysis-Based Oxygen-Sensing Mechanisms in Plants and Animals.
In plants, the ERF-VII transcription factors are highly unstable in normoxia because their N-terminal Cys residue is oxidized by plant cysteine oxidases (PCOs), leading to arginylation of the ethylene response factor-group VII (ERF-VII) with subsequent proteasomal degradation. An oxygen-sensing mechanism mirroring that of plants was recently discovered also in animal cells. Here, the regulator of G-protein signaling 4/5 (RGS4/5) proteins also display an N-terminal Cys residue that is oxidized 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 oxidized 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 hypoxia inducible transcription factor (HIF)-1, catalyzed 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/).

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 following 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 systems consist of oxygen-dependent proteolysis of transcriptional regulators that are constitutively expressed (Figure 2). It is worth mentioning that the recognition of PCO functions led to the very recent discovery that the Cys-branch of the Arg/N-degron pathway, already proposed in animal cells as an additional oxygen-sensing mechanism to HIF/pVHL/PHD, is controlled by enzymatic oxidation of N-terminal cysteine in humans as well (Figure 2) [14]. Thus, future research efforts should be aimed at resolving the evolution, hierarchy, and differentiation of oxygen perception in eukaryotes. Although 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 are possibly tightly associated 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 programs in perfectly aerobic environments [15].

The discovery of these oxygen-sensing pathways are 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. Oxygen sensing is impaired in many human diseases, and enhancement of adaptive responses to hypoxia by catalytic inhibitors of oxygen-sensing components may represent a new strategy for therapeutic intervention. In the plant sector, selection for variants of ERF-VII/PCO will make breeding of flood-tolerant varieties possible, a goal of utmost importance in the context of climate change and its impact on agriculture. Moreover, these reports certainly paved the way towards the discovery of new ancillary pathways to these sensing mechanisms that conjugate cell biology with this essential element.

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Forum

3D Bioprinting in Plant Science: An Interdisciplinary Approach

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