



# Biochemistry of the hypoxia-inducible factor hydroxylases

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## Abstract

The hypoxia-inducible factors are  $\alpha,\beta$ -heterodimeric transcription factors that mediate the chronic response to hypoxia in humans and other animals. Protein hydroxylases belonging to two different structural subfamilies of the Fe(II) and 2-oxoglutarate (2OG)-dependent oxygenase superfamily modify HIF $\alpha$ . HIF $\alpha$  prolyl-hydroxylation, as catalysed by the PHDs, regulates HIF $\alpha$  levels and, consequently,  $\alpha,\beta$ -HIF levels. HIF $\alpha$  asparaginyl-hydroxylation, as catalysed by factor inhibiting HIF (FIH), regulates the transcriptional activity of  $\alpha,\beta$ -HIF. The activities of the PHDs and FIH are regulated by O<sub>2</sub> availability, enabling them to act as hypoxia sensors. We provide an overview of the biochemistry of the HIF hydroxylases, discussing evidence that their kinetic and structural properties may be tuned to their roles in the HIF system. Avenues for future research and therapeutic modulation are discussed.

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## Keywords

Hypoxia-inducible factor (HIF), HIF prolyl-hydroxylases (PHDs/EGLNs), JmjC demethylases, Factor inhibiting HIF (FIH), Asparaginyl hydroxylase, Ankyrin repeat domain, Oxygen/hypoxia sensing, JmjC histone demethylases (JmjC KDMs), Epigenetics.

## Introduction

Aerobic life forms need to maintain a sufficient supply of dioxygen (O<sub>2</sub>) to their cells. To ensure this, different mechanisms for sensing and responding to limiting O<sub>2</sub>

availability, termed hypoxia, appear to have evolved [1]. Because of the central importance of O<sub>2</sub> in enabling cellular energy production by the mitochondrial electron transport chain, such mechanisms must be robust and capable of operating over different timescales and under different conditions. In the case of humans and many other animals, the latter include changes in the environment, both of the entire organism (e.g., movement to a different altitude) and within an organism (e.g., in different tissues/stresses). There remain considerable gaps in our knowledge of the biochemistry of the hypoxic responses in animals, e.g., how a response is achieved on a timescale of seconds and how robust and context dependent responses are achieved. Nonetheless, considerable progress has been made in understanding how animals sense hypoxia and induce a response at the transcriptional level. There is strong in vitro and physiological evidence that Fe(II) and 2-oxoglutarate (2OG)-dependent oxygenases (OGO) are important in hypoxia sensing in animals at the transcriptional level and beyond [2,3]. Here we review biochemical perspectives on the roles of the OGO that hydroxylate the  $\alpha$ -subunits of heterodimeric hypoxia-inducible factors ( $\alpha,\beta$ -HIFs).

## Hypoxia-inducible factors

Pioneering studies on the regulation of the erythropoietin gene (*EPO*) which is upregulated in hypoxia, led to the finding that it is regulated by the  $\alpha,\beta$ -HIFs [1,4]. Crucially, levels of HIF $\alpha$ , but not HIF $\beta$ , were observed to be regulated by O<sub>2</sub> availability [5]. In humans there are 3 HIF $\alpha$  isoforms, all of which, like HIF1 $\beta$ , contain a basic helix-loop-helix domain (bHLH) and a Per-Arnt-Sim domain (PAS), which enable DNA binding and dimerization, respectively [4–6]. HIF $\alpha$  proteins contain regions termed oxygen dependent degradation domains (ODDs) that are important in their O<sub>2</sub> mediated degradation (Figure 1a, S3.A). HIF1 $\alpha$  and HIF2 $\alpha$  have two such domains, that is the N-terminal and C-terminal ODDs, namely NODD and CODD (corresponding to residues 344-503:1 $\alpha$ -NODD, 558-574:1 $\alpha$ -CODD, 398-415:2 $\alpha$ -NODD and 523-541:2 $\alpha$ -CODD). HIF3 $\alpha$  only has a CODD type ODD (residues 484-585). HIF1 $\alpha$  and HIF2 $\alpha$  also contain two transcriptional activation domains, that is an N-terminal (N-TAD, residues 531-575:1 $\alpha$ -NTAD and 415-530:2 $\alpha$ -NTAD) that overlaps with the CODD domain, and a C-terminal (CAD,

residues 786-826:1 $\alpha$ -CAD and 823-866:2 $\alpha$ -CAD) transcriptional activation domain; the latter of which (CAD) is important in the hypoxic response, and which is lacking in HIF3 $\alpha$  [6]. Note that HIF3 $\alpha$  has an N-TAD domain (residues 454-506) (Fig S3.A).

Under hypoxic conditions HIF $\alpha$  levels rise, HIF $\alpha$  moves to the nucleus, dimerises with HIF $\beta$  and binds to hypoxic response elements (HRE) in chromatin, so promoting transcription of  $\alpha$ , $\beta$ -HIF target genes (Figure 1a) [4,5]. Exactly how the different forms and combinations of HIF $\alpha$  and  $\beta$  interact with each other and interface with the myriad of variables involved in the regulation of eukaryotic transcription in vivo is far from understood.

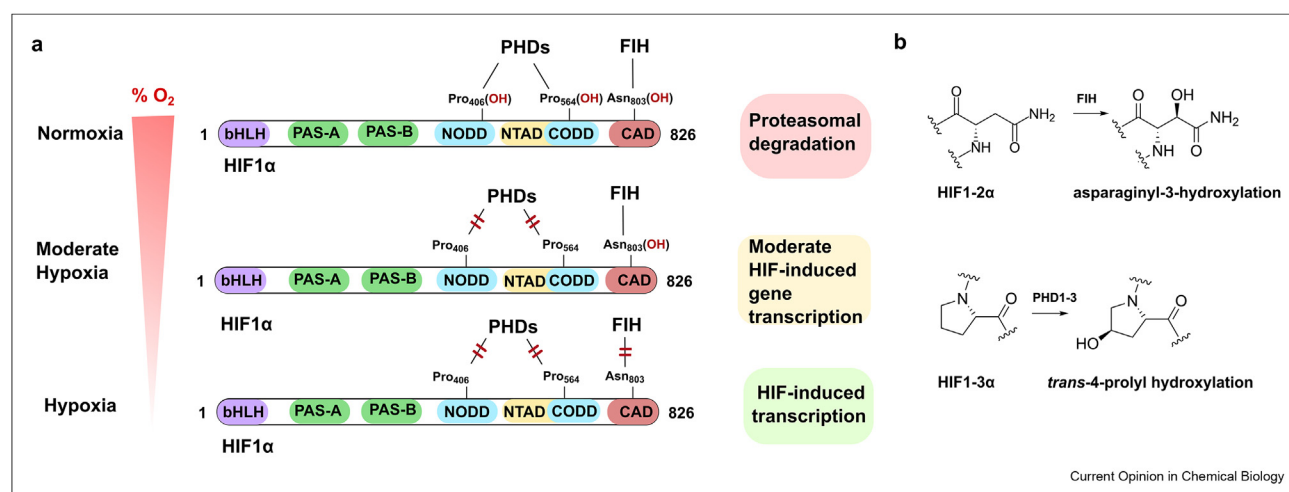
The available evidence is that HIF1 $\alpha$  and HIF2 $\alpha$  are more important in human biology than HIF3 $\alpha$ . Cellular studies have revealed the potential of the  $\alpha$ , $\beta$ -HIF heterodimer to upregulate the expression of multiple genes [2]. Further, HIF1 $\alpha$  and HIF2 $\alpha$  promote the expression of specific sets of HIF target genes to different extents. Notably HIF2 $\alpha$  selectively promotes *EPO* expression, whereas both HIF1 and 2 $\alpha$  can promote vascular endothelial growth factor gene (*VEGF*) expression [7,8]. A truncated form of HIF3 $\alpha$  (inhibitory PAS protein IPAS) is reported to dimerise with HIF1 $\alpha$  thus inhibiting expression promoted by HIF1 $\alpha$  [9]. More recent studies have shown some HIF3 $\alpha$  splice variants can promote expression of target genes, illustrating just one aspect of the complexity in the HIF system [10].

The complexities of the HIF system have not, however, precluded its manipulation for therapeutic benefit as evidenced by small-molecule drug mediated upregulation of HIF $\alpha$ /*EPO* levels for treatment of anaemia in chronic kidney disease (see below) and suppression of HIF2 $\alpha$  mediated expression for treatment of kidney cancer associated with mutation of the gene encoding for the von Hippel Lindau protein (pVHL, VHL1) [11], which is a key player in the O<sub>2</sub> enabled degradation of HIF $\alpha$  isoforms.

## O<sub>2</sub> mediated HIF $\alpha$ hydroxylation regulates HIF-dependent transcription

Following demonstration of a link between atmospheric O<sub>2</sub> and interactions of HIF $\alpha$  and pVHL [12], prolyl-residue hydroxylation in the ODDs was shown to signal for HIF $\alpha$  degradation [3,13,14]. O<sub>2</sub> dependent C4-prolyl-hydroxylation of conserved 'LXXLAP' motifs in HIF $\alpha$  isoforms was shown to strongly promote their binding to the pVHL-elonginB-elonginC (pVCB) complex, which catalyses their ubiquitination so targeting HIF $\alpha$  for proteasomal degradation (Figure 1a) [13,15]. From a chemical perspective it is notable that the preferred conformation of the proline pyrrolidine ring changes from C4-endo to C4-exo following C4-hydroxylation (Fig S1.A,B) [16]. The C4-endo conformation is observed in PHD2.HIF $\alpha$  substrate complex structures (Figure 3d, S1.A) [17,18], whilst the C4-exo conformation is observed in complexes of VCB with C4-hydroxylated HIF $\alpha$  targets (Fig S1.B) [19–21]. Recent studies have revealed differences in the binding strength of different HIF $\alpha$  ODDs to the pVCB complex,

Figure 1



**Schematic representation of key elements of the HIF mediated hypoxia sensing/response system.** (a) Prolyl-hydroxylation promotes HIF1-3 $\alpha$  degradation via the ubiquitin-proteasome pathway as catalysed by PHD1-3. HIF induced gene transcription is inhibited by asparaginyl-hydroxylation as catalysed by FIH, in both normoxia and moderate hypoxia. bHLH: basic helix-loop-helix domain, PAS: Per-Arnt-Sim domain, CODD: C-terminal oxygen-dependent degradation domain, NODD: N-terminal oxygen-dependent degradation domain, NTAD: N-terminal transactivation domain, CAD: C-terminal transactivation domain. (b) FIH catalysed asparaginyl-3-hydroxylation of HIF1-2 $\alpha$  isoforms and PHD catalysed *trans*-4-prolyl hydroxylation of HIF1-3 $\alpha$ .

highlighting just one interface for complexity in the HIF system [22]. Despite this and other complexities, it is striking that the addition of a single 16 Da oxygen atom can apparently signal for the efficient degradation of the macromolecular HIF $\alpha$  proteins, an observation that has subsequently been exploited in medicinal chemistry.

HIF $\alpha$  prolyl-hydroxylation is catalysed by a structurally conserved family of OGO, in humans PHD1,2,3 (or EGLN2,1,3) (Figure 1a,b) [2,3]. PHD inhibitors competing with 2OG have been developed for upregulation of EPO for treatment of anaemia (Fig. S2) [23]. Another OGO, namely factor inhibiting HIF (FIH), which, unlike the PHDs, is dimeric, catalyses asparaginyl-hydroxylation of the CADs of HIF1 $\alpha$  and HIF2 $\alpha$  [24]. By contrast with PHD catalysis which promotes a protein-protein interaction, C3-asparaginyl-hydroxylation of HIF $\alpha$  hinders the interaction between the  $\alpha$ , $\beta$ -HIF heterodimer and the transcriptional coactivators and histone acetyl transferases CBP/p300, so suppressing HIF mediated transcription (Figure 1a,b) [24,25]. This mechanism links the apparently relatively 'functionally simple' HIF $\alpha$  hydroxylations with the highly complex mechanisms of 'epigenetic' chromatin regulation involving histone modifications. The link between FIH and 'epigenetic' regulation is highlighted by the fact that FIH, but not the PHDs, belongs to the JmjC structural subfamily of OGO that was subsequently shown to also include the N<sup>e</sup>-methyl lysine (and likely arginine) residue histone demethylases (JmjC KDMs) [26,27].

The genes encoding for PHD3 (in particular) and PHD2, but likely not PHD1 or FIH are themselves HIF regulated [28–31] along with some, but not all, JmjC KDMs, suggesting a mechanism for OGO mediated feedback regulation [32,33]. In this regard, KDM3A (JMJD1A) is of particular interest as has been reported to be involved in regulation of some HIF target genes [34].

### HIF prolyl-hydroxylases (PHDs)

There are ~60–70 human OGO, of which in addition to the PHDs, there are 3 other types of assigned prolyl-hydroxylases, i.e., the procollagen C4 and C3 prolyl hydroxylases and the C3 prolyl hydroxylase OGFO (Figure 2, Table S1). Of the human prolyl hydroxylases, the C-terminally located PHD catalytic domains appear most similar to the  $\alpha$ -subunit of procollagen C4-prolyl-hydroxylase, the human version of which is an  $\alpha_2\beta_2$  heterotetramer, with its  $\beta$ -subunit being identical in sequence to protein disulfide isomerase [35]. The catalytic domains of the human PHDs likely have similar overall folds, but they have distinctive features, including in active site bordering regions which are important in substrate selectivity (Figure 4, Fig S3.C). PHD1-3 Differ most notably in their N-terminal

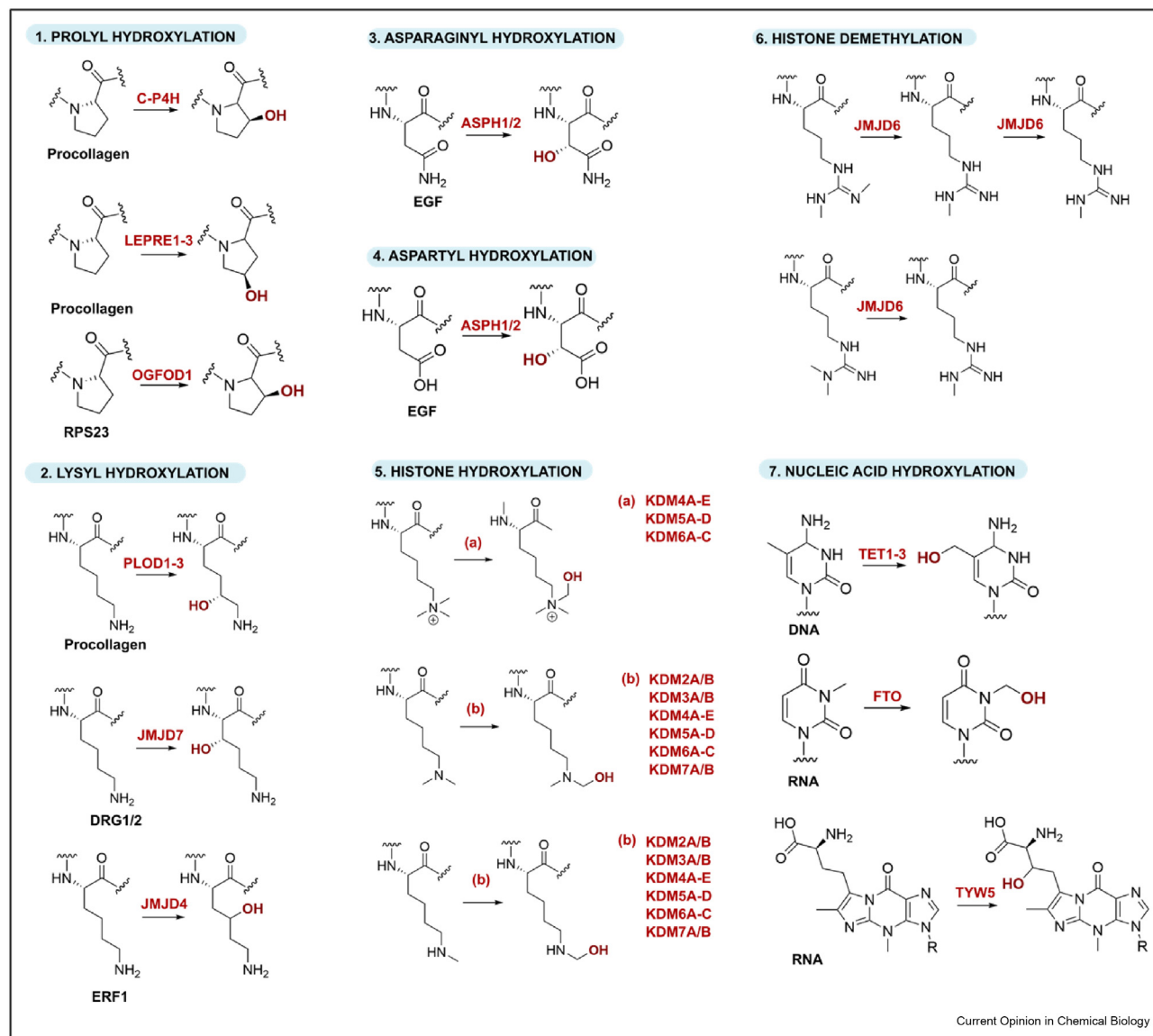
regions; PHD3 (293 residues) has a relatively short N-terminal region compared to PHD1-2 (both ~400 residues) (Fig S3.B,C) [36]. The N-terminal regions of PHD1 and 2 are not well conserved, with the latter containing a conserved zinc-finger domain of the myeloid nervy DEAF-1 (MYND) type, which has been reported to bind to 'PXLG' motifs [37]. Such motifs are present in the ribosomal chaperone nascent polypeptide complex- $\alpha$  (NACA) and its interaction with PHD2 is proposed to recruit the latter to the translational machinery in order to co-translationally hydroxylate HIF $\alpha$  [38].

PHD2, with its characteristic and functionally relevant MYND finger, is likely conserved in all animals, including the simple animal *Trichoplax adhaerens*; PHD1/3 may have evolved from PHD2 during genome duplications in animal evolution [39]. Bioinformatic studies imply that at least one form of HIF $\alpha$  and pVHL are present in all animals [39]. By contrast FIH appears to be present in all metazoans but is sporadically present in earlier eukaryotic organisms. PHD-related OGO are present in cellular slime mould *Dictyostelium discoideum* and in the proteozoan parasite *Toxoplasma gondii* where they hydroxylate S-phase kinase-associated protein 1 (Skp1) [40]. Notably, PHD-like OGO are found in bacteria such as *Pseudomonas aeruginosa*, *Vibrio cholera* and *Bacillus Anthracis* where they catalyse C4-prolyl-hydroxylation of the elongation factor thermally unstable (EF-Tu) [41–43]. The identification of these bacterial PHD-like OGOs raises the possibility that the HIF system may have its origin in modifications to the prokaryotic translation machinery.

### The substrate selectivities of the PHDs and FIH are different

The three human PHDs are proposed to have, at least partially, non-redundant roles [44,45]. Consistent with this, the expression levels of PHD1-3 are tissue dependent, with PHD2 typically being most abundant. PHD1 is the only isoform reported to be expressed in the testes and studies conducted on murine tissue and cultured cells show PHD3 to be highly expressed in the heart [46,47]. Although the experiments are challenging, including because of the tissue (and environment) varying expression levels of the PHDs and HIF isoforms, there is evidence that the selectivity of the PHD1-3 towards HIF $\alpha$  isoforms can vary. Such information is important in defining the specific roles of individual PHDs and may help to rationalise the effects of clinically relevant mutations to HIF $\alpha$  and the PHDs. In some contexts, PHD2 has more influence on HIF1 $\alpha$ -mediated expression compared to HIF2 $\alpha$  mediated expression, whilst PHD3 has more influence on HIF2 $\alpha$  than HIF1 $\alpha$  mediated expression [44,48]. Consistent with cellular results [3,49,50], studies with isolated enzymes have shown that PHD1-2 can hydroxylate

Figure 2



**Selected reactions catalysed by human 2OG oxygenases.** Reactions are coupled with 2OG decarboxylation to give  $\text{CO}_2$  and succinate. Enzymes are in red. Abbreviations: C-P4H, procollagen C-4 prolyl hydroxylase; LEPRE, procollagen prolyl 3-hydroxylase; OGFD1, 2-oxoglutarate- and iron-dependent oxygenase domain containing 1; RPS23, ribosomal protein S23; PLOD, procollagen C-5 lysyl hydroxylase; JMJD, jumonji C domain containing protein; DRG, developmentally regulated GTP-binding protein; ERF, eukaryotic release factor; ASPH, aspartyl/asparaginyl hydroxylase; EGF, epidermal growth factor; KDM, N<sup>ε</sup>-methyl-lysine demethylase; TET, ten-eleven translocation; FTO, fat-, mass-, and obesity-associated protein; TYW5, tRNA-wybutosine synthesizing enzyme 5. See Table S1 for more details.

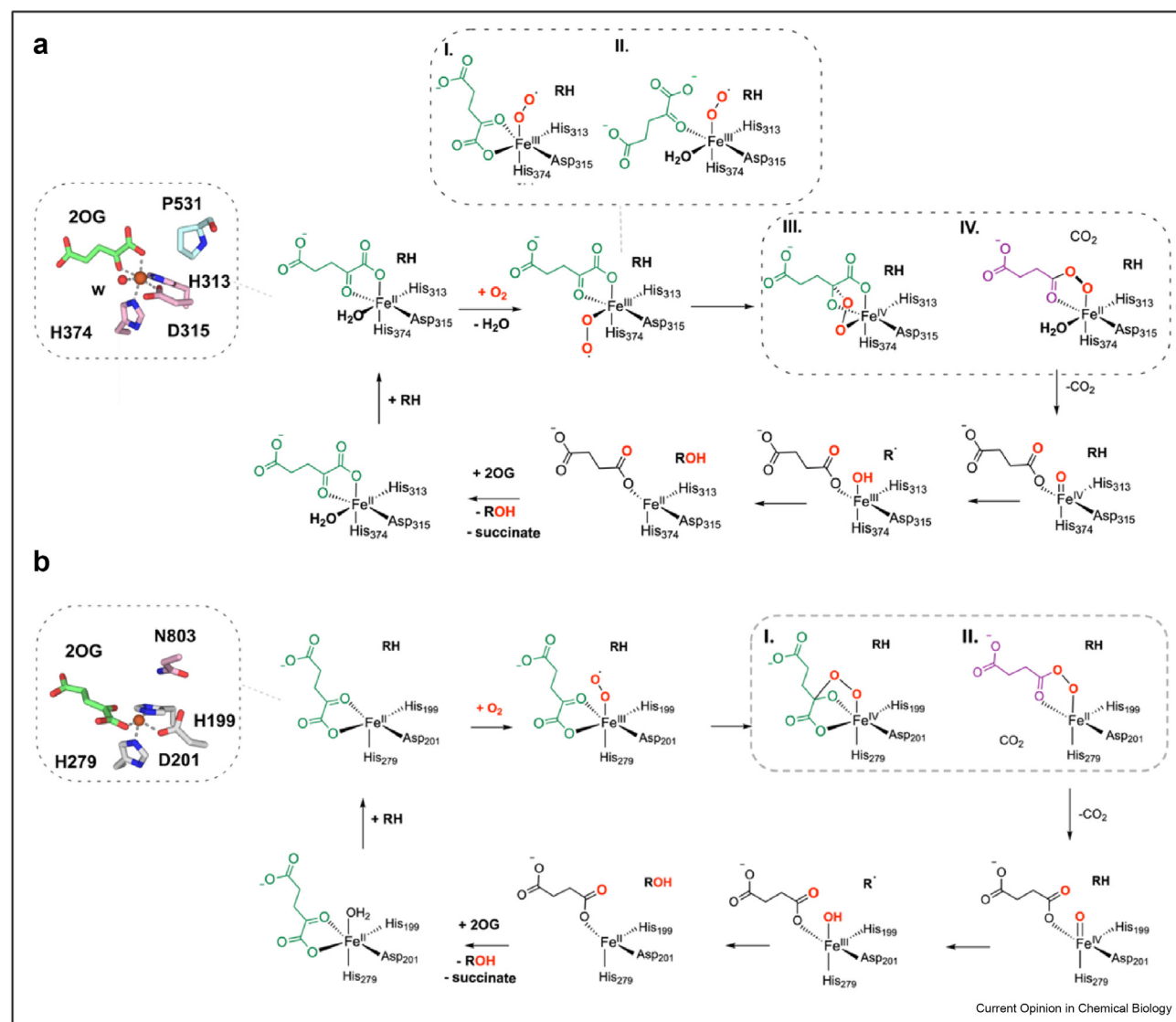
HIF1-3 $\alpha$ -CDD and HIF1-2 $\alpha$ -NODD, whilst PHD3 hydroxylates HIF1-3 $\alpha$ -CDD, HIF2 $\alpha$ -NODD, but does not hydroxylate HIF1 $\alpha$ -NODD, at least efficiently [3,49,50] (PHD3 hydroxylates HIF1 $\alpha$ -NODD at low levels in cells when Pro564<sup>HIF1 $\alpha$ -CDD</sup> is mutated to a glycine, supporting the preference of PHD3 for the CDD over the NODD domain [51]). PHD2 and PHD3 show higher activity with HIF1 $\alpha$ -CDD when compared with PHD1 both with isolated enzyme and

cellular studies [52–54]. As yet, how these differences in selectivity relate to healthy physiology is unclear, though erythrocytosis-associated and breast cancer PHD2 variants [55–57] are reported to manifest altered isoform selectivity towards different HIF hydroxylation sites [58].

Following the identification of the PHDs as HIF $\alpha$  prolyl-hydroxylases, a question arising was whether they



Figure 3

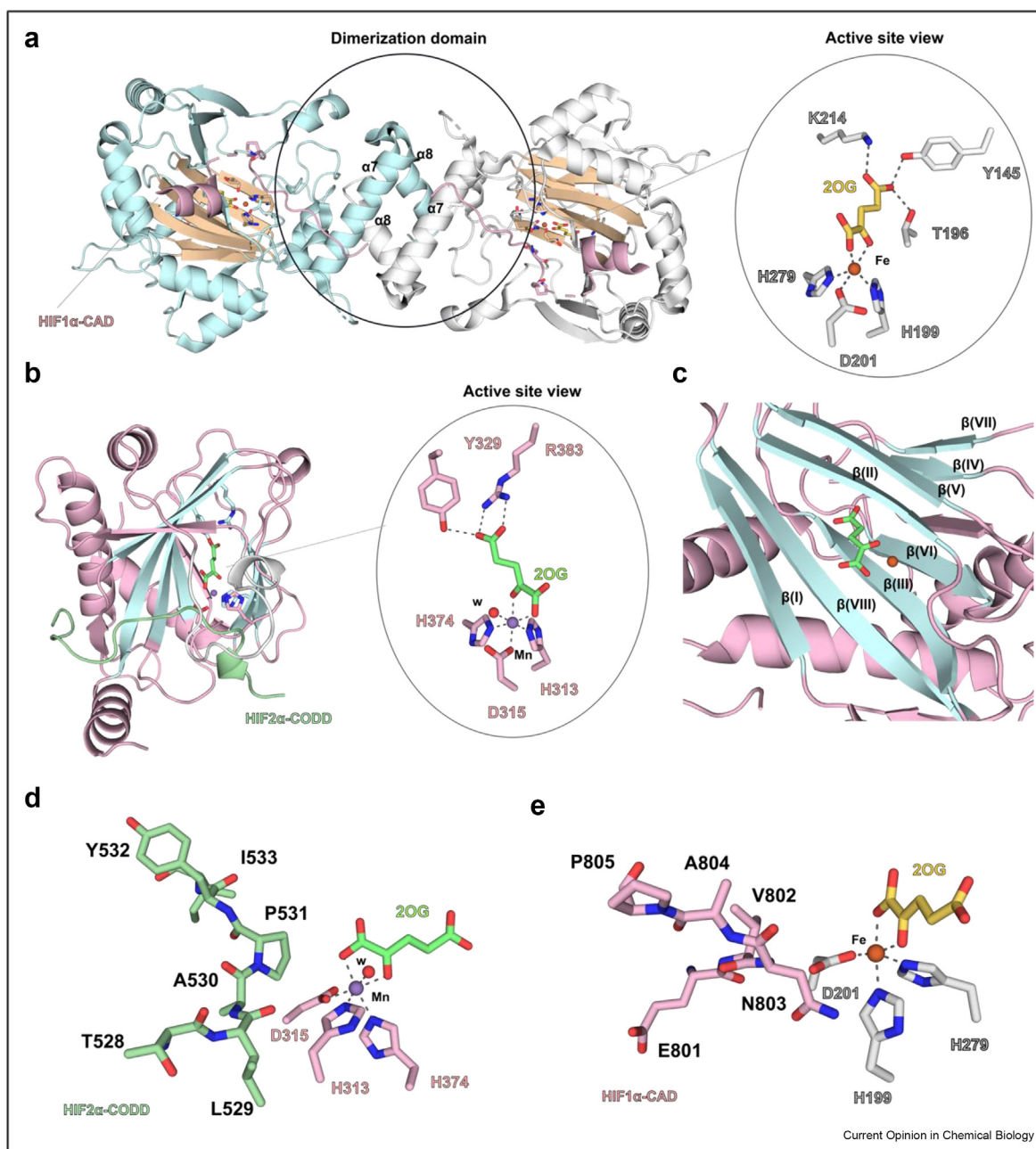


**Proposed mechanisms for PHD2 and FIH.** With both the PHDs and FIH, the active site Fe(II) is coordinated by two histidine and an aspartate residue; 2OG binds to the Fe(II) in a bidentate manner. **(a)** With PHD2, the 2OG C1 carboxylate is observed by crystallography to coordinate *trans* to His374 [58]. The precise mode of O<sub>2</sub> binding/superoxo formation for the PHDs is uncertain; possibilities other than that shown in the catalytic cycle have been proposed (I and II) [73]. 2OG decarboxylation may occur through a cyclic intermediate (III) [67,74] to give a succinyl peroxide intermediate (as observed for some 2OG oxygenases) (IV) [75], leading to the formation of an Fe(IV)=O species responsible for substrate hydroxylation. **(b)** The FIH.Fe(II).2OG.substrate complex typically presents a vacant coordination site [64] where O<sub>2</sub> is proposed to bind to give a Fe(III)-superoxo intermediate that can react to give a Fe(IV)=O species responsible for substrate hydroxylation [64,76].

accept other substrates and, if so, what roles these may have in hypoxia sensing. To date >20 non-HIF substrates for the PHDs have been reported (Table S2). However, a biochemical study with isolated PHDs does not provide support for the (mostly) cell based reported assignments [59]. There is the possibility that there are factors absent in the isolated enzymes assays that enable activity in cells. Combined biochemical and *in vivo* studies on PHD-like enzymes in other organisms

clearly reveals the potential for (folded) non-HIF $\alpha$  protein substrates to be accepted by the PHDs [40]. Thus, although it seems very possible that human PHDs accept non-HIF $\alpha$  substrates, further work is required for definitive assignments. Whilst the existence of non-HIF substrates for PHDs remains controversial, much stronger evidence demonstrating the existence of non-HIF substrate have been reported for FIH.

Figure 4



**Overview of FIH.Fe.2OG.HIF1 $\alpha$ -CAD (PDB: 1H2L) and PHD2.Mn.2OG.HIF2 $\alpha$ -CODD (PDB: 7Q5V) complex structures and active site views. (a)** The FIH.Fe.2OG.HIF1 $\alpha$ -CAD<sub>795-822</sub> structure, showing the dimerization domain (black circle) and double stranded beta helix (DBSH) core fold (wheat cartoon), HIF1 $\alpha$ -CAD<sub>795-822</sub> (light pink cartoon), 2OG (yellow sticks), H199, H279, T196, K214, D201 and Y145 (white sticks), Fe (orange sphere). **(b)** The monomeric PHD2<sub>181-407</sub>.Mn.2OG.HIF2 $\alpha$ -CODD<sub>523-542</sub> structure and active site showing the DBSH fold (light cyan cartoon),  $\beta$ 2- $\beta$ 3 loop (white cartoon), HIF2 $\alpha$ -CODD<sub>523-542</sub> (pale green cartoon), 2OG (green sticks), H374 and R383, Y329, D315 and H313 (light pink sticks), Mn (substituting for Fe, purple sphere), water (red sphere). **(c)** Close up of the DBSH core fold (with its  $\beta$ -strands labelled I to VIII) in the PHD2.Fe.2OG complex structure (PDB: 3OUJ), with 2OG (green sticks); Fe (orange sphere) [77]. **(d)** Close up of HIF2 $\alpha$ -CODD (residues 529-533) bound to PHD2.Fe.2OG (PDB: 7Q5V). Colours: active site Fe-binding triad (light pink sticks); 2OG (green sticks), HIF2 $\alpha$ -CODD (pale green sticks), Mn and water (purple and red spheres). **(e)** Close up of HIF1 $\alpha$ -CAD (residues 801-805) bound to FIH.Fe.2OG (PDB: 1H2L). Colours: active site triad (white sticks), 2OG (yellow sticks), HIF1 $\alpha$ -CAD (light pink sticks), Fe (orange sphere).

FIH is proposed to be highly promiscuous and is able to hydroxylate asparagine and other residues in ankyrin repeat domains (ARDs) and some other proteins (Table S3) [60]. Interestingly, mice lacking FIH manifest both increased glycolysis and aerobic metabolism [61]. However, the role of FIH catalysed non-HIF substrate hydroxylation is unclear, as the case for at least two other promiscuous human 2OG dependent protein hydroxylases (JMJD6 and AsfH) (Table S1). The rarely complete extent of ARD hydroxylation suggests biophysical protein stabilisation due to FIH-mediated hydroxylation is unlikely to be of general importance [62]; there is also no validated evidence for 'switch-like' signalling roles in FIH catalysed ARD hydroxylation, as proposed for CAD hydroxylation in the HIF system. One possibility is that non-HIF substrates compete with HIF $\alpha$  so tuning the role of FIH in the HIF-mediated response [63]. The question also arises as to how sufficient free FIH is available to catalyse efficient hydroxylation of the relatively low levels of HIF $\alpha$  compared to the totality of other apparent FIH substrates. It is possible that targeting factors are involved; in this regard the dimeric nature of FIH may be important [64].

### Are the biochemical properties of the HIF hydroxylases tailored for their sensing roles?

Following the proposal of HIF hydroxylases as hypoxia/ $O_2$  sensors, ongoing work is addressing whether their biochemical properties are tailored to their sensing roles. For them to act as  $O_2$  sensors their overall activity in cells must be limited by  $O_2$  availability. Although multiple factors may be involved in cells, the available evidence is that PHD2 (and, possibly, PHD1/3) has kinetic properties that are suited to its proposed sensing role. PHD2 has a high  $K_M$  for  $O_2$  and PHD2.Fe(II).2OG.HIF $\alpha$  complexes reacts slowly with  $O_2$  (Table S4) [65], by contrast, FIH has more typical kinetic properties, i.e., a lower  $K_M$  for  $O_2$  and FIH.Fe(II).2OG.HIF $\alpha$  complexes react relatively faster with  $O_2$ , at least with the investigated substrates (Table S5) [66].

Both the PHDs and FIH appear to follow the overall general consensus mechanism for OGO, though there are variations in detail (Figure 3) [67,68]. Thus, 2OG and active site HIF $\alpha$  substrate binding is followed by that of  $O_2$ . There is, however, a striking difference in the coordination mode of the 2OG C1 carboxylate and of the Asp of the HXD ... H Fe(II) binding motifs by FIH and PHD2 (Figure 3b, 4a). In the case of FIH, the 2OG C1 carboxylate coordinates the Fe(II) *trans* to His199, leaving a water occupied or vacant coordination site adjacent to the substrate [64]. By contrast in the case of PHD2 the 2OG C1 carboxylate occupies the position adjacent to the substrate [18] (Figure 3a, 4b), likely slowing reaction with  $O_2$  and necessitating a modified mechanism.

Consistent with the biochemical/structural observations and a need for sufficient HIF $\alpha$  to be present, FIH is more active in cells than the PHDs at relatively low  $O_2$  concentrations (Figure 1) [24,69]. Cellular studies also show that the regulation of HIF target genes by FIH appears to be cell type dependent; one role of FIH might be to tune the overall HIF-mediated response [70]. Structural analyses imply substantial induced fit binding helping to isolate the active site, contrasting with FIH where the active/substrate binding groove is more open [71,72]; these observations possibly relate to the different substrate selectivities of the PHDs and FIH, including with respect to the likely requirement of FIH to partially unfold its ARD substrates.

### Conclusions and future prospects

Research on the mechanisms by which humans respond to limited  $O_2$  availability has led to the identification of key roles for the 2OG dependent PHDs and FIH in regulating HIF mediated transcription [2]. The available evidence is that both the PHDs (at least PHD2) and FIH may be special OGOs, though in different ways, that is the PHDs in term of their kinetics and FIH in term of its promiscuity. There is considerable scope for further work in further exploring these proposals, ranging from single-turnover time resolved experimental and modelling studies to understand exactly how  $O_2$  binds and reacts with PHD2 to cellular and physiological work to understand the exact role(s) of FIH. Inhibitors of the PHDs that upregulate *EPO* have been approved for the treatment of anaemia in chronic kidney disease (Fig. S2) [78,79]. However, the approved compounds are rather blunt instruments, blocking PHD catalysis by competing with 2OG at the PHD active site [48,80]. The development of new types of PHD/FIH inhibitors (and enhancers), e.g. those that reduce, but do not ablate PHD catalysis, either by allosteric binding to the PHD catalytic domain or by interacting with the PHD2 MYND finger, are of interest. Given the role of FIH in the HIF system in metabolic regulation, exploring selective inhibitors of FIH is also of interest [81]. With respect to inhibition, it should, however, be noted that Co(II) ions were once used to treat anaemia and inhibit the PHDs [82,83]. Thus, whilst highly selective inhibitors of the PHDs (including of individual isoforms) and FIH are of research interest, they are not necessarily safer or better mimics of the natural hypoxic response than more broadly acting treatments.

Important remaining questions include the potential physiological importance of OGO other than the HIF hydroxylases in the hypoxic response, with the JmjC KDMs and other chromatin modifying enzymes being of particular interest. On the basis of cellular studies various JmjC KDMs, including KDM4A, KDM5A, and KDM6A have been shown to have potential to modify

chromatin in a  $O_2$ -dependent manner, with a gradual loss of their demethylase activity being observed at decreasing concentrations of  $O_2$  [34,84–87]. KDM3A-catalysed demethylation of a mono- $N^{\epsilon}$ -methylated lysine, of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 $\alpha$ ), has also been reported to be inhibited at low  $O_2$  levels [85]. Further work is required to investigate the in vivo/physiological relevance of JmjC OGO in hypoxia/oxygen-sensing. The complexity of JmjC KDM biochemistry in cells, including with respect to the multiplicity of histone H3 N-terminal tail modifications, coupled with possibilities for redundancy and adaptation in ‘epigenetic’ regulation means studies on this are experimentally non-trivial. Studies in model organisms, such as yeasts, may be productive.

There is also a lack of understanding of how Fe is delivered to and maintained in the Fe(II) form of the active site of OGO (and other Fe-utilising enzymes) within cells. In this regard, the redox-active small molecule ascorbate (vitamin C) is particularly interesting as it promotes catalysis by both the procollagen prolyl hydroxylases and the PHDs [88–90]. The interface between disease perturbed small-molecule metabolism and OGO is also of interest. Mutations to genes encoding for TCA cycle and related enzymes (e.g., isocitrate dehydrogenase 1 and 2; IDH1,2) result in elevated levels of metabolites that may inhibit OGO (e.g., 2-hydroxoglutarate), including the HIF hydroxylases, potentially in a pro-oncogenic manner [91–93]. Work in the field is of interest to investigate the roles of wild-type and mutant OGO in cancer. It is clinically relevant because of the increasing use of mutant IDH inhibitors [94]. Finally, we note that, at least in isolated form, some OGO can employ 2-oxoacid cosubstrates other than 2OG, suggesting there is the possibility for cosubstrate promiscuity to mimic the protein substrate promiscuity of FIH [95].

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

No data was used for the research described in the article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpa.2024.102428>.

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Papers of particular interest, published within the period of review, have been highlighted as:

\* of special interest

\*\* of outstanding interest

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