

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

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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 [1]) (Figure 1). Subsequently as part of the evolution of endosymbiosis with an ancient
26 cyanobacterial group before 1 Ga early eukaryotic algae gained the ability to photosynthesise,
27 leading to further increases in O_2 levels that peaked at over 30% during the Carboniferous
28 (~360 to 300 Ma). Endosymbiosis with purple non-sulphur bacteria (that became
29 mitochondria), that predated chloroplast endosymbiosis, may have allowed early eukaryotes
30 to tolerate O_2 and use the energy of mitochondrial aerobic respiration to become multicellular.
31 Various hypotheses have been advanced that increased O_2 levels were either highly
32 poisonous and catastrophic for early anaerobic eukaryotes or that these organisms were
33 already pre-adapted to deal with reactive oxygen species that aided evolution of O_2 tolerance
34 (discussed in [2]).

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 [3, 4]. It is clear that for such an essential component of intracellular

38 biochemistry, sensing and response to changing O₂ levels must be an important feature of
39 multicellular eukaryotes. In this review we focus on biochemical pathways that evolved in
40 plants and animals to sense and respond to reduced O₂ levels (hypoxia). Analogous pathways
41 evolved in both lineages, that target nuclear-located processes for response. We highlight the
42 different evolutionary trajectories of pathways, the importance of dioxygenases as conserved
43 sensors of hypoxia, the physiological importance of oxygen-sensing and avenues for
44 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 [4]. The NTAD of HIF α contains conserved proly residues that are
55 hydroxylated using O₂ by proly 4-hydroxylases (PHD, also known as EGLN) [5, 6]. This
56 modification then permits binding of the E3 ubiquitin ligase von Hippel-Lindau protein (pVHL)
57 to initiate polyubiquitylation [7, 8], leading to HIF α degradation. Hypoxia limits PHD activity,
58 precluding pVHL binding, thus allowing association with HIF β and re-localisation to the
59 nucleus [4] (Figure 2). The CTAD-containing HIF α variants can also be hydroxylated on
60 asparaginy residues by Factor Inhibiting HIF1 α (FIH), which limits HIF α association with
61 transcriptional co-factors [9]. This separate O₂-triggered modification also therefore
62 contributes to inhibition of HIF activity through a parallel hydroxylation-dependent but non-
63 proteasomal route.

64 In flowering plants, the group VII ETHYLENE RESPONSE FACTOR transcription
65 factors (ERFVII) control anaerobic gene expression under hypoxia [10]. Following co-
66 translational Methionine excision, in high O₂ levels the N-terminal Cysteine of ERFVII is
67 converted to Cys-sulfinic acid by PLANT CYSTEINE OXIDASEs (PCOs), which leads to
68 amino-terminal (Nt)-arginylation by ATE [11, 12]. Nt-Arg-ERFVII are then targeted for
69 proteasomal degradation by the E3 ligase PROTEOLYSIS (PRT)6 [13, 14]. This pathway also
70 requires nitric oxide (NO) [15]. Thus, similarly to HIF α regulation, coupling protein turnover to
71 O₂ availability results in ERFVII stabilisation under hypoxia (Figure 2). Recently, a mammalian
72 protein with high similarity to PCO, cysteamine (2-aminoethanethiol) dioxygenase (ADO), was
73 characterised and shown to control O₂-dependent turnover of non-nuclear REGULATOR OF
74 G PROTEIN SIGNALLING (RGS) 4,5,16 proteins via the mammalian Arg/N-degron pathway

75 [16]. This highlights an alternative mechanism for O₂-sensitive proteolysis in mammals,
76 equivalent to the predominant system in plants.

77 There is evidence that alternative pathways can also target HIF α and ERFVIs for
78 degradation, revealing additional proteolytic mechanisms for fine-tuning their stability [4, 17-
79 20]. Furthermore, animal PHD and plant PCO enzymes also have non-HIF and -ERFVII
80 targets, respectively. In *Arabidopsis thaliana*, the PCO targets LITTLE ZIPPER (ZPR)2 and
81 Polycomb Repressive Complex (PRC)2 component VERNALIZATION (VRN)2 are subject to
82 ubiquitin-mediated degradation [21, 22], whereas hydroxylation of candidate non-canonical
83 PHD/FIH substrates, such as IKK β , p53, and OTUB1, can have different effects on protein
84 activity and interactions [23].

85

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

87 The enzymes catalysing both prolyl-/asparaginyl-hydroxylation (PHD, FIH) and Nt-
88 cysteine oxidation (PCO, ADO) belong to the non-heme iron(Fe²⁺)-dependent dioxygenase
89 family, so called because their catalytic sites contain a redox active iron directly coordinated
90 to the protein, and incorporate both atoms from O₂ into substrates [24]. PHDs function as
91 physiological O₂ sensors due to their high K_mO₂ values, which for the dominant PHD2 isoform
92 (dependent on the length of peptide studied) has variably been reported from less than 100 μ M
93 to 1700 μ M, much higher than *in vivo* O₂ concentrations [25, 26]. In contrast, FIH has a higher
94 affinity for O₂ than PHDs, indicating that greater decreases in O₂ availability would be required
95 before its activity is inhibited [27]. PHD/FIH incorporate one oxygen atom into the target HIF α
96 prolyl or asparaginyl residue, whilst the second decarboxylates 2-oxo-glutarate (2-OG) to
97 produce CO₂ and succinate [23, 28] (Figure 2). PCOs and ADO also have high K_mO₂ values
98 above typical plant and animal tissue O₂ concentrations, but in contrast to PHDs they are not
99 2-OG dependent, they integrate both atoms directly into Nt-Cys to generate Cys-sulfinic acid
100 [12, 16, 29].

101 Metazoans encode multiple PHD isoforms, which are differentially expressed and have
102 varying subcellular localisations, although the main mammalian PHD2 variant is cytosolic and
103 constitutively expressed [4, 30]. Flowering plant PCOs have different sensitivities to O₂ and
104 pH, and divergent substrate preferences based on assessment of their activities on peptide
105 sequences [29]. Of the five PCOs in *A. thaliana*, PCO4 is the most catalytically potent
106 suggesting that it may be the dominant variant. Apparently without an active oxygen-transport
107 system, strong gradients of hypoxia exist in plant tissues (obvious examples include tubers
108 and seeds) [3, 31] and it may be that PCOs with different affinities for O₂ operate in different
109 tissues/at different developmental time points. Interestingly, a subset of these oxygen-sensing
110 enzymes in animals and plants are transcriptionally induced by low- O₂ levels, suggesting that

111 homeostatic mechanisms for dampening the hypoxic response have evolved in both kingdoms
112 [4, 11].

113 In addition to PHD and PCO/ADO proteins, there are many other non-heme iron(Fe^{2+})-
114 dependent dioxygenases in animals and plants [24, 32], although several of these, including
115 collagen prolyl hydroxylases and certain JmjC (Jumonji C) domain lysine demethylases
116 (KDMs), are unlikely to sense physiological changes due to their high O_2 affinities [23, 33].
117 Nonetheless, it was recently shown that some histone-specific KDMs (KDM5A and 6A) do
118 have $K_m\text{O}_2$ values in the requisite range for sensing intracellular O_2 , and are able to directly
119 modulate the methylation status of chromatin dependent on O_2 availability [33, 34] (Figure 2).
120 Under hypoxic conditions, KDM activity is reduced, resulting in enhanced global levels of
121 histone methylation, regulating gene expression and cell fate. The activity of a separate non-
122 histone KDM (KDM3A), which is involved in the demethylation of the transcriptional co-
123 activator PGC-1 α , also connects O_2 availability to the regulation of genes linked to
124 mitochondrial biogenesis [35], suggesting others await discovery.

125

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

127 Components of the Arg/N-degron pathway are conserved in eukaryotes, though
128 distinct evolutionary trajectories are observed. Whereas ATE activity is highly conserved
129 across all major groups, E3 ligase functions for recognising distinct destabilising residues
130 (carried out by UBR-type proteins in non-plants) were split early in plant evolution [36] (Figure
131 1). ERFVIIs are not present in the genome of basal land-plants *Physcomitrella patens* or
132 *Marchantia polymorpha*, and VRN2 and ZPR2 appeared with angiosperms [21, 22]. As the
133 nature of Nt-Cys oxidation was for several years obscure, a major advance was the
134 identification of the PCOs in *A. thaliana* [11]. This showed that Nt-Cys oxidation required PCO
135 enzyme activity, and genetic removal of *PCO* function leads to ERFVII stabilisation and
136 enhanced hypoxia tolerance. The identification of ADO indicates that oxygen-sensing via this
137 pathway is ancient, predating the split between animal and plant groups (>1 Ga) [16] (Figure
138 1), and may indicate that a major mechanism of oxygen-sensing in early eukaryotes was
139 through cysteine dioxygenase control of Nt-Cys oxidation, during periods of earth history with
140 comparatively low O_2 levels. Alternatively, it may suggest that originally the major function of
141 the pathway was NO sensing, and became coupled to O_2 as atmospheric levels rose. PCO-
142 type Nt-cysteine dioxygenases have not been found in fungi, that diverged from animals after
143 plants, indicating loss of the capacity of this group to oxidise Nt-Cys and use this pathway for
144 oxygen-sensing [16]. Genes encoding Met-Cys initiating RGS proteins are only present in
145 vertebrate genomes, and those encoding IL-32 only in mammalian lineages (Figure 1).

146 Although the PCO/ADO branch of the N-degron pathways is ancient in eukaryotes, the
147 HIF pathway is only present in metazoan animals (choanoflagellates, closest extant relatives

148 to animals, do not contain bHLH-PAS domain proteins, [37]) (Figure 1). A functioning HIF
149 system was identified in the placozoan *Trichoplax adhaerens*, representing one of the simplest
150 multicellular animals [38]. A recent analysis of representatives of basal metazoa groups
151 porifera (sponges) and ctenophores failed to identify pVHL or PHD-like proteins, or hypoxia-
152 regulated gene expression [37]. One feature of the evolution of the HIF system appears to be
153 increased diversification of components in derived evolutionary groups. Whereas *T.*
154 *adhaerens* contains single proteins for each component of the pathway mammals contain
155 multiple variants of HIF α and PHD [38]. The appearance and diversification of a functional HIF
156 pathway, that correlates with large increases in atmospheric and oceanic O₂, may have
157 influenced the concomitant explosion of animal diversity and size beginning around the
158 Cambrian period (~540 Ma) (Figure 1).

159

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

161 Key observations related to major consequences of oxygen-sensing have been the
162 identification of nuclear changes in response to hypoxia. In both plants and animals these
163 converge on transcription of hypoxia-related genes and chromatin structure. In plants an
164 evolutionarily-conserved core set of hypoxia-related genes are activated by ERFVILs in
165 response to hypoxia-induced stabilisation, through a conserved Hypoxia Responsive
166 Promoter Element (HRPE) [10]. Similarly, animal Hypoxia Response Elements (HREs) are
167 bound by HIF factors to enhance low O₂ responsiveness [39]. Low O₂ levels also influence
168 chromatin structure, through the stabilisation of components of chromatin modifying
169 complexes (VRN2 as part of PRC2 [21]), via enhanced expression of chromatin modifiers
170 (gene activation by HIF [40]), or directly through repression of histone H3 demethylation
171 activity of KDMs [33, 34]. In both animal and plant responses, genes encoding biochemical
172 pathways associated with enhanced tolerance of hypoxia are important targets (including
173 fermentative metabolism, glycolysis and an inhibition of mitochondrial oxidative
174 phosphorylation), and the control of pathways with oxygen-requiring reactions or that occur in
175 hypoxic niches are also important [22, 41, 42]. Two animal cytoplasmic substrates of ADO
176 have been identified, RGS4,5,16 and INTERLEUKIN (IL)-32 [16, 43], that gives the possibility
177 of more rapid response to declining O₂ than transcriptional circuits, since their immediate
178 stabilisation would trigger a change more quickly than responses dependent on increased
179 protein production through HIF control of gene expression. Both IL-32 and RGS4/5 are
180 transcriptional targets of HIF, indicating a possible interaction between the two sensing
181 systems [16]. Moving forward it will be important to decipher the comparative timescales
182 through which PHD/FIH, KDM and ADO activity leads to cellular changes, as this likely
183 contributes to physiologically relevant fine tuning of the overall hypoxia response.

184 Analyses of physiological functions reveal the broad reach of oxygen-sensing systems,
185 and specific roles are related to the different lifestyles of plants and animals. As plants are
186 sessile a key function of oxygen-sensing is related to perception of waterlogging and flooding
187 [13, 14]. Both stabilised ERFVIs and VRN2 enhance survival of hypoxia [13, 14, 21]. It was
188 recently shown that the plant Cys-initiating substrate ZPR2 is stabilised by the hypoxic
189 environment of the shoot apical meristem, regulating the production of new leaves [22], and
190 VRN2 also accumulates in hypoxic meristems, where it modulates flowering time and root
191 development [44]. In addition, hypoxia-enhanced stability of ERFVIs was shown to repress
192 chlorophyll synthesis (an O₂-requiring pathway) in dark grown seedlings [41], as well as lateral
193 root development [45].

194 The HIF pathway plays major roles in O₂-homeostasis, including erythropoiesis
195 (development of red blood cells) and angiogenesis (development of new blood vessels)
196 (reviewed in [46]). Similar to ZPR2/VRN2 in plant meristems, HIF1 α is stabilised within hypoxic
197 hematopoietic stem cells (that give rise to blood cells) [42]. Stabilised HIF1/2 α enhance
198 expression of growth regulators (erythropoietin (EPO) and angiogenic growth factors) and
199 associated components (for example systems for iron uptake and utilisation [46]). An
200 important role of the HIF system is in adaptation of animals to high altitude, where the partial
201 pressure of O₂ is reduced. Genome wide association studies identified allele signatures in
202 human populations associated with life at high altitudes in the Tibetan Plateau (average
203 altitude 4000 m, *p*O₂ 13 kPa) for both HIF2 α and PHD2. For example, in modern Tibetan
204 populations a variant of *EGLN1/PHD2* (Asp4Glu; Cys127Ser) was shown to have a lower
205 *K_mO₂* suggesting that it promotes increased degradation of HIF at high altitude (lower *p*O₂)
206 thus reducing HIF levels to those equivalent to low altitudes [47]. Interestingly one allele of
207 *EPAS1/HIF2A* enriched in Tibetan populations appears to have been derived from ancient
208 hominid Denisovans [48]. Many studies demonstrate wider roles for the HIF system, indicating
209 that oxygen-sensing by this pathway influences many aspects of cellular biochemistry, growth
210 and development (discussed in [49]).

211 Since the PCO/ADO pathway also acts as an NO sensor [15, 43], the stability of both
212 animal and plant substrates also regulates responses to intracellular NO levels that
213 accompany internal and external stress. For example, destruction of RGS proteins to induce
214 cardiomyocyte proliferation can also be induced by endothelium-derived NO [50]. Stabilisation
215 of ERFVIs by reduced NO enhances hypoxia tolerance and tolerance to other abiotic stresses
216 (including high salinity) [51, 52]. It is still unclear exactly where NO acts within the pathway.
217 Although an in vitro reconstituted mammalian system was shown to be NO dependent [43], in
218 vitro activity of PCO/ADO on peptides does not require NO [16, 29]. It is possible therefore
219 that NO influences the activities of enzyme components of the pathway in vivo (ATE,
220 PCO/ADO or UBR1/PRT6), and it was shown that PRT6 contains an NO binding domain [53].

221 Factors other than hypoxia can influence oxygen-sensing pathways. A sub-pool of
222 ERFVIIIs is stable and sequestered at the plasma membrane through association with ACYL
223 CoA BINDING PROTEINS (ACBP) during normoxia [14, 54]. Zinc excess in the soil
224 (detrimental to plant growth), inhibits PCO enzymes thus causing stabilisation of ERFVIIIs [55].
225 Non-canonical mechanisms also control HIF stability; for example, increases in succinate
226 during the progression of certain types of cancer can allosterically inhibit PHD activity to trigger
227 HIF accumulation under normoxia [56, 57]. The possible mechanisms influencing O₂-
228 responsive factors, and therefore the breadth of possible affected physiological processes will
229 be much wider than those specifically related to O₂ or NO.

230

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

232 Oxygen-sensing pathways represent key cellular targets for counteracting diseases and
233 enhancing stress resilience. HIF signalling controls a range of cellular responses, and also
234 drives tumorigenesis and the maintenance of tumour microenvironment in certain cancers
235 [58]. Thus, interventions that impact the HIF pathway have the capacity to treat pathologies
236 associated with these processes. *EPO*, a target of HIF, is down-regulated in patients with
237 chronic kidney disease (CKD) due to reduced O₂ consumption [59]. Several PHD inhibitor
238 molecules (PHIs) have been developed that stimulate increased EPO production in CKD
239 patients to counteract renal anaemia [60], acting as 2-OG mimetics or iron-chelators to inhibit
240 enzymatic activity and increase HIF stability in normoxia [59]. Chemicals that disrupt other
241 aspects of HIF signalling have also been identified as potent repressors of cancer progression
242 [61]. For example, cancers in patients with VHL disease result from ectopic accumulation of
243 HIF2 α [58], and a novel drug that specifically disrupts the HIF2 α /HIF2 β dimer to downregulate
244 HIF2 signalling was recently shown to limit tumour progression [62]. The development of
245 inhibitory molecules that target discrete HIF or PHD isoforms, as well as other regulatory
246 points in the HIF signalling pathway, will help to increase therapeutic specificity and efficacy
247 of such treatments.

248 Genetic manipulation of O₂-signalling components in crop species can increase resistance
249 to waterlogging-induced hypoxia, as shown in barley through genetic reduction in *HvPRT6*
250 expression/activity [63], whilst ERFVIIIs provide increased tolerance to multiple abiotic
251 stresses [52] and biotic stresses where pathogen-associated hypoxia is an integral factor [64-
252 66]. In rice (*Oryza sativa*), the ERFVII SUB1A-1 is a major regulator of submergence tolerance
253 that has been bred into high yielding varieties [67]. SUB1A-1 is naturally uncoupled from O₂-
254 dependent degradation despite containing Cys2 and downstream Lys residues [13, 68]
255 suggesting that the plant oxygen-sensing system has been targeted by natural selection for
256 adaptation in wetland environments, and that biotechnological approaches could be used to
257 achieve similar outcomes in flooding-susceptible crops.

258

259

260 **Conclusions and unresolved questions**

261 Where to look for undiscovered oxygen-sensors? Based on structures and domains of
262 already identified proteins there are clear candidates to test as novel components of oxygen-
263 sensing pathways. Plant and animal genomes contain Jumonji C domain-containing KDMs in
264 addition to those already shown to act as oxygen-sensors. Determining those with a
265 physiologically relevant (high) K_mO_2 would be a first step in defining potential roles as sensors.
266 Although plants do not contain HIF α -like sequences, both plants and animals contain
267 hundreds of proteins initiating Met-Cys, that could be substrates of PCO/ADO action, in
268 addition to endopeptidase substrates cleaved to reveal Nt-Cys. Cys2 is evolutionarily
269 constrained in most eukaryote proteomes [69] suggesting that this is an important determinant
270 related to O₂/NO-sensing. In addition, recently it was hypothesised that mechanisms other
271 than PCO-regulated destabilisation may act to promote oxygen-sensing in plants, in several
272 cases backed-up by experimental data [70].

273 Why is N-degron mediated oxygen-sensing not the primary system in metazoans as it is
274 in angiosperms? The HIF system evolved only in one lineage of animals, whereas the
275 PCO/ADO pathway evolved early in eukaryotes (Figure 1). Perhaps the unavoidable link of
276 the PCO/ADO pathway to a requirement for NO made this pathway unsuitable, or possibly it
277 was not suitable for large mobile organisms. Lack of transcriptional response to hypoxia in the
278 marine sponge *Tethya wilhelma* indicates that the PCO/ADO pathway does not perform this
279 function in basal animals, though complete anoxia did result in large changes in gene
280 expression [37]. It is unclear what advantage the coupling of NO- and oxygen-sensing in this
281 pathway has; it may be a remnant of evolutionary drivers early in eukaryote history, where O₂
282 levels were low, which might also suggest early Nt-cysteine dioxygenases had high affinities
283 for O₂, making the pathway primarily important for responding to changes in levels of
284 intracellular NO.

285 There are several striking commonalities in the major oxygen-sensing systems of
286 angiosperms and metazoans. Both require dioxygenases with O₂-sensitivity within a
287 physiological range, both directly target nuclear-factors for UPS-mediated destruction, and
288 both result in large changes in gene expression with downstream physiological consequences
289 providing homeostatic control of O₂ response. An important goal of future research will be to
290 define the links between O₂ affinity of pathway dioxygenases and their expression patterns,
291 allowing an understanding of how these enzymes sense all physiologically possible internal
292 O₂ tensions. The complete gamut of influenced processes and interactions is yet to be
293 resolved, at the intracellular level there are clearly similarities of interactions between oxygen-
294 sensing pathways and mitochondrial function (key for oxidative phosphorylation), well

295 understood for the HIF system, but requiring more understanding for the PCO/ADO pathway
296 in animals and plants. It is likely that many components of known oxygen-sensing pathways
297 remain to be discovered, including dioxygenases with novel activities, and PHD/ADO/PCO
298 targets. An important goal of future research will be to investigate the use of these components
299 to enhance tolerance to hypoxia for both medical and agricultural interventions.

300

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306

307 **Glossary of abbreviations:**

308

309	ACBP	Acyl-CoA-binding domain-containing protein
310	ADO	cysteamine (2-aminoethanethiol) dioxygenase
311	ATE	ARGINYL TRANSFERASE
312	bHLH	basic Helix Loop Helix DNA binding domain
313	CKD	Chronic Kidney Disease
314	EPAS	Endothelial PAS domain-containing protein
315	EPO	Erythropoietin
316	ERFVII	Group VII ETHYLENE RESPONSE FACTOR
317	FIH	Factor Inhibiting HIF1 α
318	HIF	Hypoxia Inducible Factor
319	HRE	Hypoxia Response Element
320	HRPE	Hypoxia Responsive Promoter Element
321	IKK β	Inhibitor of nuclear factor kappa-B kinase subunit beta
322	KDM	JmjC (Jumonji C) domain lysine demethylase
323	NO	Nitric oxide
324	Nt-	Amino terminus of the protein
325	NTAD/CTAD	N- and C-terminal transactivation domains of HIF
326	OTUB1	Ovarian tumor domain containing ubiquitin aldehyde binding protein 1
327	PAS	Per-Arnt-Sim domain
328	PCO	PLANT (Nt-)CYSTEINE OXIDASE
329	PHD(EGLN)	proly 4-hydroxylases/ Egl nine homolog
330	PHI	PHD inhibitor molecule
331	PRC2	Polycomb Repressive Complex 2
332	PRT6	PROTEOLYSIS6 E3 ligase N-recognin
333	pVHL	von Hippel-Lindau protein E3 ligase
334	RGS	REGULATOR OF G PROTEIN SIGNALLING
335	SUB1A	SUMERGENCE1A
336	UBR	Ubiquitin protein ligase E3 component N-recognin
337	VRN2	VERNALIZATION2
338	ZPR2	LITTLE ZIPPER2

339

340

341

342 **Figure 1:**

343

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

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

352

353 **Figure 2:**

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

355 Mammalian HIF α and plant ERFVII transcription factors are stable under hypoxia where they
356 drive hypoxic gene expression through binding to genes bearing specific promoter elements
357 (HRE, HRPE). In oxygenated environments, prolyl residues in HIF α are hydroxylated by 2-
358 OG dependent PHD dioxygenases prior to ubiquitylation (Ub) by the pVHL E3 ubiquitin ligase,
359 whilst the N-terminal Cys of ERFVII is converted to Cys-sulfinic acid by 2-OG-independent
360 PCO dioxygenases, prior to ATE-mediated arginylation that permits recognition by the PRT6
361 E3 ubiquitin ligase. ZPR2 stability is also regulated via PCO in plants to control shoot
362 meristem function. The recently discovered ADO pathway in mammals is equivalent to the
363 PCO pathway in plants and regulates the stability of non-nuclear RSG and IL-32 substrates
364 that do not directly modulate gene expression. Mammals and angiosperms have contrasting
365 oxygen-regulated mechanisms controlling histone modifications. In humans, KDM
366 dioxygenases demethylate histones in high O₂, but are inhibited under hypoxia; KDMs are
367 also found in plants, but their oxygen-sensitivity is yet to be established. In plants, stability of
368 the VRN2 subunit of PRC2, a major histone methylating complex, is regulated via PCOs
369 similarly to ERFVII. Acronyms and proteins names are defined in the main text and glossary.
370 Hatched blue box highlights the conserved N-degron-based O₂ sensing pathways in mammals
371 and plants.

372

373 **References**

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