Prolyl hydroxylases as regulators of cell metabolism

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Abstract
Cellular response to oxygen depletion is mediated by HIF (hypoxia-inducible factor). HIF is a heterodimer consisting of a constitutively expressed subunit (HIFα) and an oxygen-regulated subunit (HIFα). HIFα stability is regulated by prolyl hydroxylation by PHD (prolyl hydroxylase domain-containing protein) family members. PHD activity depends on the availability of molecular oxygen, making PHDs the oxygen-sensing system in animal cells. However, PHDs have recently been shown to respond to stimuli other than oxygen, such as 2-oxoglutarate (α-ketoglutarate), succinate or fumarate, as illustrated by the pseudo-hypoxic response in succinate dehydrogenase- or fumarate dehydrogenase-deficient tumours. Moreover, HIFα is not the sole PHD effector, suggesting that PHDs have functions that extend beyond oxygen sensing. Currently, we are investigating the role of PHDs in the cellular response to amino acid deprivation, a process regulated by mTOR (mammalian target of rapamycin). The precise mechanism whereby amino acids are signalling to mTOR is not fully understood. Given that 2-oxoglutarate is a limiting co-substrate for PHD activity during normoxia and that 2-oxoglutarate levels depend on amino acid availability, it is possible that PHD activity depends not only on oxygen, but also on amino acid availability, suggesting a global metabolic sensor function for PHDs which could be signalling not only to HIF, but also to mTOR.

Prolyl hydroxylases, HIF (hypoxia-inducible factor) 1 and hypoxia response
The cellular response to low oxygen at the molecular level is mediated by the transcription factor HIF. Under low oxygen, HIF induces the expression of several sets of genes, including genes involved in glucose metabolism, erythropoiesis, angiogenesis, vascular tone, cell proliferation and apoptosis [1]. HIF is currently the focus of considerable research efforts because of its connection with tumorigenesis [2]. Indeed, HIF is overexpressed in many different types of cancer, and this often correlates with increased angiogenesis, malignant progression and treatment failure [2,3]. Although HIF is not considered to be an oncogene, as it is not able to drive tumorigenesis on its own, HIF induction is particularly advantageous in the hypoxic regions of solid tumours, as it mediates adaptation to hypoxia through increased glucose metabolism and angiogenesis. HIF is therefore a potentially good target in cancer therapy [2].

HIF is a heterodimeric protein formed by a constitutively expressed subunit (HIFα) and an oxygen-regulated subunit (HIFα). HIFα availability is regulated post-transcriptionally by proteasomal degradation. In the presence of molecular oxygen, HIFα is hydroxylated on proline residues in the ODDD (oxygen-dependent degradation domain) by PHDs (prolyl hydroxylase domain-containing proteins) [4–6]. This hydroxylation allows the interaction of the ODDD with the pVHL (von Hippel–Lindau protein)–ubiquitin E3 ligase, promoting the ubiquitination and subsequent proteasomal degradation of HIFα. Under low oxygen, the hydroxylation does not occur (PHDs have low affinity for oxygen [7]), thus preventing the interaction with pVHL. As a result, HIFα is stabilized, forms a heterodimer with HIFβ and promotes the expression of target genes. In humans, the PHD family is composed of three different 2-oxoglutarate (α-ketoglutarate) dioxygenases (PHD1, PHD2 and PHD3) that require iron and ascorbate as cofactors. For the hydroxylation reaction, oxygen atoms are obtained from molecular oxygen, whereas 2-oxoglutarate provides electrons and is then decarboxylated to succinate.

The paradox of the prolyl hydroxylase family
Prolyl hydroxylases were first discovered in Caenorhabditis elegans and later described in many other organisms, including mammals [8,9], Drosophila and, more recently, Dictyostelium [10], the fission yeast (Schizosaccharomyces pombe) [11] or even photosynthetic organisms such as Chlamydomonas reinhardtii [12]. The existence of PHD members in species which have been described not to have HIF, or even in photosynthetic (oxygen-generating) organisms, implies the existence of HIF-independent functions of PHDs. For example, in some of the aforementioned species, such as Dictyostelium or Chlamydomonas reinhardtii, PHDs...
have been shown to play an important role in development or cell wall assembly respectively.

A second interesting aspect of PHDs is their apparent unnecessary redundancy. Although, as already mentioned, there are three different members of the PHD family, only PHD2 has been confirmed to be involved in the oxygen regulation of HIF1α, with PHD1 and PHD3 displaying only partial additive effects on HIF1α stability [13,14]. All three members are encoded by five exon-genomes, and the resulting proteins share high similarity in the catalytic (C-terminal) part of the protein. The N-terminal region varies considerably among the three isoforms. It is also interesting to note that alternative splicing of exon 4 and 5 has been described for the PHDs [7]. Splicing of exons 4 and 5 alters the structure of the catalytic core, but the effect of such splicing on the prolyl hydroxylase activity is unclear [7,15]. An alternative translational initiation codon has also been described in PHD1, generating another isoform of the protein [16]. The function and regulation of all of these spliced isoforms remain unknown.

Finally, a biochemical aspect of PHDs points to their possible role in processes different from oxygen sensing. As mentioned above, PHDs use not only oxygen as substrate, but also 2-oxoglutarate as an electron donor. As a result, 2-oxoglutarate is oxidized into succinate. As the end-product of this reaction, succinate can inhibit PHD activity. Indeed, accumulation of succinate as a result of mutations in succinate dehydrogenase is enough to inhibit PHD activity under normoxia, leading to HIF1 stabilization in what is described as pseudo-hypoxia [17]. Interestingly, by artificially increasing 2-oxoglutarate levels inside the cell, it is possible to reactivate PHD activity under pseudo-hypoxic conditions [18]. These findings highlight the ability of PHDs to respond not only to oxygen availability, but also to the availability of 2-oxoglutarate, a compound which plays a central role in several metabolic processes.

2-Oxoglutarate can be considered as a hub in cellular metabolism. It is a key intermediate in the tricarboxylic acid cycle, involved in transamination processes and is the molecule resulting from glutaminolysis. The latter connects 2-oxoglutarate directly to amino acid metabolism, being the point of anaplerotic re-feeding of the tricarboxylic acid cycle by glutamine, an extremely important process for cellular energy especially in cancer cells [19]. Glutamine is metabolized inside the cell by glutaminase which catalyses a first deamination step, generating glutamate. Glutamate in turn can be converted into 2-oxoglutarate by a second deamination process catalysed by the enzyme GDH (glutamate dehydrogenase), a step that can also occur through transamination. GDH is positively regulated by leucine. 2-Oxoglutarate then enters the tricarboxylic acid cycle to allow the synthesis of oxaloacetate. Oxaloacetate in turn reacts with acetyl-CoA to generate citrate.

The close connection between 2-oxoglutarate and glutamine (the most important amino acid from an energetic point of view) has already been proven by recent work showing that glutamine starvation causes a quick drop in intracellular levels of different tricarboxylic acid cycle intermediates [20]. This demonstrated the necessity of glutamine to sustain the levels of these intermediates. It would therefore be expected that cellular levels of 2-oxoglutarate would be seriously compromised in the absence of anaplerotic flux from glutamine toward the tricarboxylic acid cycle. As PHDs are strictly dependent on the availability of 2-oxoglutarate for prolyl hydroxylation reactions, one would anticipate a dependence on immediate amino acid availability (especially glutamine and leucine, as leucine regulates GDH activity) for PHD activity.

PHDs and other metabolic processes: the mTOR (mammalian target of rapamycin) connection

The serine/threonine kinase mTOR functions as a master regulator of cellular metabolism. It integrates different stimuli emanating from growth factor signals, amino acid levels, oxygen availability and internal energetic status [21,22], and controls cell growth. Whereas the mechanisms by which growth factors, oxygen and energetic status control mTOR activity are relatively well understood, the pathway by which amino acids activate mTOR activity is still under discussion.

mTOR has been described as functioning in two different complexes: the rapamycin-sensitive mTORC1 (mTOR complex 1) and the rapamycin-insensitive mTORC2. mTORC1 is known to respond to amino acid availability [23] and to control protein synthesis and cell-cycle progression, mainly through regulation of protein translation and ribosome biogenesis [21,24].

mTORC2 is less well studied than mTORC1 and has been shown to be involved in actin reorganization [25], and in the phosphorylation and activation of Akt [26], pointing to a possible role in cell survival and proliferation.

One fundamental question remains: how do cells detect amino acid availability and transmit the signal to mTOR in order to regulate cellular growth? Several mechanisms have already been proposed. First, the GTPase Rheb was implicated. The interaction between Rheb and mTORC1 is critical for mTORC1 activity, and was shown to depend on amino acid availability [27]. An RNAi (RNA interference) screen in Drosophila implicated the MAPK (mitogen-activated protein kinase) signalling pathway in mTOR regulation, and identified MAP4K3 (mitogen-activated protein kinase kinase kinase 3) as an amino-acid-responsive regulator of the mTOR pathway [28]. Moreover, the class III phosphoinositide 3-kinase hVps34 (human vacuolar protein sorting 34) was proposed to transduce the activation signal from amino acids to mTORC1 through a mechanism involving changes in calcium levels [29]. However, this model is controversial at present, as genetic studies in Drosophila have failed to give similar results [30]. Finally, two independent studies have...
shown, in human cell lines and in a Drosophila genetic model, that Rag (Ras-related GTPase) proteins are necessary and sufficient to mediate amino acid signalling to mTORC1 [31,32].

The identification of various independent mechanisms signalling from amino acids to mTORC1 may simply be a reflection of the complexity of the system, and the need to integrate inputs relating to a wide array of cellular conditions. However, none of the previously proposed mechanisms has addressed how amino acid availability is sensed in the cell. Being dependent on glutaminolytic flux, 2-oxoglutarate can represent an internal indicator for the catabolism of amino acids. Since PHD activity is sensitive to 2-oxoglutarate flux, PHDs are potential candidates for 2-oxoglutarate sensors, and therefore for amino acid sensors. Indeed, initial observations showed that PHD activity is dependent on amino acid availability (R.V. Durán, H. Boulahbel and E. Gottlieb, unpublished work). The next step would be to elucidate any functional connection between PHDs and mTOR to confirm whether PHDs are indeed the signal transducers between amino acids and mTOR (Figure 1). This potential PHD-mediated regulation of mTOR will provide an excellent model to trace amino acid signalling pathways inside the cell and would open a new field of investigation in which prolyl hydroxylation plays a central role, beyond HIF regulation, in the control of cellular metabolism.

References


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