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# Every breath you take: new insights into plant and animal oxygen sensing

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## Abstract

Responses to hypoxia are regulated by oxygen-dependent degradation of kingdom-specific proteins in animals and plants. Masson *et al.* (2019) identified and characterised the mammalian counterpart of an oxygen-sensing pathway previously only observed in plants. Alongside other recent findings identifying novel oxygen sensors, this provides new insights into oxygen-sensing origins and mechanisms in eukaryotes.

## Main text

Oxygen is essential to almost all life on earth, being required for respiration and a wide range of cellular biochemistry. Multicellular eukaryotes evolved in response to the oxidation events that increased oxygen levels during earth history, and mechanisms for directly sensing and responding to oxygen availability are found broadly across taxa. In metazoan animals and higher plants, transcriptional responses to reduced oxygen (hypoxia) are achieved by oxygen-dependent degradation of transcription factors through analogous but mechanistically distinct post-translational modifications. A recent study published in *Science* showed that an oxygen-sensing enzyme first described in plants is also present in humans, revealing a conserved

mechanism that transduces responses to hypoxia in both kingdoms (Masson *et al.*, 2019).

In animals, gene expression in response to hypoxia is coordinated by the HIF1α transcription factor. In the presence of oxygen, HIF1α is modified by HIF prolyl hydroxylases, which are low-$O_2$ affinity (high $K_mO_2$) 2-OG-dependent dioxygenases that catalyse *trans*-4 prolyl hydroxylation of HIF1α, triggering its ubiquitin-mediated turnover (Figure 1) (Kaelin & Ratcliffe, 2008). When oxygen is limiting hydroxylation is perturbed, and HIF1α accumulates and binds with HIF1β to modulate gene transcription. In contrast, in flowering plants, group VII ETHYLENE RESPONSE FACTOR (ERFVII) transcription factors are regulators of hypoxia-regulated transcriptional reprogramming (Gibbs *et al.*, 2011; Licausi *et al.*, 2011). PLANT CYSTEINE OXIDASEs (PCOs) are monomeric, non-heme iron-dependent dioxygenases that in normoxia convert the amino-terminal (Nt) Cysteine of ERFVIIs to Cys-sulfinic acid (Weits *et al.*, 2014), which targets them for degradation via the PROTEOLYSIS (PRT)6 N-degron pathway, equivalent to the non-plant Arg/N-degron pathway (Figure 1). When oxygen levels decline ERFVIIs are stabilised, induce gene expression critical for coordinating developmental responses to hypoxia, and enhance flooding survival.

Interestingly, whereas the HIF-based sensing system is confined to the metazoan (multicellular animal) lineage, degradation based on a Cys N-degron is conserved across eukaryotes, indicating that the plant oxygen-sensing mechanism has more ancient evolutionarily origins. Supporting this, it was previously reported that certain Cys-initiating REGULATOR OF G PROTEIN SIGNALING proteins (RGS4, 5, 16), which control angiogenesis in mammals, are targets for Cys N-degron-mediated destruction in the presence of both oxygen and nitric oxide (NO) (Hu *et al.*, 2005). However, a direct mechanism connecting their turnover to oxygen levels remained elusive. Masson *et al.* (2019) identified and characterised a human enzyme, previously assigned as cysteamine (2-aminoethanethiol) dioxygenase (ADO), with high sequence similarity to the PCO enzymes of plants. Using a range of genetic, biochemical and cross-kingdom complementation experiments, it was shown that ADO enzymes, like PCOs, have conserved functions as high $K_mO_2$ N-terminal cysteine dioxygenases, catalysing identical oxygen-dependent modifications on the exposed

Nt-cysteine of target proteins to permit subsequent Nt-arginylation and destruction through the mammalian Arg/N-degron pathway (Masson *et al.*, 2019). This reveals for the first time that direct enzyme-mediated oxygen-sensing through Cys N-degrons occurs in both kingdoms.

In addition to connecting ADO activity to the regulation of RGS protein stability, Masson *et al.* investigated other potential Met-Cys-initiating targets of this enzyme in humans, uncovering the atypical cytokine Interleukin (IL)-32 as a physiological substrate. IL-32 has previously been linked to the regulation of angiogenic growth factors. Alongside RGS proteins, this suggests that oxygen-sensing via the mammalian Arg/N-degron pathway may have evolved to control cardiovascular development in response to hypoxia, which may occur within faster timescales than can be achieved through the HIF-dependent system. Two recent reports in plants have identified novel oxygen-regulated physiological targets of PCOs, the transcriptional regulator LITTLE ZIPPER 2 (ZPR2), which coordinates hypoxic control of shoot apical meristem activity, and VERNALIZATION 2 (VRN2), an angiosperm-specific equivalent of the polycomb repressive complex 2 (PRC2) component Su(z)12 (Gibbs *et al.*, 2018; Weits *et al.*, 2019). Collectively these studies raise the possibility that other Cys-initiating targets of PCO/ADO exist. Such proteins would represent a diverse oxygen-targeted sub-proteome that could simultaneously orchestrate cellular responses to hypoxia. Interestingly, a unique feature of this N-degron pathway in plants and mammals is the requirement for NO, in addition to oxygen, to achieve Cys N-degron substrate destruction (Figure 1A), thereby providing sensing capacities for both gases in a single pathway (Hu *et al.*, 2005; Gibbs *et al.*, 2014)

As a component of the PRC2, VRN2 may connect oxygen availability to chromatin methylation status in plants, as its increased stability under hypoxia is proposed to enhance H3K27me3 levels. Remarkably, two studies published earlier this year identified another class of direct oxygen-sensing enzyme in human cells that promotes histone demethylation under normoxia (Batie *et al.*, 2019; Chakraborty *et al.*, 2019). The Jmjc domain-containing histone Lysine demethylases KDM5A and KDM6A, which like HIF prolyl-hydroxylases and ADO/PCO are 2-OG-dependent dioxygenases, were shown to function as direct oxygen sensors. Reduced oxygen availability leads to decreased KDM demethylase activity, and increased histone H3 methylation,

promoting global chromatin remodelling to modulate gene expression and regulate cell fate. All three studies reveal that hypoxia can therefore influence histone modifications by directly affecting protein stability or activity. KDM-type enzymes are also found in plants and fungi, and the plant enzymes influence growth and development, though no oxygen-regulated functions have been investigated. This suggests that, similar to N-degron-mediated hypoxia signalling, oxygen sensing by chromatin may predate the evolution of the HIF signalling pathway.

Collectively these recent studies uncover distinctions and commonalities in the ways that different eukaryote lineages have evolved to sense and respond to oxygen. The conserved Cys N-degron pathway emerged as the predominant system for hypoxia-regulated transcriptional responses in higher plants, whereas the HIF-based mechanism evolved later as the core transcriptional transduction system in metazoa. Furthermore, mechanisms controlling histone methylation in response to oxygen availability have emerged in both lineages. It is interesting to note that in animals, there is a hierarchical order to the different systems: RGS and KDM proteins are transcriptionally regulated by HIF, indicating complex interplay amongst these separate transduction pathways for controlling global hypoxia responses. The identification of new hypoxia-responsive enzymes and oxygen-regulated targets highlights the importance of sensing this essential gas in both animals and plants, and should facilitate future research efforts to understand hypoxia signalling, and how it influences development, disease and stress survival across kingdoms.

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## Figure Legend

**Mechanisms and functions of oxygen-sensing systems in higher eukaryotes**. **(A)** Oxygen ($O_2$) regulated destruction of substrates of the PCO/ADO branch of the

PRT6/Arg/N-degron pathway, of HIF1$\alpha$, and oxygen-regulated activation of KDM histone demethylase activity. **(B)** Functions of stabilised oxygen-sensitive substrates (KDMs are inactive) in hypoxia. Colour of substrates refers to the pathway in A by which they are regulated. MetAP, methionine amino-peptidase; NO, nitric oxide; ATE1, arginyl-transferase 1; pVHL, Von Hippel–Lindau tumor suppressor; UBR1, ubiquitin system recognition component 1. Three letter amino-acid abbreviations are used. Other abbreviations are given in the main text.

## References


**Batie M, Frost J, Frost M, Wilson JW, Schofield P, Rocha S. 2019.** Hypoxia induces rapid changes to histone methylation and reprograms chromatin. *Science* **363**(6432): 1222-1226.

**Chakraborty AA, Laukka T, Myllykoski M, Ringel AE, Booker MA, Tolstorukov MY, Meng YJ, Meier SR, Jennings RB, Creech AL, et al. 2019.** Histone demethylase KDM6A directly senses oxygen to control chromatin and cell fate. *Science* **363**(6432): 1217-1222.

**Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J, et al. 2011.** Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* **479**(7373): 415-418.

**Gibbs DJ, Md Isa N, Movahedi M, Lozano-Juste J, Mendiondo GM, Berckhan S, Marin-de la Rosa N, Vicente Conde J, Sousa Correia C, Pearce SP, et al. 2014.** Nitric Oxide Sensing in Plants Is Mediated by Proteolytic Control of Group VII ERF Transcription Factors. *Mol Cell*.

**Gibbs DJ, Tedds HM, Labandera A-M, Bailey M, White MD, Hartman S, Sprigg C, Mogg SL, Osborne R, Dambire C, et al. 2018.** Oxygen-dependent proteolysis regulates the stability of angiosperm polycomb repressive complex 2 subunit VERNALIZATION 2. *Nat Commun* **9**(1): 5438.

**Hu RG, Sheng J, Qi X, Xu Z, Takahashi TT, Varshavsky A. 2005.** The N-end rule pathway as a nitric oxide sensor controlling the levels of multiple regulators. *Nature* **437**(7061): 981-986.

**Kaelin WG, Jr., Ratcliffe PJ. 2008.** Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* **30**(4): 393-402.

**Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LA, Perata P, van Dongen JT. 2011.** Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature* **479**(7373): 419-422.

**Masson N, Keeley TP, Giuntoli B, White MD, Puerta ML, Perata P, Hopkinson RJ, Flashman E, Licausi F, Ratcliffe PJ. 2019.** Conserved N-terminal cysteine dioxygenases transduce responses to hypoxia in animals and plants. *Science* **365**(6448): 65-69.

**Weits DA, Giuntoli B, Kosmacz M, Parlanti S, Hubberten HM, Riegler H, Hoefgen R, Perata P, van Dongen JT, Licausi F. 2014.** Plant cysteine oxidases control the oxygen-dependent branch of the N-end-rule pathway. *Nat Commun* **5**: 3425.

**Weits DA, Kunkowska AB, Kamps NCW, Portz KMS, Packbier NK, Nemec Venza Z, Gaillochet C, Lohmann JU, Pedersen O, van Dongen JT, et al. 2019.** An apical hypoxic niche sets the pace of shoot meristem activity. *Nature* **569**(7758): 714-717.