

Comparative biology of oxygen sensing in plants and animals

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1 **Comparative biology of oxygen-sensing in plants and animals**

2

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7

8 **Abstract:**

9 Aerobic respiration is essential to almost all eukaryotes and sensing oxygen is a key
10 determinant of survival. Analogous but mechanistically different oxygen-sensing pathways
11 were adopted in plants and metazoan animals, that include ubiquitin-mediated degradation of
12 transcription factors and direct sensing via non-heme iron(Fe^{2+})-dependent-dioxygenases.
13 Key roles for oxygen-sensing have been identified in both groups, with downstream signalling
14 focussed on regulated gene transcription and chromatin modification to control development
15 and stress responses. Components of sensing systems are promising targets for human
16 therapeutic intervention and developing stress resilient crops. Here we review current
17 knowledge about the origins, commonalities and differences between oxygen-sensing in
18 plants and animals.

19

20 **Introduction:**

21 Molecular oxygen (O_2) is necessary for many core biochemical pathways and most
22 importantly as the final electron acceptor in mitochondrial electron transport, and is therefore
23 essential to the vast majority of eukaryotes. Oxygen first appeared in quantity on earth as a
24 result of the evolution of oxygenic photosynthesis at least 2.3 Ga (billion years ago) (reviewed
25 in {Fischer, 2016 #3073}) (Figure 1). Subsequently as part of the evolution of endosymbiosis
26 with an ancient cyanobacterial group before 1 Ga early eukaryotic algae gained the ability to
27 photosynthesise, leading to further increases in O_2 levels that peaked at over 30% during the
28 Carboniferous (~360 to 300 Ma). Endosymbiosis with purple non-sulphur bacteria (that
29 became mitochondria), that predated chloroplast endosymbiosis, may have allowed early
30 eukaryotes to tolerate O_2 and use the energy of mitochondrial aerobic respiration to become
31 multicellular. Various hypotheses have been advanced that increased O_2 levels were either
32 highly poisonous and catastrophic for early anaerobic eukaryotes or that these organisms
33 were already pre-adapted to deal with reactive oxygen species that aided evolution of O_2
34 tolerance (discussed in {Lane, 2016 #3079}).

35

36 Oxygen varies in the environment (for example declining with increased altitude, or as
37 a result of submergence in water or under the soil surface) and also internally during
development or disease {van Dongen, 2015 #1896; Kaelin, 2008 #1520}. It is clear that for

38 such an essential component of intracellular biochemistry, sensing and response to changing
39 O₂ levels must be an important feature of multicellular eukaryotes. In this review we focus on
40 biochemical pathways that evolved in plants and animals to sense and respond to reduced O₂
41 levels (hypoxia). Analogous pathways evolved in both lineages, that target nuclear-located
42 processes for response. We highlight the different evolutionary trajectories of pathways, the
43 importance of dioxygenases as conserved sensors of hypoxia, the physiological importance
44 of oxygen-sensing and avenues for identification of novel sensors and pathway components.

45

46 **The ubiquitin proteasome system as a hub for oxygen-sensing across kingdoms**

47 In metazoans and angiosperms major mechanisms directing changes in gene
48 expression under hypoxia are controlled by hypoxia-responsive transcription factors. Their
49 stability is intrinsically linked to O₂ levels and in oxygenated environments they are
50 polyubiquitylated and rapidly degraded by the 26S proteasome (Figure 2). In metazoans, the
51 Hypoxia Inducible Factor (HIF, also known as EPAS) heterodimer consists of HIF α and β
52 bHLH-PAS domain subunits (Figure 2). Three HIF α proteins are found in mammals; HIF1 α
53 and HIF2 α contain N- and C-terminal transactivation domains (NTAD and CTAD), whilst
54 HIF3 α lacks the latter {Kaelin, 2008 #1520}. The NTAD of HIF α contains conserved proly
55 residues that are hydroxylated using O₂ by proly 4-hydroxylases (PHD, also known as EGLN)
56 {Ivan, 2001 #3107;Jaakkola, 2001 #3108}. This modification then permits binding of the E3
57 ubiquitin ligase von Hippel-Lindau protein (pVHL) to initiate polyubiquitylation {Iliopoulos, 1996
58 #3105;Maxwell, 1999 #3118 }, leading to HIF α degradation. Hypoxia limits PHD activity,
59 precluding pVHL binding, thus allowing association with HIF β and re-localisation to the
60 nucleus {Kaelin, 2008 #1520} (Figure 2). The CTAD-containing HIF α variants can also be
61 hydroxylated on asparaginyI residues by Factor Inhibiting HIF1 α (FIH), which limits HIF α
62 association with transcriptional co-factors {Lando, 2002 #3112}. This separate O₂-triggered
63 modification also therefore contributes to inhibition of HIF activity through a parallel
64 hydroxylation-dependent but non-proteasomal route.

65 In flowering plants, the group VII ETHYLENE RESPONSE FACTOR transcription
66 factors (ERFVIIs) control anaerobic gene expression under hypoxia {Gasch, 2016 #2022}.
67 Following co-translational Methionine excision, in high O₂ levels the N-terminal Cysteine of
68 ERFVIIs is converted to Cys-sulfinic acid by PLANT CYSTEINE OXIDASEs (PCOs), which
69 leads to amino-terminal (Nt)-arginylation by ATE {Weits, 2014 #1764;White, 2017 #2111}. Nt-
70 Arg-ERFVIIs are then targeted for proteasomal degradation by the E3 ligase PROTEOLYSIS
71 (PRT)6 {Gibbs, 2011 #1529;Licausi, 2011 #1530}. This pathway also requires nitric oxide (NO)
72 {Gibbs, 2014 #1765}. Thus, similarly to HIF α regulation, coupling protein turnover to O₂
73 availability results in ERFVII stabilisation under hypoxia (Figure 2). Recently, a mammalian
74 protein with high similarity to PCO, cysteamine (2-aminoethanethiol) dioxygenase (ADO), was

75 characterised and shown to control O₂-dependent turnover of non-nuclear REGULATOR OF
76 G PROTEIN SIGNALLING (RGS) 4,5,16 proteins via the mammalian Arg/N-degron pathway
77 {Masson, 2019 #2903}. This highlights an alternative mechanism for O₂-sensitive proteolysis
78 in mammals, equivalent to the predominant system in plants.

79 There is evidence that alternative pathways can also target HIF α and ERFVIs for
80 degradation, revealing additional proteolytic mechanisms for fine-tuning their stability {Gibbs,
81 2015 #1922;Kaelin, 2008 #1520;Isaacs, 2002 #3106;Kong, 2006 #3110;Papdi, 2008 #1500}.
82 Furthermore, animal PHD and plant PCO enzymes also have non-HIF and -ERFVII targets,
83 respectively. In *Arabidopsis thaliana*, the PCO targets LITTLE ZIPPER (ZPR)2 and Polycomb
84 Repressive Complex (PRC)2 component VERNALIZATION (VRN)2 are subject to ubiquitin-
85 mediated degradation {Gibbs, 2018 #2614;Weits, 2019 #2868}, whereas hydroxylation of
86 candidate non-canonical PHD/FIH substrates, such as IKK β , p53, and OTUB1, can have
87 different effects on protein activity and interactions {Strowitzki, 2019 #3128}.

88

89 **The key role of non-heme iron(Fe²⁺)-dependent dioxygenases in oxygen-sensing**

90 The enzymes catalysing both prolyl-/asparaginyl-hydroxylation (PHD, FIH) and Nt-
91 cysteine oxidation (PCO, ADO) belong to the non-heme iron(Fe²⁺)-dependent dioxygenase
92 family, so called because their catalytic sites contain a redox active iron directly coordinated
93 to the protein, and incorporate both atoms from O₂ into substrates {White, 2016 #3135}. PHDs
94 function as physiological O₂ sensors due to their high K_mO₂ values, which for the dominant
95 PHD2 isoform (dependent on the length of peptide studied) has variably been reported from
96 less than 100 μ M to 1700 μ M, much higher than *in vivo* O₂ concentrations {Ehrismann, 2007
97 #3102;Koivunen, 2006 #3146}. In contrast, FIH has a higher affinity for O₂ than PHDs,
98 indicating that greater decreases in O₂ availability would be required before its activity is
99 inhibited {Koivunen, 2004 #3145}. PHD/FIH incorporate one O₂ atom into the target HIF α prolyl
100 or asparaginyl residue, whilst the second decarboxylates 2-oxo-glutarate (2-OG) to produce
101 CO₂ and succinate {Strowitzki, 2019 #3128;Yeh, 2017 #3139} (Figure 2). PCOs and ADO also
102 have high K_mO₂ values above typical plant and animal tissue O₂ concentrations, but in contrast
103 to PHDs they are not 2-OG dependent, they integrate both O₂ atoms directly into Nt-Cys to
104 generate Cys-sulfinic acid {Masson, 2019 #2903;White, 2018 #2531;White, 2017 #2111}.

105 Metazoans encode multiple PHD isoforms, which are differentially expressed and have
106 varying subcellular localisations, although the main mammalian PHD2 variant is cytosolic and
107 constitutively expressed {Metzen, 2003 #3121;Kaelin, 2008 #1520}. Flowering plant PCOs
108 have different sensitivities to O₂ and pH, and divergent substrate preferences based on
109 assessment of their activities on peptide sequences {White, 2018 #2531}. Of the five PCOs in
110 *A. thaliana*, PCO4 is the most catalytically potent suggesting that it may be the dominant

111 variant. Without an active oxygen-transport system, strong gradients of hypoxia exist in plant
112 tissues (obvious examples include tubers and seeds) {van Dongen, 2015 #1896;Considine,
113 2017 #3147} and it may be that PCOs with different affinities for O₂ operate in different
114 tissues/at different developmental time points. Interestingly, a subset of these oxygen-sensing
115 enzymes in animals and plants are transcriptionally induced by low- O₂ levels, suggesting that
116 homeostatic mechanisms for dampening the hypoxic response have evolved in both kingdoms
117 {Kaelin, 2008 #1520;Weits, 2014 #1764}.

118 In addition to PHD and PCO/ADO proteins, there are many other non-heme iron(Fe²⁺)-
119 dependent dioxygenases in animals and plants {McDonough, 2010 #3119;White, 2016
120 #3135}, although several of these, including collagen prolyl hydroxylases and certain JmjC
121 (Jumonji C) domain lysine demethylases (KDMs), are unlikely to sense physiological changes
122 due to their high O₂ affinities {Strowitzki, 2019 #3128;Chakraborty, 2019 #3091}. Nonetheless,
123 it was recently shown that some histone-specific KDMs (KDM5A and 6A) do have *K_mO₂* values
124 in the requisite range for sensing intracellular O₂, and are able to directly modulate the
125 methylation status of chromatin dependent on O₂ availability {Batie, 2019 #3092;Chakraborty,
126 2019 #3091} (Figure 2). Under hypoxic conditions, KDM activity is reduced, resulting in
127 enhanced global levels of histone methylation, regulating gene expression and cell fate. The
128 activity of a separate non-histone KDM (KDM3A), which is involved in the demethylation of
129 the transcriptional co-activator PGC-1α, also connects O₂ availability to the regulation of genes
130 linked to mitochondrial biogenesis {Qian, 2019 #3124}, suggesting others await discovery.

131

132 **Evolutionary origins of the different oxygen sensing systems**

133 Components of the Arg/N-degron pathway are conserved in eukaryotes, though
134 distinct evolutionary trajectories are observed. Whereas ATE activity is highly conserved
135 across all major groups, E3 ligase functions for recognising distinct destabilising residues
136 (carried out by UBR-type proteins in non-plants) were split early in plant evolution {Till, 2019
137 #3042} (Figure 1). ERFVIIIs are not present in the genome of basal land-plants *Physcomitrella*
138 *patens* or *Marchantia polymorpha*, and VRN2 and ZPR2 appeared with angiosperms {Gibbs,
139 2018 #2614;Weits, 2019 #2868}. As the nature of Nt-Cys oxidation was for several years
140 obscure, a major advance was the identification of the PCOs in *A. thaliana* {Weits, 2014
141 #1764}. This showed that Nt-Cys oxidation required PCO enzyme activity, and genetic
142 removal of PCO function leads to ERFVII stabilisation and enhanced hypoxia tolerance. The
143 identification of ADO indicates that oxygen-sensing via this pathway is ancient, predating the
144 split between animal and plant groups (>1 Ga) {Masson, 2019 #2903} (Figure 1), and may
145 indicate that a major mechanism of oxygen-sensing in early eukaryotes was through cysteine
146 dioxygenase control of Nt-Cys oxidation, during periods of earth history with comparatively

147 low O₂ levels. Alternatively, it may suggest that originally the major function of the pathway
148 was NO sensing, and became coupled to O₂ as atmospheric levels rose. PCO-type Nt-
149 cysteine dioxygenases have not been found in fungi, that diverged from animals after plants,
150 indicating loss of the capacity of this group to oxidise Nt-Cys and use this pathway for oxygen-
151 sensing {Masson, 2019 #2903}.

152 Although the PCO/ADO branch of the N-degron pathways is ancient in eukaryotes, the
153 HIF pathway is only present in metazoan animals (choanoflagellates, closest extant relatives
154 to animals, do not contain bHLH-PAS domain proteins, {Mills, 2018 #3063}) (Figure 1). A
155 functioning HIF system was identified in the placozoan *Trichoplax adhaerens*, representing
156 one of the simplest multicellular animals {Loenarz, 2011 #3066}. A recent analysis of
157 representatives of basal metazoa groups porifera (sponges) and ctenophores failed to identify
158 pVHL or PHD-like proteins, or hypoxia-regulated gene expression {Mills, 2018 #3063}. One
159 feature of the evolution of the HIF system appears to be increased diversification of
160 components in derived evolutionary groups. Whereas *T. adhaerens* contains single proteins
161 for each component of the pathway mammals contain multiple variants of HIF α and PHD
162 {Loenarz, 2011 #3066}. The appearance and diversification of a functional HIF pathway, that
163 correlates with large increases in atmospheric and oceanic O₂, may have influenced the
164 concomitant explosion of animal diversity and size beginning around the Cambrian period
165 (~540 Ma) (Figure 1).

166

167 **Integration of oxygen-sensing with downstream signalling and physiology**

168 Key observations related to major consequences of oxygen-sensing have been the
169 identification of nuclear changes in response to hypoxia. In both plants and animals these
170 converge on transcription of hypoxia-related genes and chromatin structure. In plants an
171 evolutionarily-conserved core set of hypoxia-related genes are activated by ERFVIs in
172 response to hypoxia-induced stabilisation, through a conserved Hypoxia Responsive
173 Promoter Element (HRPE) {Gasch, 2016 #2022}. Similarly, animal Hypoxia Response
174 Elements (HREs) are bound by HIF factors to enhance low O₂ responsiveness {Mole, 2009
175 #3068}. Low O₂ levels also influence chromatin structure, through the stabilisation of
176 components of chromatin modifying complexes (VRN2 as part of PRC2 {Gibbs, 2018 #2614}),
177 via enhanced expression of chromatin modifiers (gene activation by HIF {Xia, 2009 #3069}),
178 or directly through repression of histone H3 demethylation activity of KDMs {Batie, 2019
179 #3092;Chakraborty, 2019 #3091}. In both animal and plant responses, genes encoding
180 biochemical pathways associated with enhanced tolerance of hypoxia are important targets
181 (including fermentative metabolism, glycolysis and an inhibition of mitochondrial oxidative
182 phosphorylation), but the control of pathways with O₂-requiring reactions or that occur in
183 hypoxic niches are also important {Abbas, 2015 #1923;Weits, 2019 #2868; Takubo, 2010

184 #3140}. Two animal cytoplasmic substrates of ADO have been identified, RGS4,5,16 and
185 INTERLEUKIN (IL)-32 {Hu, 2005 #1341;Masson, 2019 #2903}, that gives the possibility of
186 more rapid response to declining O₂ than transcriptional circuits, since their immediate
187 stabilisation would trigger a change more quickly than responses dependent on increased
188 protein production through HIF control of gene expression. Both IL-32 and RGS4/5 are
189 transcriptional targets of HIF, indicating a possible interaction between the two sensing
190 systems {Masson, 2019 #2903}. Moving forward it will be important to decipher the
191 comparative timescales through which PHD/FIH, KDM and ADO activity leads to cellular
192 changes, as this likely contributes to physiologically relevant fine tuning of the overall hypoxia
193 response.

194 Analyses of physiological functions reveal the broad reach of oxygen-sensing systems,
195 and specific roles are related to the different lifestyles of plants and animals. As plants are
196 sessile a key function of oxygen-sensing is related to perception of waterlogging and flooding
197 {Gibbs, 2011 #1529;Licausi, 2011 #1530}. Both stabilised ERFVILs and VRN2 enhance
198 survival of hypoxia {Gibbs, 2011 #1529;Gibbs, 2018 #2614;Licausi, 2011 #1530}. It was
199 recently shown that the plant Cys-initiating substrate ZPR2 is stabilised by the hypoxic
200 environment of the shoot apical meristem, regulating the production of new leaves {Weits,
201 2019 #2868}, and VRN2 also accumulates in hypoxic meristems, where it modulates flowering
202 time and root development {Labandera, #3148}. In addition, hypoxia-enhanced stability of
203 ERFVILs was shown to repress chlorophyll synthesis (an O₂-requiring pathway) in dark grown
204 seedlings {Abbas, 2015 #1923}, as well as lateral root development {Shukla, 2019 #2889}.

205 The HIF pathway plays major roles in O₂-homeostasis, including erythropoiesis
206 (development of red blood cells) and angiogenesis (development of new blood vessels)
207 (reviewed in {Samanta, 2017 #3070}). Similar to ZPR2/VRN2 in plant meristems, HIF1 α is
208 stabilised within hypoxic hematopoietic stem cells (that give rise to blood cells) {Takubo, 2010
209 #3140}. Stabilised HIF1/2 α enhance expression of growth regulators (erythropoietin (EPO)
210 and angiogenic growth factors) and associated components (for example systems for iron
211 uptake and utilisation {Samanta, 2017 #3070}). An important role of the HIF system is in
212 adaptation of animals to high altitude, where the partial pressure of O₂ is reduced. Genome
213 wide association studies identified allele signatures in human populations associated with life
214 at high altitudes in the Tibetan Plateau (average altitude 4000 m, pO_2 13 kPa) for both HIF2 α
215 and PHD2. For example, in modern Tibetan populations a variant of *EGLN1/PHD2* (Asp4Glu;
216 Cys127Ser) was shown to have a lower K_mO_2 suggesting that it promotes increased
217 degradation of HIF at high altitude (lower pO_2) thus reducing HIF levels to those equivalent to
218 low altitudes {Lorenzo, 2014 #2028}. Interestingly one allele of *EPAS1/HIF2A* enriched in
219 Tibetan populations appears to have been derived from ancient hominid Denisovans {Huerta-
220 Sanchez, 2014 #2071}. Many studies demonstrate wider roles for the HIF system, indicating

221 that oxygen-sensing by this pathway influences many aspects of cellular biochemistry, growth
222 and development (discussed in {Pugh, 2017 #3099}).

223 Since the PCO/ADO pathway also acts as an NO sensor {Gibbs, 2014 #1765;Hu, 2005
224 #1341}, the stability of both animal and plant substrates also regulates responses to
225 intracellular NO levels that accompany internal and external stress. For example, destruction
226 of RGS proteins to induce cardiomyocyte proliferation can also be induced by endothelium-
227 derived NO {Jaba, 2013 #1608}. Stabilisation of ERFVILs by reduced NO enhances hypoxia
228 tolerance and tolerance to other abiotic stresses (including high salinity) {Hartman, 2019
229 #2983;Vicente, 2017 #2298}. It is still unclear exactly where NO acts within the pathway.
230 Although an in vitro reconstituted mammalian system was shown to be NO dependent {Hu,
231 2005 #1341}, in vitro activity of PCO/ADO does not require NO {Masson, 2019 #2903;White,
232 2018 #2531}. It is possible therefore that NO influences the activities of enzyme components
233 of the pathway in vivo (ATE, PCO/ADO or UBR1/PRT6), and it was shown that PRT6 contains
234 an NO binding domain {Zarban, 2019 #2907}.

235 Factors other than hypoxia can influence oxygen-sensing pathways. A sub-pool of
236 ERFVILs is stable and sequestered at the plasma membrane through association with ACYL
237 CoA BINDING PROTEINS (ACBP) during normoxia {Licausi, 2011 #1530;Schmidt, 2018
238 #2615}. Zinc excess in the soil (detrimental to plant growth), inhibits PCO enzymes thus
239 causing stabilisation of ERFVILs {Dalle Carbonare, 2019 #2904}. Non-canonical mechanisms
240 also control HIF stability; for example, increases in succinate during the progression of certain
241 types of cancer can allosterically inhibit PHD activity to trigger HIF accumulation under
242 normoxia {Iommarini, 2017 #3098;Selak, 2005 #3149}. The possible mechanisms influencing
243 O₂-responsive factors, and therefore the breadth of possible affected physiological processes
244 will be much wider than those specifically related to O₂ or NO.

245

246 **Pathologies and interventions of oxygen-sensing in plants and animals**

247 Oxygen-sensing pathways represent key cellular targets for counteracting diseases and
248 enhancing stress resilience. HIF signalling controls a range of cellular responses, and also
249 drives tumorigenesis and the maintenance of tumour microenvironment in certain cancers
250 {Huang, 2017 #3104}. Thus, interventions that impact the HIF pathway have the capacity to
251 treat pathologies associated with these processes. *EPO*, a target of HIF, is down-regulated in
252 patients with chronic kidney disease (CKD) due to reduced O₂ consumption {Schodel, 2019
253 #3067}. Several PHD inhibitor molecules (PHIs) have been developed that stimulate
254 increased *EPO* production in CKD patients to counteract renal anaemia {Myllyharju, 2013
255 #3122}, acting as 2-OG mimetics or iron-chelators to inhibit enzymatic activity and increase
256 HIF stability in normoxia {Schodel, 2019 #3067}. Chemicals that disrupt other aspects of HIF

257 signalling have also been identified as potent repressors of cancer progression {Fallah, 2019
258 #3143}. For example, cancers in patients with VHL disease result from ectopic accumulation
259 of HIF2 α {Huang, 2017 #3104}, and a novel drug that specifically disrupts the HIF2 α /HIF2 β
260 dimer to downregulate HIF2 signalling was recently shown to limit tumour progression {Chen,
261 2016 #3101}. The development of inhibitory molecules that target discrete HIF or PHD
262 isoforms, as well as other regulatory points in the HIF signalling pathway, will help to increase
263 therapeutic specificity and efficacy of such treatments.

264 Genetic manipulation of O₂-signalling components in crop species can increase resistance
265 to waterlogging-induced hypoxia, as shown in barley through genetic reduction in *HvPRT6*
266 expression/activity {Mendiondo, 2016 #1869}, whilst ERFVIIs provide increased tolerance to
267 multiple abiotic stresses {Vicente, 2017 #2298} and biotic stresses where pathogen-
268 associated hypoxia is an integral factor {Valeri, #3144;Gravot, 2016 #2030;Kerpen, 2019
269 #2610}. In rice (*Oryza sativa*), the ERFVII SUB1A-1 is a major regulator of submergence
270 tolerance that has been bred into high yielding varieties {Xu, 2006 #1508}. SUB1A-1 is
271 naturally uncoupled from O₂-dependent degradation despite containing Cys2 and downstream
272 Lys residues {Gibbs, 2011 #1529;Lin, 2019 #2609} suggesting that the plant oxygen-sensing
273 system has been targeted by natural selection for adaptation in wetland environments, and
274 that biotechnological approaches could be used to achieve similar outcomes in flooding-
275 susceptible crops.

276

277

278 **Conclusions and unresolved questions**

279 Where to look for undiscovered oxygen-sensors? Based on structures and domains of
280 already identified proteins there are clear candidates to test as novel components of oxygen-
281 sensing pathways. Plant and animal genomes contain Jumonji C domain-containing KDMs in
282 addition to those already shown to act as oxygen-sensors. Determining those with a
283 physiologically relevant (high) K_mO_2 would be a first step in defining potential roles as sensors.
284 Although plants do not contain HIF α -like sequences, both plants and animals contain
285 hundreds of proteins initiating Met-Cys, that could be substrates of PCO/ADO action, in
286 addition to endopeptidase substrates cleaved to reveal Nt-Cys. Cys2 is evolutionarily
287 constrained in most eukaryote proteomes {Gibbs, 2014 #1744} suggesting that this is an
288 important determinant related to O₂/NO-sensing. In addition, recently it was hypothesised that
289 mechanisms other than PCO-regulated destabilisation may act to promote oxygen-sensing in
290 plants, in several cases backed-up by experimental data {Holdsworth, 2017 #2899}.

291 Why is N-degron mediated oxygen-sensing not the primary system in metazoans as it is
292 in angiosperms? The HIF system evolved only in one lineage of animals, whereas the
293 PCO/ADO pathway evolved early in eukaryotes (Figure 1). Perhaps the unavoidable link of

294 the PCO/ADO pathway to a requirement for NO made this pathway unsuitable, or possibly it
295 was not suitable for large mobile organisms. Lack of transcriptional response to hypoxia in the
296 marine sponge *Tethya wilhelma* indicates that the PCO/ADO pathway does not perform this
297 function in basal animals, though complete anoxia did result in large changes in gene
298 expression {Mills, 2018 #3063}. It is unclear what advantage the coupling of NO- and oxygen-
299 sensing in this pathway has; it may be a remnant of evolutionary drivers early in eukaryote
300 history, where O₂ levels were low, which might also suggest early Nt-cysteine dioxygenases
301 had high affinities for O₂, making the pathway primarily important for responding to changes
302 in levels of intracellular NO.

303 There are several striking commonalities in the major oxygen-sensing systems of
304 angiosperms and metazoans. Both require dioxygenases with O₂-sensitivity within a
305 physiological range, both directly target nuclear-factors for UPS-mediated destruction, and
306 both result in large changes in gene expression with downstream physiological consequences
307 providing homeostatic control of O₂ response. An important goal of future research will be to
308 define the links between O₂ affinity of pathway dioxygenases and their expression patterns,
309 allowing an understanding of how these enzymes sense all physiologically possible internal
310 O₂ tensions. The complete gamut of influenced processes and interactions is yet to be
311 resolved, at the intracellular level there are clearly similarities of interactions between oxygen-
312 sensing pathways and mitochondrial function (key for oxidative phosphorylation), well
313 understood for the HIF system, but requiring more understanding for the PCO/ADO pathway
314 in animals and plants. It is likely that many components of known oxygen-sensing pathways
315 remain to be discovered, including dioxygenases with novel activities, and PHD/ADO/PCO
316 targets. An important goal of future research will be to investigate the use of these components
317 to enhance tolerance to hypoxia for both medical and agricultural interventions.

318

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324

325 **Glossary of abbreviations:**

326

327	ACBP	Acyl-CoA-binding domain-containing protein
328	ADO	cysteamine (2-aminoethanethiol) dioxygenase
329	ATE	ARGINYL TRANSFERASE
330	bHLH	basic Helix Loop Helix DNA binding domain
331	CKD	Chronic Kidney Disease
332	EPAS	Endothelial PAS domain-containing protein

333	EPO	Erythropoietin
334	ERFVII	Group VII ETHYLENE RESPONSE FACTOR
335	FIH	Factor Inhibiting HIF1 α
336	HIF	Hypoxia Inducible Factor
337	HRE	Hypoxia Response Element
338	HRPE	Hypoxia Responsive Promoter Element
339	IKK β	Inhibitor of nuclear factor kappa-B kinase subunit beta
340	KDM	JmjC (Jumonji C) domain lysine demethylase
341	NO	Nitric oxide
342	Nt-	Amino terminus of the protein
343	NTAD/CTAD	N- and C-terminal transactivation domains of HIF
344	OTUB1	Ovarian tumor domain containing ubiquitin aldehyde binding protein 1
345	PAS	Per-Arnt-Sim domain
346	PCO	PLANT (Nt-)CYSTEINE OXIDASE
347	PHD(EGLN)	prolyl 4-hydroxylases/ Egl nine homolog
348	PHI	PHD inhibitor molecule
349	PRC2	Polycomb Repressive Complex 2
350	PRT6	PROTEOLYSIS6 E3 ligase
351	pVHL	von Hippel-Lindau protein E3 ligase
352	RGS	REGULATOR OF G PROTEIN SIGNALLING
353	SUB1A	SUMERGENCE1A
354	UBR	Ubiquitin protein ligase E3 component N-recognin
355	VRN2	VERNALIZATION2
356	ZPR2	LITTLE ZIPPER2

357
358

359

360 **Figure 1:**

361

362 **Evolutionary history of core components of the HIF and PCO/ADO oxygen-sensing**
363 **pathways.**

364 Ages of key evolutionary events, and predicted O₂ levels at distinct ages of earth history are
365 indicated (Billion years ago; Ga). GOE, Great Oxidation Event, first appearance of significant
366 atmospheric O₂ levels. Possible times of appearance of oxygen-sensing pathway components
367 (ovals with gene name indicated) are shown based on presence of similar protein sequences
368 or functional testing in extant taxonomic groups, and important functional diversification
369 indicated. Animal-specific components are in greys, plant-specific in greens.

370

371 **Figure 2:**

372 **A comparison of major oxygen-sensing systems in metazoans and flowering plants.**

373 Mammalian HIF α and plant ERFVII transcription factors are stable under hypoxia where they
374 drive hypoxic gene expression through binding to genes bearing specific promoter elements
375 (HRE, HRPE). In oxygenated environments, prolyl residues in HIF α are hydroxylated by 2-
376 OG dependent PHD dioxygenases prior to ubiquitylation (Ub) by the pVHL E3 ubiquitin ligase,
377 whilst the N-terminal Cys of ERFVII is converted to Cys-sulfinic acid by 2-OG-independent

378 PCO dioxygenases, prior to ATE-mediated arginylation that permits recognition by the PRT6
379 E3 ubiquitin ligase. ZPR2 stability is also regulated via PCO in plants to control shoot
380 meristem function. The recently discovered ADO pathway in mammals is equivalent to the
381 PCO pathway in plants and regulates the stability of non-nuclear RSG and IL-32 substrates
382 that do not directly modulate gene expression. Mammals and angiosperms have contrasting
383 oxygen-regulated mechanisms controlling histone modifications. In humans, KDM
384 dioxygenases demethylate histones in high O₂, but are inhibited under hypoxia; KDMs are
385 also found in plants, but their oxygen-sensitivity is yet to be established. In plants, stability of
386 the VRN2 subunit of PRC2, a major histone methylating complex, is regulated via PCOs
387 similarly to ERFVIIIs. Acronyms and protein names are defined in the main text and glossary.
388 Hatched blue box highlights the conserved N-degron-based O₂ sensing pathways in mammals
389 and plants.

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391 **References**

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