# HIF at a glance

**M. Christiane Brahimi-Horn* and Jacques Pouysségur**

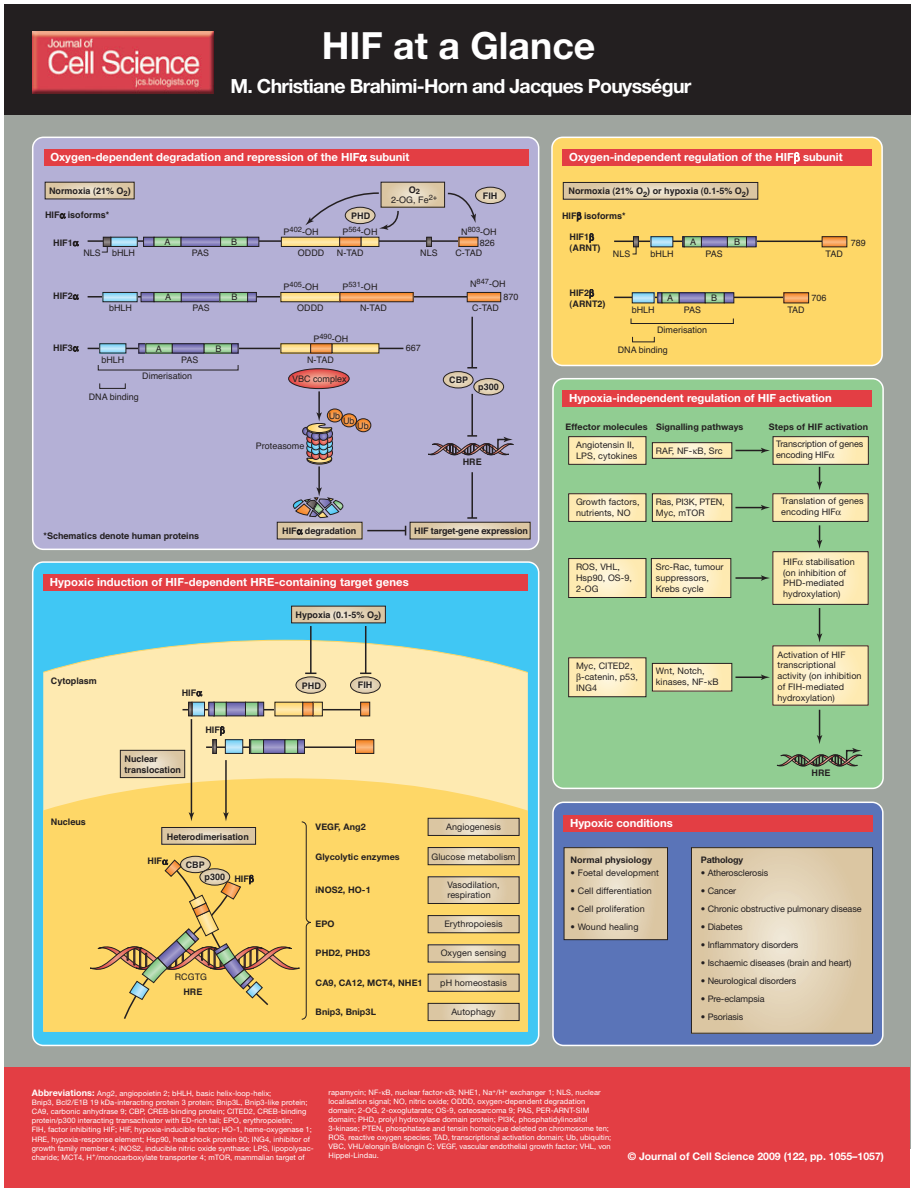
Institute of Developmental Biology and Cancer Research, University of Nice, CNRS UMR 6543, Centre A. Lacassagne, 33 Avenue Valombrose, 06189 Nice, France

*Author for correspondence
(e-mail: brahimi@unice.fr)

*Journal of Cell Science 122, 1055-1057*
*Published by The Company of Biologists 2009*
*doi:10.1242/jcs.035022*

Hypoxia-inducible factor (HIF) is a sequence-specific DNA-binding protein that can promote or repress the transcription of a broad range of genes that are involved in maintaining biological homeostasis. HIF is mostly non-functional in oxygenated cells but becomes active under specific conditions, including low-oxygen (hypoxic) stress. HIF is a heterodimeric complex that is composed of an oxygen-destructible α-subunit and an oxygen-indestructible β-subunit. Three isoforms of the α-subunit and two isoforms of the β-subunit are thought to be involved in the in vivo response to hypoxia.

HIF transcriptional activity requires inhibition of the post-transcriptional hydroxylation of the α-subunit, which targets HIF for proteasomal degradation and thereby causes its inactivation. In addition to hypoxic stress, several other conditions activate HIF transcriptional activity, including autocrine stimulation by growth factors, the loss of tumour-suppressor function and the gain of oncogene function. Overall, the genes targeted by HIF help cells to adapt to, and thereby survive in, a stressful microenvironment. Adaptation can be beneficial to human physiology in the context of foetal development and in many pathophysiological conditions, such as ischaemic disorders, but can be detrimental in the context of tumourigenesis. In this article and its accompanying poster, we briefly cover the major structural and functional characteristics of HIF and its involvement in conditions of normal physiology and in disease.




*(See poster insert)*

## Normoxic degradation and inactivation of HIFα through post-translational modification

The activities of three well-described HIFα isoforms (HIF1α, HIF2α and HIF3α) are regulated in a similar manner by post-translational prolyl hydroxylation of the oxygen-dependent degradation domain (ODDD) (Schofield and Ratcliffe, 2004). Prolyl hydroxylation targets the proteins for proteasomal degradation by promoting their interaction with von Hippel-Lindau (VHL), a component of an E3 multiprotein ubiquitin-ligase complex known as VHL/elongin B/elongin C (VBC). VBC covalently links a chain of ubiquitin (Ub) moities to HIFα and causes it to dock onto the multisubunit proteolytic proteasomal complex, which selectively degrades ubiquitin-conjugated proteins (Kaelin and Ratcliffe, 2008). Additional hydroxylation of an asparaginyl moiety at the end of the C-terminus of the HIF1α and HIF2α subunits abrogates HIF activation by inhibiting the binding of coactivators such as p300 and its paralogue CREB-binding protein (CBP), which also act as histone acetyltransferases. These hydroxylation events are catalysed, respectively, by prolyl hydroxylase domain proteins (PHDs), of which there are three major isoforms (Schofield and Ratcliffe, 2004), and factor inhibiting HIF (FIH) (Peet and Linke, 2006). These enzymes are dioxygenases and belong to the largest known family of non-haem oxidising enzymes (International Union of Biochemistry number EC 1.14.11.2). Their activity is dependent on substrates oxygen and 2-oxoglutarate (2-OG, a Krebs cycle intermediate) and on cofactor $Fe^{2+}$.

### Hypoxic stabilisation of HIFα and nuclear translocation

Under hypoxic conditions, PHDs and FIH are inactive because of the lack of sufficient oxygen. Both of these enzymes use oxygen and 2-OG as substrates in the hydroxylation reaction; however, they are very different with respect to the concentration of oxygen required for their activity. In vitro, PHD has a Michaelis constant ($K_m$) for oxygen that is three times greater than that of FIH (Koivunen et al., 2004). Thus, as the oxygen concentration decreases, PHDs become inactive and the HIFα protein is consequently stabilised. Because HIFα has a nuclear localisation signal (NLS) in its C-terminal region, when stable it can rapidly bind to nuclear pore proteins and translocate into the nucleus (Kallio et al., 1998). However, HIFα may remain at least partially inactive until a further decrease in the oxygen concentration occurs, which inactivates FIH and fully activates HIF transcriptional activity.

### Dimerisation of HIFα and HIFβ and DNA binding

Once in the nucleus, the HIFα and HIFβ subunits interact with one another and bind to specific DNA sequences (Brahimi-Horn and Pouysségur, 2005). Dimerisation between the HIFα and HIFβ subunits occurs through the basic helix-loop-helix (bHLH) and PER-ARNT-SIM (PAS) A and B domains located in the N-terminal region of each subunit, whereas DNA binding occurs through the bHLH domains. The specific DNA sequences that are targeted by HIF, known as hypoxia-response elements (HREs), are composed of 5′-RCGTG-3′ (where R is either an A or G) and are mostly found in the promoter, intron and/or enhancer regions of target genes (Pugh et al., 1991; Semenza et al., 1991; Wenger et al., 2005).

### Induction of HIF target-gene expression and functional consequences

The transcriptional activity of the heterodimer is controlled by transcriptional-activation domains (TADs) found in the C-terminus; HIF1α and HIF2α subunits each have two TADs, known as the N-TAD and C-TAD. The activity of the C-TAD can be inhibited by FIH hydroxylation, but the activity of the N-TAD is independent of FIH. Thus, HIFα has bifunctional transcriptional activity, which, depending on the activity of FIH, allows for differential gene activation that is either N- or C-TAD controlled or N- and C-TAD controlled (Dayan et al., 2006). In addition, although the HIF1α and HIF2α isoforms have distinct target-gene preferences, substantial overlap in their preference for a target gene indicates that these two isoforms might act cooperatively (Gordan and Simon, 2007; Ratcliffe, 2007). In this respect, and in general, little is known about the less well-characterised HIF3α isoform, which can act as a dominant-negative of HIF transcriptional activity (Makino et al., 2001).

The expression of more than 60 well-defined gene products is increased by HIF (Semenza, 2003). One of the best characterised is the gene that encodes vascular endothelial growth factor A (VEGF-A), which induces vascular endothelial tip cells to migrate to hypoxic areas and promotes blood vessel growth. This phenomenon is known as angiogenesis, which is an adaptive response that attempts to compensate for the low oxygen level in tissues (Ferrara et al., 2003). Other HIF-induced genes are involved in metabolism, vasodilation, erythropoiesis, pH homeostasis, oxygen sensing and autophagy, among others (Semenza, 2003). In addition, the expression of a broad range of gene products can be repressed by HIF (Manalo et al., 2005).

### Interpathway signalling and HIF function

Although hypoxia is considered to be the main stimulus that drives HIF function, a number of non-hypoxic effectors and signalling pathways can influence HIF function, directly or indirectly, at different stages of its activation. Notably, many of the effectors and intermediates of these HIF-inducing pathways are themselves HIF target-gene products. Effectors that have been implicated in stimulating or suppressing an immune response (such as lipopolysaccharide and cytokines) and that activate the nuclear factor-κB (NF-κB) signalling pathway promote HIF1α transcription (Gorlach and Bonello, 2008; Rius et al., 2008; van Uden et al., 2008), whereas stimulation with autocrine growth factors [including epithelial growth factor (EGF), fibroblast growth factor 2 (FGF2) and insulin-like growth factor (IGF)] enhance translation of the HIF1α protein (Semenza, 2003). The loss of function of tumour suppressors (such as ING4, p53, PTEN and VHL) and the gain of function of oncogenes (such as those encoding AKT, MYC, mTOR, PI3K, RAF, RAS, SCR) also regulate different steps that lead to HIF activation (Dang et al., 2008; Semenza, 2003).

### HIF in physiology and pathophysiology

As hypoxia can occur in both physiological and pathophysiological conditions, HIF plays a key role in both instances. Foetal development is highly dependent on establishing an efficient haematopoietic and vascular system, both of which are controlled by HIF target-gene products such as erythropoietin (Semenza, 2003), VEGF (Simon and Keith, 2008) and angiopoietin 2 (Ang2) (Simon et al., 2008). In addition, cell proliferation and differentiation, which constitute a major part of organismal structuring, require adequate nutrient availability and appropriate metabolic processes (Sainson and Harris, 2006). HIF fulfils this requirement by regulating the expression of proteins that control cellular glucose uptake and metabolism, and that influence the mammalian target of rapamycin (mTOR) pathway, which responds to the nutritional status by regulating protein synthesis (Pouysségur et al., 2006).

Hypoxia is also a characteristic of several disease states in which there is an inadequate supply of oxygen owing to a defective or inadequate vasculature; this occurs particularly in ischemic disorders of the heart and brain and in cancer (Loor and Schumacker, 2008). Massive proliferation of cells in a solid tumour creates distance between tumour cells and the blood vessels that carry oxygen and nutrients, thereby leading to a hypoxic microenvironment that activates HIF (Brahimi-Horn et al., 2007). HIF, in turn, initiates several adaptive survival processes that maintain metabolic equilibrium, pH homeostasis (Chiche et al., 2009) and autophagy (Bellot et al., 2009), thereby reinforcing tumour growth and promoting metastasis (Erler et al., 2006).

### Conclusion

The transcription factor HIF has a central role in oxygen sensing and is therefore vital for survival of the organism. The molecular blockade of HIF activity involves a cascade of enzyme-mediated events, including prolyl hydroxylation, asparaginyl hydroxylation and ubiquitylation, which modify the

α-subunit at the post-translational level when sufficient oxygen is available. When oxygen concentrations are low, these enzyme-mediated events are inhibited and active HIF can induce gene transcription that might or might not be beneficial to an organism, depending on the pathophysiological context. The development of pharmacological approaches that activate or inhibit HIF or its target-gene products may provide therapeutic benefit in ischaemic disorders or cancer, respectively (Melillo, 2007).

The laboratory is funded by grants from the Ligue Nationale Contre le Cancer (Equipe labellisée), the Association pour la Recherche contre le Cancer (ARC), METOXIA (EU program FP7), the Centre A. Lacassagne, the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé et de la Recherche Médicale (Inserm), the University of Nice (http://www.unice.fr/isdbc/) and the Institut National du Cancer (INCa). We apologise to the many research groups whose work was only cited indirectly by reference to review articles.

## References

**Bellot, G., Garcia-Medina, R., Gounon, P., Chiche, J., Roux, D., Pouysségur, J. and Mazure, N. M.** (2009). Hypoxia-induced autophagy is mediated through HIF induction of BNIP3 and BNIP3L via their BH3 domains. *Mol. Cell. Biol.* (in press).

**Brahimi-Horn, M. C. and Pouysségur, J.** (2005). The hypoxia-inducible factor and tumor progression along the angiogenic pathway. *Int. Rev. Cytol.* **242**, 157-213.

**Brahimi-Horn, M. C., Chiche, J. and Pouysségur, J.** (2007). Hypoxia and cancer. *J. Mol. Med.* **85**, 1301-1307.

**Chiche, J., Ilc, K., Laferriere, J., Trottier, E., Dayan, F., Mazure, N. M., Brahimi-Horn, M. C. and Pouyssegur, J.** (2009). Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. *Cancer Res.* **69**, 358-368.

**Dang, C. V., Kim, J., Gao, P. and Yustein, J.** (2008). The interplay between MYC and HIF in cancer. *Nat. Rev. Cancer* **8**, 51-55.

**Dayan, F., Roux, D., Brahimi-Horn, M. C., Pouysségur, J. and Mazure, N. M.** (2006). The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. *Cancer Res.* **66**, 3688-3698.

**Erler, J. T., Bennewith, K. L., Nicolau, M., Dornhofer, N., Kong, C., Le, Q. T., Chi, J. T., Jeffrey, S. S. and Giaccia, A. J.** (2006). Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* **440**, 1222-1226.

**Ferrara, N., Gerber, H. P. and LeCouter, J.** (2003). The biology of VEGF and its receptors. *Nat. Med.* **9**, 669-676.

**Gordan, J. D. and Simon, M. C.** (2007). Hypoxia-inducible factors: central regulators of the tumor phenotype. *Curr. Opin. Genet. Dev.* **17**, 71-77.

**Gorlach, A. and Bonello, S.** (2008). The cross-talk between NF-kappaB and HIF-1: further evidence for a significant liaison. *Biochem. J.* **412**, e17-e19.

**Kaelin, W. G. and Ratcliffe, P.** (2008). Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* **30**, 393-402.

**Kallio, P. J., Okamoto, K., O'Brien, S., Carrero, P., Makino, Y., Tanaka, H. and Poellinger, L.** (1998). Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha. *EMBO J.* **17**, 6573-6586.

**Koivunen, P., Hirsila, M., Gunzler, V., Kivirikko, K. I. and Myllyharju, J.** (2004). Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases. *J. Biol. Chem.* **279**, 9899-9904.

**Loor, G. and Schumacker, P. T.** (2008). Role of hypoxia-inducible factor in cell survival during myocardial ischemia-reperfusion. *Cell Death Differ.* **15**, 686-690.

**Makino, Y., Cao, R., Svensson, K., Bertilsson, G., Asman, M., Tanaka, H., Cao, Y., Berkenstam, A. and Poellinger, L.** (2001). Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* **414**, 550-554.

**Manalo, D. J., Rowan, A., Lavoie, T., Natarajan, L., Kelly, B. D., Ye, S. Q., Garcia, J. G. and Semenza, G. L.** (2005). Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* **105**, 659-669.

**Melillo, G.** (2007). Targeting hypoxia cell signaling for cancer therapy. *Cancer Metastasis Rev.* **26**, 341-352.

**Peet, D. and Linke, S.** (2006). Regulation of HIF: asparaginyl hydroxylation. *Novartis Found. Symp.* **272**, 37-49; discussion 49-53; 131-140.

**Pouysségur, J., Dayan, F. and Mazure, N. M.** (2006). Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* **441**, 437-443.

**Pugh, C. W., Tan, C. C., Jones, R. W. and Ratcliffe, P. J.** (1991). Functional analysis of an oxygen-regulated transcriptional enhancer lying 3′ to the mouse erythropoietin gene. *Proc. Natl. Acad. Sci. USA* **88**, 10553-10557.

**Ratcliffe, P.** (2007). HIF-1 and HIF-2: working alone or together in hypoxia? *J. Clin. Invest.* **117**, 862-865.

**Rius, J., Guma, M., Schachtrup, C., Akassoglou, K., Zinkernagel, A. S., Nizet, V., Johnson, R. S., Haddad, G. G. and Karin, M.** (2008). NF-kB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1a. *Nature* **453**, 807-812.

**Sainson, R. C. A. and Harris, A. L.** (2006). Hypoxia-regulated differentiation: let's step it up a Notch. *Trends Mol. Med.* **12**, 141-143.

**Schofield, C. J. and Ratcliffe, P. J.** (2004). Oxygen sensing by HIF hydroxylases. *Nat. Rev. Mol. Cell. Biol.* **5**, 343-354.

**Semenza, G. L.** (2003). Targeting HIF-1 for cancer therapy. *Nat. Rev. Cancer* **3**, 721-732.

**Semenza, G. L., Nejfelt, M. K., Chi, S. M. and Antonarakis, S. E.** (1991). Hypoxia-inducible nuclear factors bind to an enhancer element located 3′ to the human erythropoietin gene. *Proc. Natl. Acad. Sci. USA* **88**, 5680-5684.

**Simon, M. C. and Keith, B.** (2008). The role of oxygen availability in embryonic development and stem cell function. *Nat. Rev. Mol. Cell. Biol.* **9**, 285-296.

**Simon, M. P., Tournaire, R. and Pouysségur, J.** (2008). The angiopoietin-2 gene of endothelial cells is up-regulated in hypoxia by a HIF binding site located in its first intron and by the central factors GATA-2 and Ets-1. *J. Cell Physiol.* **217**, 809-818.

**van Uden, P., Kenneth, N. S. and Rocha, S.** (2008). Regulation of hypoxia-inducible factor-1alpha by NF-kappaB. *Biochem. J.* **412**, 477-484.

**Wenger, R. H., Stiehl, D. P. and Camenisch, G.** (2005). Integration of oxygen signalling at the consensous HRE. *Sci. STKE* **2005(360)**, re 12.

***Cell Science at a Glance on the Web***

*Electronic copies of the poster insert are available in the online version of this article at jcs.biologists.org. The JPEG images can be downloaded for printing or used as slides.*