ELSEVIER

Seminars in Cell & Developmental Biology 16 (2005) 474–486

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

www.elsevier.com/locate/semcdb

Review

Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression

Thomas Kietzmann [a], Agnes Görlach [b,*]

[a] *Faculty of Chemistry, Department of Biochemistry, Erwin-Schrödinger-Strasse, University of Kaiserslautern, 67663 Kaiserslautern, Germany*
[b] *Experimental Pediatric Cardiology, Department of Pediatric Cardiology and Congenital Heart Disease, German Heart Center Munich at the Technical University Munich, Lazarettstrasse 36, D-80636 Munich, Germany*

Available online 17 May 2005

Abstract

Reactive oxygen species (ROS) have long been considered as cytotoxic. However, recent evidence indicates a prominent role of ROS as signaling molecules in the response to hormones, growth and coagulation factors, cytokines and other factors as well as to changes in oxygen tension. The hypoxia-inducible transcription factors (HIFs) are key players in the cellular response to changes in oxygen tension. Recently, HIFs have also been shown to respond to the above-mentioned non-hypoxic stimuli. In this article, the role of ROS in the regulation of HIF-1 under hypoxic and non-hypoxic conditions is summarized.

Keywords: Hypoxia; Reactive oxygen species; HIF; Oxygen sensing; NADPH oxidase

Contents

1. Introduction

The provision of oxygen is mandatory for all aerobic living organisms since energy generation is coupled to a redox process where the energy substrates serve as electron donors and molecular O_2 as electron acceptor. However, when O_2 is not completely reduced to water, the electron transfer results in the production of oxygen-containing free radicals and subsequently also of other reactive compounds, which are referred to as reactive oxygen species (ROS). High amounts of ROS are known to cause damage to proteins, DNA and lipids. These cytotoxic properties of ROS explain the evolution of non-enzymatic and enzymatic detoxification mecha-

* Corresponding author. Tel.: +49 89 1218 2700; fax: +49 89 1218 2633.
E-mail address: goerlach@dhm.mhn.de (A. Görlach).

1084-9521/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2005.03.010

nisms. However, there is evidence that ROS can also act as signaling molecules mediating changes in O_2 tension as well as the response to hormones, growth factors and mechanical or chemical stress. Within the last decade, it became evident that a family of hypoxia-inducible transcription factors (HIFs) are key players in the cellular response to limited O_2 supply. More recently, HIFs have also been shown to respond to growth and coagulation factors, hormones, cytokines or stress factors under non-hypoxic conditions. Interestingly, ROS appear to play an important role in both the hypoxic and non-hypoxic signaling processes which control the activity of HIFs. In the following, we will therefore summarize the role of ROS in the regulation of HIF activity.

2. The nature of hypoxia-inducible transcription factors

Studies investigating the hypoxia-dependent expression of the erythropoietin (EPO) gene led to the identification, purification and cloning of the hypoxia-inducible transcription factor-1 (HIF-1) [1–4]. HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β (arylhydrocarbon receptor-nuclear translocator (ARNT) [5]). Both constitute of amino-terminally located basic helix–loop–helix and Per–ARNT–Sim (PAS) domains which are involved in DNA binding and heterodimerization of the two HIF subunits. While ARNT is constitutively expressed, the HIF-1α subunit carries the sensitivity towards O_2.

In addition, two other HIF-α subunits (HIF-2α and HIF-3α) as well as two other ARNT isoforms (ARNT2 and ARNT3) have been described (reviewed by Wenger [6]). Whereas HIF-1α appears to be ubiquitously expressed, HIF-2α expression was thought to be limited to the endothelium [7]. However, newer studies indicate that HIF-2α is also rather ubiquitously expressed [8] but partially differs in its target gene specificity from HIF-1α [9,10]. In contrast to HIF-1α and HIF-2α, HIF-3α appears not to be as efficient in mediating the hypoxic response or even to act as a negative regulator [11,12]. Likewise, a splice variant of mouse HIF-3α, designated inhibitory PAS protein (IPAS) has been shown to bind to HIF-1α, thereby inhibiting its activity [13,14]. However, HIF-1 is considered to be the major regulator of about 100 physiologically important genes and was found to be conserved from *Caenorhabditis elegans* via *Drosophila melanogaster* to *Homo sapiens* [4,15–17] suggesting that the HIF-1 system played an essential role during evolution.

Although evidence exists that HIF-1α is regulated by hypoxia at the translational level [4,11,18–21], the predominant mode of HIF-1α regulation by hypoxia occurs post-translationally. This is brought about by the HIF-1α carboxy-terminal half which contains an oxygen-dependent degradation domain (ODD) [22] and two transactivation domains (TADs) referred to as amino-terminal (TADN) and carboxy-terminal TAD (TADC) which are connected by an inhibitory domain [23,24]. The ODD and the TADN partially overlap and are mainly responsible for HIF-1α protein stability. Under normoxia, HIF-α subunit destabilization is mediated by O_2-dependent hydroxylation of at least two proline residues (P402, P564) within the ODD [25,26]. This process allows binding of the von Hippel-Lindau tumor suppressor protein (pVHL) [27–30]. The VHL protein is found in a multiprotein complex with elongins B/C, Cul2, and Rbx1 forming an E3 ubiquitin ligase complex called VEC. This modular enzyme then initiates degradation by the ubiquitin-proteasome pathway [28,31–35]. So far, three HIF-α prolyl hydroxylase domain containing proteins (PHD1–3) have been identified [36–38] and an additional prolyl hydroxylase enzyme (PH4) appears to regulate HIF-1α, although experimental support for such a role is limited [39]. Another hydroxylase named factor inhibiting HIF (FIH) [40–42] prevents the recruitment of the coactivator CREB binding protein (CBP)/p300 by hydroxylating an asparaginyl residue (N803) in the TADC [43]. In addition, TADC was modified by redox factor-1 (Ref-1) [44] and interacted with other coactivators such as steroid receptor coactivator-1 (SRC-1) and transcription intermediary factor-2 (TIE-2) in a redox-dependent manner [45,46], thus suggesting a role for ROS in modulating HIF-1 activity.

Although HIF-1 has initially been described as a transcription factor regulated by hypoxia, there is now increasing evidence that HIF-1 is also responsive to a variety of non-hypoxic stimuli among them hormones such as insulin [47–49], growth factors such as PDGF, TGF-β and IGF-1 [50–52], coagulation factors such as thrombin [50], vasoactive peptides such as angiotensin II [51], cytokines [53], or carbachol which activates muscarinic acetylcholine receptors [54]. Moreover, HIF-1 has also been shown to be activated by metal ions such as cobalt, chromium and arsenite [55,56] as well as by mechanical stress [57]. However, the mechanisms by which these non-hypoxic stimuli induce HIF-1α are not completely known, yet, although some evidence points to a role of ROS as messengers regulating HIF activity.

3. ROS act as signaling molecules

In mammalian cells, ROS can be formed in response to toxic reagents or as by-products of O_2-utilizing enzymes such as those in the mitochondrial respiratory chain, the arachidonic acid pathway, the cytochrome P450 family, glucose oxidase, amino acid oxidases, xanthine oxidase, NADH/NADPH oxidases, or NO synthases [58,59]. Transfer of one electron to O_2 results in the production of superoxide anion radicals ($O_2^{\bullet -}$) which can further give rise to the formation of other ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$) (Fig. 1).

Under normal physiological conditions, formation of ROS is counterbalanced by the endogenous antioxidant defense systems such as superoxide dismutases (SOD), glutathione peroxidases (GPX), catalase, peroxiredoxins, thioredoxin and exogenously taken up micronutrients and vitamins

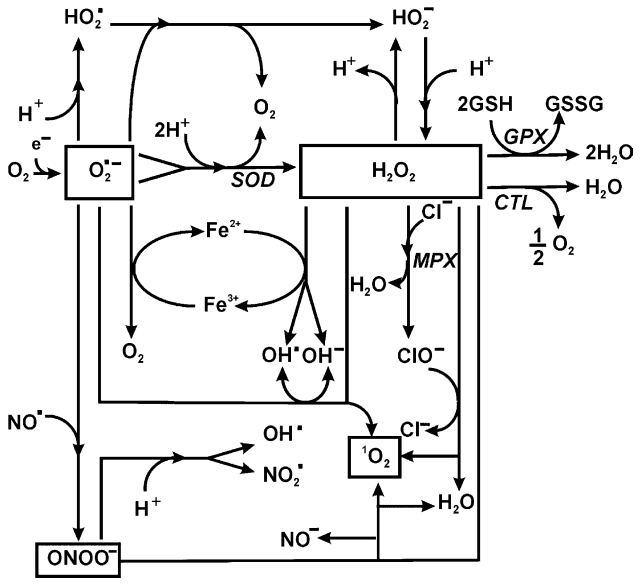

Fig. 1. Major pathways of ROS generation. When O_2 accepts an electron, superoxide anion radicals ($O_2^{\bullet -}$) are formed. $O_2^{\bullet -}$ molecules are then dismutated by the action of superoxide dismutase (SOD) or spontaneously to form hydrogen peroxide (H_2O_2). The $O_2^{\bullet -}$ can also be protonated to hydroperoxyl radicals ($HO_2^{\bullet}$). Then, $O_2^{\bullet -}$ and $HO_2^{\bullet}$ disproportionate to the basic hydrogen peroxide (HO_2^-) which exists at neutral pH in its protonated form H_2O_2. H_2O_2 can also take part in a Fenton reaction forming hydroxyl radicals ($OH^{\bullet}$) and hydroxyl anions (HO^-) in the presence of iron (Fe^{2+}) which can be reduced by $O_2^{\bullet -}$. $O_2^{\bullet -}$ reacts with H_2O_2 or $OH^{\bullet}$ to produce singlet oxygen ($^1O_2$). $^1O_2$ can also be generated from H_2O_2 and hypochlorite (ClO^-) which is formed in the myeloperoxidase (MPX) reaction. $O_2^{\bullet -}$ and nitric oxide ($NO^{\bullet}$) form peroxynitrite anions ($ONOO^-$) which break into nitrogen dioxide ($NO_2^{\bullet}$) and $OH^{\bullet}$. Further, $ONOO^-$ can react with H_2O_2 to form $^1O_2$, nitroxyl anion (NO^-) and water. Since H_2O_2 gives rise to the formation of the highly reactive $OH^{\bullet}$, ClO^- and $^1O_2$, it is usually detoxified in cells by the action of glutathione peroxidase (GPX) or catalase (CTL).

[60,61]. When ROS production exceeds the antioxidant capacity, this oxidative stress can cause damage of DNA, proteins and polysaccharides as well as lipid peroxidation, uncontrolled proliferation and apoptosis [60,62]. In contrast to this harmful condition, there is now compelling evidence that various stimuli including growth factors, hormones, vasoactive factors, metal ions, etc. can stimulate the enzymatic generation of lower levels of ROS and that these ROS act as second messengers in response to these factors [63,64].

One prominent example of an enzyme which actively generates $O_2^{\bullet -}$ is the NADPH oxidase. This enzyme, initially described to be responsible for the respiratory burst in neutrophils, is composed of two membrane-bound subunits, gp91phox and p22phox, which form the heme-containing catalytic core of the enzyme termed cytochrome b558. Activation of the enzyme requires translocation of the cytoplasmic subunits p67phox, p47phox, p40phox, Rac1 or Rac2 to the membrane and their assembly with cytochrome b558, thus allowing transfer of electrons from NADPH to oxygen resulting in the rapid elevation of $O_2^{\bullet -}$ levels [63–67].

NADPH oxidases have also been identified in non-phagocytic cells including vascular cells and tumor cells [63,66,68,69]. These non-phagocyte NADPH oxidases generate much lower amounts of ROS than the neutrophil enzyme, which may be due to several isoforms of NADPH oxidase subunits. Two groups of gp91phox isoforms have been described according to structural and evolutionary relationships. The five homologues very similar to gp91phox have been termed NOX (NADPH oxidase), where gp91phox is now NOX2, and NOX1 as well as NOX3–NOX5 comprise the new homologues. An evolutionary more distinct group has been named Duox (dual oxidase) [63–67]. In addition, isoforms of p47phox and p67phox termed NOXO1 and NOXA1 have also been characterized, thus further confirming the presence of distinct NADPH oxidases in non-phagocytic cells [66].

Whereas $O_2^{\bullet -}$ is less likely to act as second messenger since it is not freely diffusible, its dismutation product H_2O_2 is more suitable to fulfill such a function. Due to its freely diffusible non-charged character, it can participate in two- and one-electron transfer reactions in the cells. Usually, H_2O_2 is degraded by GPX in the cytosol and mitochondria or by catalase in peroxisomes. However, in the presence of Fe(II), it can also be converted non-enzymatically into hydroxyl anions and highly reactive hydroxyl radicals ($OH^{\bullet}$) in a Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$) [70] which can affect Fe–S clusters or cysteine residues within adjacent proteins. Thus, whereas high amounts of ROS can damage various cellular components, low amounts of ROS can serve signaling functions which trigger the activation of specific pathways and the modulation of gene expression and protein function.

4. ROS and HIF-1α are involved in hypoxic signaling processes

Since the formation of ROS requires molecular oxygen, it has been suggested that ROS may be involved in the response to hypoxia. Indeed, there has been a long-lasting debate about the role of ROS in oxygen sensing stimulated by the ideas that: (a) a heme protein functioning as an oxidase generates ROS as signaling molecules; (b) ROS derived from the mitochondrial respiratory chain could contribute to oxygen signaling; or (c) ROS production may be linked to the activity of signaling components upstream of HIF-1α such as hydroxylases or kinases.

4.1. Heme-containing oxidases modulate ROS levels and HIF-1α under hypoxia

Spectrophotometrical analyses of carotid body preparations and HepG2 cells demonstrated the presence of a non-mitochondrial, b-type cytochrome similar to the cytochrome b558 of the NADPH oxidase from neutrophils. It was thus suggested that a heme-containing oxidase similar to the NADPH oxidase is able to convert O_2 to $O_2^{\bullet -}$ and subsequently to H_2O_2 and that these ROS act as sig-

naling molecules mediating the response to hypoxia [68]. In addition, a pO_2 and NAD(H) regulated non-mitochondrial oxidase activity was found in microsomes containing a cytochrome b558 [71]. Moreover, a cytosolic cytochrome b-type NAD(P)H oxidoreductase was proposed to act as a candidate oxygen sensor [72]. Accordingly, inhibition of heme synthesis and addition of CO to the atmosphere prevented oxygen-dependent expression of the EPO [73] and phosphoenolpyruvate carboxykinase (PCK) gene [74,75]. In addition, application of H_2O_2 repressed the hypoxia-induced EPO production in HepG2 cells and decreased the upregulation of aldolase A, glucokinase and tyrosine hydroxylase by hypoxia in hepatocytes or PC12 cells, respectively [76–79]. Reciprocally, addition of H_2O_2 prevented hypoxic downregulation of the glucagon-dependent PCK expression in primary hepatocytes [77]. The sensitivity of HIF-1 target genes towards application of exogenous oxidants under hypoxia implicated that also HIF-1 itself is redox-sensitive.

First evidence, that this is indeed the case came from experiments in which treatment of purified HIF-1 with H_2O_2 and the oxidative agent diamide abolished HIF-1 DNA-binding activity. In addition, alkylation of free sulfhydryl groups with *N*-ethyl-maleimide led to a loss of DNA-binding activity. This effect could be circumvented by prior addition of dithiothreitol [80] suggesting that HIF-1 DNA-binding activity requires reducing conditions. Indeed, redox factor-1 (Ref-1), a dual function DNA repair endonuclease and redox regulatory protein and its regulator thioredoxin (Trx) have been shown to enhance HIF-1 activity and protein levels under normoxia and hypoxia, respectively [44,81]. In contrast, a redox-inactive Trx (C32S/C35S) markedly decreased HIF-1α protein levels [81]. Subsequently, Ref-1 was found to bind to TADN and TADC with a predominant effect on TADC [45,46]. Within this domain, the oxidation/reduction states of cysteine 800 in HIF-1α and cysteine 848 in HIF-2α are critical for transactivation and recruitment of CBP/p300, SRC-1 and Tie-2 [44–46]. Mutation of cysteine 800 prevented the decrease in HIF-1α TADC activity in response to hydroxyl radicals ($OH^{\bullet}$) formed in a Fenton reaction at the endoplasmic reticulum [82] further indicating that reducing conditions may promote HIF-1α activity under hypoxic conditions.

Further evidence that H_2O_2 and other ROS play an important role in oxygen sensing and HIF regulation came from experiments where addition of H_2O_2 to cells grown under hypoxia resulted in destabilization of the HIF-1α protein in Hep3B cells [44] and of the HIF-2α protein in HeLa cells [83]. Consistently, increased $O_2^{\bullet -}$ production by the xanthine/xanthine oxidase system decreased hypoxia-induced HIF-1α protein levels in renal medullary interstitial cells [84]. The redox-sensitive regulation of HIF-1 activity was also found in nuclear extracts from *D. melanogaster* SL2 cells [16,17] and in the salmonid cell lines RTG-2 and CHSE-214 [85] indicating again evolutionary conservation.

The concept that $O_2^{\bullet -}$ producing NADPH oxidases may be involved in oxygen signaling pathways has elicited a variety of studies in oxygen-sensitive tissues such as the carotid body, neuroepithelial bodies and pulmonary arteries. In mice lacking the gp91phox (NOX2) subunit of the neutrophil NADPH oxidase, oxygen sensing of the pulmonary airway chemoreceptors was prevented [86] whereas hypoxia-dependent electrophysiologic responses in the carotid body and pulmonary artery smooth muscle cells remained intact [87]. In contrast, in mice lacking the NADPH oxidase subunit p47phox, the ventilatory and chemoreceptor responses to hypoxia were elevated whereas EPO production of the kidneys remained unchanged [88]. The involvement of NADPH oxidases in hypoxia and HIF-1 signaling was further supported by in vitro studies. In B-cell lines deficient in p22phox or gp91phox, hypoxia-induced expression of VEGF and aldolase A mRNA was modulated compared to control cells [89]. Furthermore, depletion of p22phox by an anti-sense oligodeoxynucleotide increased HIF-1α levels in rat renal medullary interstitial cells [84] whereas in A549 cells overexpression of NOX1 enhanced hypoxic accumulation of HIF-1α [90].

In addition, the GTPase Rac1 which stimulates NADPH oxidase activity, was activated by hypoxia and a dominant-negative Rac1 mutant repressed hypoxia-dependent HIF-1α protein in Hep3B and HEK293 cells [91]. By contrast, a constitutively active Rac1 mutant inhibited hypoxia-induced HIF-1α accumulation in primary hepatocytes and HepG2 cells [92]. Although the reasons for these contradictory results have not been fully understood, yet, they may relate to variabilities in tissue distribution and function of the different homologues of NOX and possibly other NADPH oxidase subunits as well as to variations in oxygen and redox sensitivity of different tissues and cell types. These findings, however, confirm the complex role of NADPH oxidases and ROS in oxygen sensing and activation of HIF-1.

4.2. Mitochondria modulate ROS levels and HIF-1α under hypoxia

Mitochondria have long been implicated to play a role in the hypoxic response since inhibitors of the mitochondrial respiratory chain have been shown to mimic hypoxia in the carotid body [93–95]. Two different concepts have been proposed to explain how the response to hypoxia can be related to mitochondrial function. First, decreased mitochondrial ROS production would mediate the hypoxic response [96] or second, increased ROS generation would allow the hypoxic response [97].

The first concept is based on the assumption that under O_2 limiting conditions ROS formation is diminished. Indeed, during alveolar hypoxia pulmonary ROS production was reduced [96]. This has been supported by findings in pulmonary arteries where hypoxia and the proximal respiratory chain inhibitors rotenone and antimycin A decreased generation of ROS as measured by three different techniques (lucigenin-based chemiluminescence, the AmplexRed H_2O_2 assay kit and 2′,7′-dihydrochlorofluorescin (DCFH) fluorescence) [98]. Consistently, many other laboratories reported

decreased ROS formation under hypoxia in the lung, hepatocytes and cardiac myocytes [77,99,100].

Although somehow counterintuitive, the concept that hypoxia increases ROS production was based on findings that ROS levels were elevated under hypoxia in pulmonary myocytes [101], cardiac myocytes, Hep3B cells [97], HeLa cells [102] and adipocytes [103]. Since in hepatocytes pO_2 levels below 2–3 mmHg decrease cytochrome c oxidase activity [97], it was proposed that hypoxia is sensed by cytochrome c oxidase which then reduces its V_{max}, thereby enhancing the half-life of reduced electron carriers upstream of cytochrome aa3 such as ubisemiquinone anions. The ubisemiquinone anion may then transfer an electron to molecular O_2 yielding $O_2^{\bullet -}$ which is then dismutated to the signal molecule H_2O_2 [104]. Thus, inhibitors which block the respiratory chain upstream of ubisemiquinone such as rotenone and myxothiazol would prevent ROS formation whereas downstream inhibitors such as cyanide or azide would increase ROS levels.

Interestingly, most of the studies showing increased ROS production under hypoxia were performed by exposing cells for several hours to the non-fluorescent dye DCFH which is converted by ROS to fluorescent dichlorofluorescein (DCF) [105]. However, ROS measurements by DCF are under debate since it has been shown that DCFH can autooxidize and that deacetylation of DCFH–DA can produce H_2O_2 [106,107]. In addition, reduced hemeproteins can oxidize DCFH much better than oxidized hemeproteins [108]. In line with this, cytochrome c released from mitochondria of preapoptotic cells has been shown to catalyse DCFH oxidation [109]. Thus, prolonged hypoxia may enhance DCF fluorescence by initiating a preapoptotic response. In addition, DCFH and DCF are both substrates for transporters of the multidrug-resistance family (MDR)/transporter associated with antigen processing (TAP) subfamily including the MDR proteins. In hepatocytes, hypoxia has been shown to downregulate MDR1B expression [110]. This would result in a reduced outward transport leading to accumulation of DCF under prolonged hypoxia. Upon return to normoxia, DCF fluorescence would decrease due to reactivation of the transport. Such a scenario may help to explain observations that in spite of the apparent irreversibility of DCFH conversion into DCF, DCF fluorescence increased in embryonic cardiomyocytes exposed to decreasing O_2 tensions, but decreased upon return of the cells to normoxia [105]. Thus, although DCFH is among the most frequently used fluorophores for ROS measurements, it should be considered that the sensitivity of this assay could be influenced by various conditions not directly related to intracellular ROS generation.

A role for mitochondrial ROS production in the regulation of HIF was also suggested from studies, where rotenone and myxothiazol decreased DCF fluorescence and prevented HIF-1α accumulation and activation of its target genes by hypoxia [97]. By contrast, in cells lacking mitochondrial DNA (Hep3Bρ0) hypoxia did not enhance DCF fluorescence and HIF-1α levels [97,111]. These data suggested that inhibition of mitochondrial ROS production would prevent the hypoxic response. However, antimycin A which enhances mitochondrial ROS production did not mimic the hypoxic response in Hep3B [97,104] and HEK293 cells [112]. The relevance of the mitochondrial respiratory chain as an ubiquitous oxygen sensor was further questioned since mitochondrial production of ROS was not involved in O_2 sensing in the airway chemoreceptor cell line H146 [113]. Similarly, H_2O_2 levels decreased under hypoxia in Hep3Bρ0 as well as in control cells suggesting that the source of H_2O_2 was not located in the mitochondria [114]. Consistently, hypoxia-dependent induction of HIF-1 was neither affected by rotenone or a lack of mitochondria in Hep3B and HeLa cells [102,114,115] nor by defects in genes encoding different components of the electron transport chain in the Chinese hamster ovary cell lines Gal32 and CCLI 6B-2 [114,115]. Further, hypoxia-mediated upregulation of GLUT1 was mediated by binding of HIF-1 to the hypoxia responsive element (HRE), whereas the induction of GLUT1 by antimycin A or rotenone involved a serum response element [116]. Moreover, inhibition of mitochondria-derived ROS did not prevent HIF-1α stabilization in gastric cancer cells infected with *Helicobacter pylori* [117].

In summary, these data support the concept that modulation of ROS levels have an important impact on the hypoxic response mediated by HIF-1α. However, the sources of ROS generation, the exact kinetics and conditions of ROS production and their specific relevance to HIF-1α activation are not completely resolved, yet.

5. The role of ROS in the regulation of HIF-1α in response to non-hypoxic stimuli

In addition to hypoxia, a variety of non-hypoxic stimuli including growth factors, hormones, vasoactive peptides and metal ions can induce HIF-1α under normoxia. Many of these factors can stimulate ROS production as part of their signaling cascades. Thus, several studies aimed to investigate the role of ROS in the control of HIF-1α under non-hypoxic conditions. Indeed, treatment with the antioxidant ascorbate inhibited ROS production and prevented accumulation of HIF-1α and activation of the HIF pathway by thrombin and $CoCl_2$ in pulmonary artery smooth muscle cells (PASMC) [118] or by IGF-1 in human prostate adenocarcinoma PC3 cells [119]. These studies confirmed previous observations in aortic smooth muscle cells where treatment with ascorbate completely prevented HIF-1α protein expression and nuclear translocation in response to thrombin, PDGF, TGF-β and activated platelets and inhibited HIF-1-dependent expression of VEGF and PAI-1 [50]. Other antioxidants including Vitamin E and pyrrolidinedithiocarbamate (PDTC) had similar effects in thrombin-treated smooth muscle cells [50], $CoCl_2$-treated Hep3B cells [104] and in *H. pylori*-infected gastric cancer cells [117]. These observations have also been confirmed in vivo since pigs receiving Vitamins C and E along with their high cholesterol diet did not show elevated ROS, HIF-1α and VEGF levels [120].

Further, an important role of the endogenous glutathione system in non-hypoxic HIF-1α regulation has been suggested since *N*-acetyl cysteine (NAC), a precursor of glutathione which is the substrate of GPX, diminished thrombin-induced ROS generation and HIF-1α protein accumulation in aortic smooth muscle cells [50]. Similarly, NAC reduced $CoCl_2$-, oxLDL-, arsenite- and HGF-dependent activation of the HIF system in osteoblasts [121], macrophages [122], human ovary cancer cells [55] or HepG2 cells [123], respectively. The importance of the glutathione system in the regulation of HIF-1 was further substantiated by studies where cytosolic GPX was overexpressed in PASMC resulting in the inhibition of HIF-1α accumulation by thrombin and $CoCl_2$ [118]. Interestingly, overexpression of GPX in PASMC [118] or treatment of alveolar type II epithelial cells with NAC enhanced HIF-1α levels under unstimulated conditions [124]. Similarly, cysteamine increased HIF-1α levels in the duodenum of rats [125]. These findings suggest that activation of the glutathione system may help to accumulate HIF-1α under steady-state conditions by a so far unknown mechanism whereas it may act as a true antioxidant in response to factors increasing ROS levels.

By contrast, cytoplasmic, mitochondrial and peroxisomal overexpression of catalase which also acts as an antioxidant by decomposing H_2O_2 had no effect on HIF-1α levels in HepG2 cells and PASMC under control conditions [115,118]. However, expression of catalase prevented the induction of HIF-1α by thrombin and $CoCl_2$ in PASMC [118]. Further, exogenous addition of catalase decreased angiotensin II-induced HIF-1α levels in smooth muscle cells [51] and chromium-induced HIF-1α levels in prostate carcinoma cells [56] as well as the induction of HIF-1α by the Epstein-Barr virus latent membrane protein 1 in epithelial cells [126] but had no effect on arsenite-induced HIF-1α levels in ovarian carcinoma cells [55].

While GPX and catalase reduced agonist-stimulated ROS levels, overexpression of Cu/Zn superoxide dismutase (Cu/ZnSOD) promoted the formation of H_2O_2 and increased HIF-1α levels in PASMC, thus mimicking the response to $CoCl_2$ or thrombin [118]. Similarly, different preparations of a cell-permeable SOD enhanced HIF-1α levels under normoxia in rat renal medullary interstitial cells and human cerebral vascular smooth muscle cells and simulated the effect of chromium in prostate carcinoma cells [127] whereas overexpression of extracellular SOD decreased $CoCl_2$- and hypoxia-induced HIF-1α levels in Hep3B cells [128]. These findings point towards an important role of H_2O_2 in the regulation of HIF-1α also under normoxic conditions although the mode of how ROS regulate HIF-1α under normoxia appears to be reciprocal to the action under hypoxia.

Whereas H_2O_2 treatment prevented hypoxic accumulation of HIF-1α and its target genes (see above), increased levels of HIF-1α protein under normoxia have been detected in H_2O_2-treated aortic smooth muscle cells [50] and Hep3B cells [104]. Further, a threshold concentration of H_2O_2 may exist since low concentrations of H_2O_2 (10–50 μM) increased HIF-1α levels, whereas high concentrations of H_2O_2 prevented HIF-1α accumulation [50]. Thus, only slight changes in the redox state may be required to activate the HIF pathway. However, the sensitivity of the HIF system towards H_2O_2 may also depend on the cell type since H_2O_2 did not activate HIF-1α in urinary bladder carcinoma ECV304 cells [50]. Moreover, the redox-sensitive NFκB pathway may be involved in the regulation of HIF-1α by TNF-α [129] or by thrombin (A. Görlach, unpublished results), thus suggesting a cross-talk between different redox-sensitive cascades in normoxic cells.

Interestingly, NADPH oxidases have been implicated as important sources of ROS in response to many factors which are also able to upregulate HIF-1α including thrombin [50], PDGF, angiotensin II [51] and mechanical stress [57]. Indeed, inhibition of this enzyme with anti-sense oligodeoxynucleotides or vectors encoding dominant-negative Rac prevented activation of the HIF pathway by thrombin in smooth muscle and endothelial cells [50] as well as by carbachol in HEK cells [54]. Consistently, overexpression of the NADPH oxidase subunit NOX1 increased HIF-1α levels in A549 cells [90]. The findings that NADPH oxidases are crucially involved in the regulation of HIF-1α was recently substantiated by the observation that both HIF-1α and VEGF levels were elevated in carotid lesions in mice overexpressing p22phox in smooth muscle cells compared to wild-type mice [130] further supporting the view that ROS derived from NADPH oxidases may be important in regulating the HIF pathway under non-hypoxic conditions in many cell types.

6. ROS and HIF proline and asparagine hydroxylases

HIF-1α is stabilized under hypoxia but rapidly degraded under normoxia. The degradation process is initiated by hydroxylation of proline 402 and proline 564 which mediate VHL binding, ubiquitination and subsequent degradation by the proteasome. In addition, a second hydroxylation occurs at asparagine 803 which prevents recruitment of CBP/p300. The hydroxylation process at the proline residues can be carried out by PHD1–3, while the asparaginyl hydroxylase activity was found to be associated with a TADC interacting protein known as factor inhibiting HIF-1 (FIH-1) [40,41,43,131,132].

Although differences in cellular expression and substrate specificity have been reported all these enzymes belong to a family of dioxygenases which are strictly dependent on the presence of O_2 and further require 2-oxoglutarate and Fe(II) at the reaction center [25,38,133,134]. In addition, ascorbate is necessary to achieve full activity, presumably to maintain iron in the Fe(II) state indicating that the catalytic hydroxylation process exerted by these enzymes requires a radical cycling system [37] and supporting the important role of ROS in regulating HIF-1α. Thus, changes in the cellular levels of any of these substrates, products or cofactors would have the potential to influence hydroxylase activity. Indeed, treat-

ment of vascular smooth muscle cells with ascorbate has been shown to mediate HIF-1α degradation [50]. This appeared to be due to increased PHD activity [119] since under physiological conditions the ascorbate levels are in the range of 25–50 μM which is far below the K_m for ascorbate of about 140–170 μM [133]. It is thus conceivable that changes in the ascorbate levels can significantly alter hydroxylase activities. The mechanism how ascorbate regulates hydroxylase activity is not completely solved, yet. On the one hand, it is proposed that ascorbate reduces Fe(III) to Fe(II) within the enzyme active site, thus rendering the enzyme active after it became inactive due to spontaneous Fe(II) to Fe(III) self-oxidation. On the other hand, ascorbate might enhance the provision of Fe(II) from an intracellular pool such as ferritin by conversion of Fe(III) into Fe(II). This may explain why the iron chelator desferrioxamine enhances HIF-1α levels whereas addition of ascorbate antagonized this effect [119]. Moreover, addition of iron to cultured human prostate adenocarcinoma PC3 cells stimulated HIF-1α degradation [119] possibly by rendering HIF-1α susceptible to further modifications exerted by hydroxyl radicals ($OH^{\bullet}$). These radicals could be generated in a Fenton reaction and would be able to reconvert ferric iron into ferrous iron or to facilitate the recycling of dehydroascorbate into ascorbate. Both processes would increase PHD activity leading to the degradation of HIF-1α. Indeed, under normoxic conditions, hydroxyl radicals colocalized with HIF-1α and VHL within the endoplasmic reticulum (ER) [82,135]. Dihydrorhodamine scavenged $OH^{\bullet}$ generation at the ER and enhanced HIF-1α protein levels and transactivation potential due to inhibition of prolyl hydroxylase activity and Ref-1-mediated reduction of Cys800 in the TADC [82]. Since PHD2, PHD3 and FIH have been shown to be distributed in the cytoplasm whereas PHD1 was exclusively detected in the nucleus it is tempting to speculate that at least some of the hydroxylases may colocalize to the ER under normoxic conditions [136]. Thus, ROS may have the potential to interfere with the complex regulation of PHDs thereby promoting a state with either higher or lower activity which may explain some of the contradictory results on the regulation of HIF-1α by ROS.

7. ROS and regulation of HIF-1α by protein kinase B and MAP kinases

In many cells, ROS have been shown to act via the activation of kinases and/or the inactivation of phosphatases. The first evidence indicating that phosphorylation reactions are involved in the regulation of HIF-1 activity were obtained by electrophoretic mobility shift assay (EMSA) which showed that treatment of nuclear extracts from hypoxic Hep3B cells with phosphatase disrupts the HIF-1/DNA complex [137]. Subsequently, several kinases including phosphatidylinositol 3-kinase (PI3-K) and protein kinase B (PKB/Akt) as well as MAP kinases have been shown to be activated under hypoxia and to influence HIF-1α [51,138–141].

A role for the PI3-K/PKB pathway in the regulation of HIF-1 was first described in NIH3T3R cells where dominant-negative vectors for PI3-K or for PKB impaired the activation of HIF-1 as well as VEGF transcription under hypoxia [138]. Further, glioblastoma cells [142] or the prostate carcinoma cells PC-3 and DU145, which are deficient of the PI(345)P_3 phosphatase and tumor suppressor PTEN [143] and thus have enhanced PKB activity displayed enhanced HIF-1α levels. In line with this, overexpression of constitutively active PKB enhanced HIF-1α levels and stimulated HIF-1-dependent PAI-1 expression in primary rat hepatocytes and HepG2 cells [49].

Moreover, many non-hypoxic stimuli of HIF-1α such as thrombin, insulin, IGF, vanadate and arsenite are able to activate the PI3-K/PKB pathway in a redox-sensitive manner. Antioxidants or inhibitors of NADPH oxidases prevented the thrombin-dependent PI3-K/PKB signaling to HIF-1α and the HIF target genes PAI-1 and VEGF in smooth muscle cells [50]. Similarly, activation of PI3-K/PKB by vanadate or arsenite was diminished by catalase in prostate cancer DU145 cells [144,145] suggesting that H_2O_2 may mediate the kinase response to HIF-1α. Indeed, H_2O_2 has been shown to activate PKB in smooth muscle and endothelial cells [146] as well as in different tumor cells under normoxia.

Although PKB induces HIF-1α stabilization [147], translation [148] or coactivator recruitment [149], HIF-1α is not a direct substrate for PKB. Instead, the PKB-dependent HIF-1α activation appears to involve the PKB targets HDM2 [150], GSK3β [151] or mammalian target of rapamycin (mTOR). The latter may act on HIF-1α stability via the ODD domain [152] as well as via p70S6 kinase and S6 kinase [48] which in turn may activate HIF-1α translation. GSK3β may also directly target the HIF-1α ODD [140] and thus contribute to the downregulation of HIF-1α after prolonged hypoxia [151]. Moreover, it was also shown that PKB may influence HIF-1α stability via its target FOXO4 [153].

In addition to PI3-K/PKB, also MAP kinases have been shown to be involved in the regulation of HIF-1α in response to hypoxia and to non-hypoxic stimuli. Indeed, ERK1/2 was activated by hypoxia in endothelial cells [154] and hepatocytes [155]. Further, ERK1/2 also contributed to angiotensin II-dependent activation of HIF-1 in smooth muscle cells [51], to IL-1β-induced HIF-1α expression in cytotrophoblast cells [156], to PGE2-mediated HIF-1α expression in colon carcinoma cells [157] or to shock wave-induced HIF-1α expression in osteoblasts [158]. Interestingly, whereas shock waves enhanced the production of $O_2^{\bullet -}$ via Rac1 subsequently leading to ERK1/2 activation and the induction of HIF-1α and VEGF levels [158] thrombin-induced activation of the NADPH oxidase did not lead to ERK1/2 and HIF-1 activation in smooth muscle cells [50] suggesting that the ROS sensitivity of ERK1/2 and the induction of HIF-1α are stimulus- and cell type-dependent.

Furthermore, overexpression of the p38 upstream kinases MKK3 and MKK6 enhanced HIF-1α under normoxic and hypoxic conditions [159] supporting a role for this kinase family in the regulation of HIF-1α. Indeed, hypoxia activates

mainly p38α and/or p38γ [160]. The p38 MAP kinases are also activated via ROS by many growth factors, hormones and extracellular stressors like viruses or metal ions. In line with this, inhibition of p38 prevented ROS-dependent induction of HIF-1α by thrombin [50]. Similarly, chromium(VI) induced HIF-1α expression and activity was mediated by H_2O_2 and subsequent activation of p38 in DU145 human prostate carcinoma cells [56]. Although the exact sites of HIF-1α phosphorylation by p38 MAP kinases as well as by ERK1/2 have not been identified, yet, but may be located in the regulatory/inhibitory domain [161], these findings demonstrate an important role of these kinases in the redox-sensitive regulation of HIF-1α under hypoxic and non-hypoxic conditions.

The activity of these kinases is tightly regulated by a variety of protein phosphatases including the dual specificity MAPK phosphatases (MKP). Importantly, phosphatases have been shown to be redox-sensitive since oxidation of their catalytic cysteines blocks the capacity of these enzymes to dephosphorylate and thus inactive their target kinases [162–164]. Thus, ROS, mainly derived from NADPH oxidases, appear to play an important role in regulating phosphatases including MKP-1 and MKP-3 as well as PP2a in response to hormones and growth factors [165]. Interestingly, despite an initial reduction in phosphatase activity [166], the expression of MKP-1 was increased by hypoxia and downregulation of MKP-1 increased HIF-1α phosphorylation and transcriptional activity [167] whereas overexpression of MKP-3 reduced HIF-1α phosphorylation in fibroblasts [168]. These findings suggest a complex regulatory pattern of HIF-1α by phosphatases depending on the expression of different phosphatases as well as on their redox state and may explain some of the apparently contradictory results on the regulation of HIF-1α by ROS under hypoxic and non-hypoxic conditions.

8. Conclusion

There is now compelling evidence that ROS play an important role in the regulation of HIF-1α under normoxia and hypoxia. Under normoxia, enhanced ROS levels in response to many agonists appear to serve as signaling molecules to upregulate HIF-1α in a variety of cell types, possibly by modulating upstream signaling pathways such as hydroxylases or kinases and phosphatases. In contrast, the role of ROS in the regulation of HIF-1α under hypoxia is less well understood. A number of investigations support the concept that heme-containing enzymes such as “low output” NADPH oxidases or a cytochrome b-type NAD(P)H oxidoreductase or mitochondria may produce ROS in dependence on the pO_2. Among the ROS generated, H_2O_2 may play a critical role by modulating asparagine and proline hydroxylase activities and thus influencing cofactor recruitment and proteasomal HIF-1α degradation, respectively. The complexity of this regulation is further potentiated by variabilities in the specific experimental system used with regard to the cell type, the amount of oxygen available or the measuring techniques. All these variables complicate a more detailed analysis of the processes how ROS affect HIF-1α under hypoxia. Cross-talks between different signaling cascades activated by high or low ROS levels may also influence the stability and/or activity of HIF-1α, thus allowing to fine tune HIF-1-dependent target gene expression in a cell-type specific manner (Fig. 2). Thus, depending on the presence of a more “oxidant” state which is prevalent in response to non-hypoxic stimuli, or on the presence of a more “reducing” state as it can be found under hypoxia, a shift of the redox state to a more reducing or a more oxidant state, respectively, may limit activation of the HIF system and may thus be beneficial for treating or preventing pathologic processes associated with enhanced HIF-1α levels.

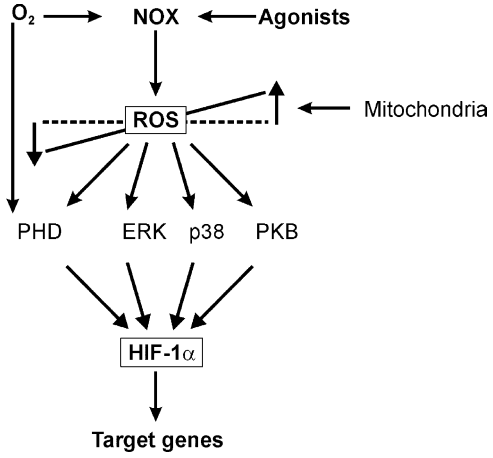

Fig. 2. Involvement of ROS in HIF-1α regulation. See text for explanation.

Acknowledgements

Work in the authors’ laboratories is supported by the Deutsche Forschungsgemeinschaft grants GO 709/4-1 and GRK438 and by the sixth framework programme of the European Commission (EUROXY) to AG and by Deutsche Forschungsgemeinschaft grants SFB 402 Teilprojekt A1 and GRK 335, Deutsche Krebshilfe 106429 and grants from Fonds der Chemischen Industrie to TK.

References

[1] Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992;12:5447–54.
[2] Beck I, Weinmann R, Caro J. Characterization of hypoxia-responsive enhancer in the human erythropoietin gene shows presence of hypoxia-inducible 120-Kd nuclear DNA-binding protein in erythropoietin-producing and nonproducing cells. Blood 1993;82:704–11.
[3] Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 1995;270:1230–7.

[4] Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix–loop–helix–PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA 1995;92:5510–4.
[5] Hoffman EC, Reyes H, Chu FF, Sander F, Conley LH, Brooks BA, et al. Cloning of a factor required for activity of the Ah (dioxin) receptor. Science 1991;252:954–8.
[6] Wenger RH. Cellular adaptation to hypoxia: O_2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O_2-regulated gene expression. FASEB J 2002;16:1151–62.
[7] Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev 1997;11:72–82.
[8] Scortegagna M, Ding K, Oktay Y, Gaur A, Thurmond F, Yan LJ, et al. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1−/− mice. Nat Genet 2003;35:331–40.
[9] Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. Mol Cell Biol 2003;23:9361–74.
[10] Sowter HM, Raval RR, Moore JW, Ratcliffe PJ, Harris AL. Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. Cancer Res 2003;63:6130–4.
[11] Kietzmann T, Cornesse Y, Brechtel K, Modaressi S, Jungermann K. Perivenous expression of the mRNA of the three hypoxia-inducible factor alpha-subunits, HIF1alpha, HIF2alpha and HIF3alpha, in rat liver. Biochem J 2001;354:531–7.
[12] Hara S, Hamada J, Kobayashi C, Kondo Y, Imura N. Expression and characterization of hypoxia-inducible factor (HIF)-3alpha in human kidney: suppression of HIF-mediated gene expression by HIF-3alpha. Biochem Biophys Res Commun 2001;287:808–13.
[13] Makino Y, Kanopka A, Wilson WJ, Tanaka H, Poellinger L. Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3alpha locus. J Biol Chem 2002;277:32405–8.
[14] Makino Y, Cao R, Svensson K, Bertilsson G, Asman M, Tanaka H, et al. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. Nature 2001;414:550–4.
[15] Jiang H, Guo R, Powell-Coffman JA. The *Caenorhabditis elegans* hif-1 gene encodes a bHLH–PAS protein that is required for adaptation to hypoxia. Proc Natl Acad Sci USA 2001;98:7916–21.
[16] Nagao M, Ebert BL, Ratcliffe PJ, Pugh CW. *Drosophila melanogaster* SL2 cells contain a hypoxically inducible DNA binding complex which recognises mammalian HIF-binding sites. FEBS Lett 1996;387:161–6.
[17] Bacon NC, Wappner P, O'Rourke JF, Bartlett SM, Shilo B, Pugh CW, et al. Regulation of the *Drosophila* bHLH–PAS protein Sima by hypoxia: functional evidence for homology with mammalian HIF-1 alpha. Biochem Biophys Res Commun 1998;249:811–6.
[18] Görlach A, Camenisch G, Kvietikova I, Vogt L, Wenger RH, Gassmann M. Efficient translation of mouse hypoxia-inducible factor-1alpha under normoxic and hypoxic conditions. Biochim Biophys Acta 2000;1493:125–34.
[19] Gross J, Rheinlander C, Fuchs J, Mazurek B, Machulik A, Andreeva N, et al. Expression of hypoxia-inducible factor-1 in the cochlea of newborn rats. Hear Res 2003;183:73–83.
[20] Pascual O, Denavit-Saubie M, Dumas S, Kietzmann T, Ghilini G, Mallet J, et al. Selective cardiorespiratory and catecholaminergic areas express the hypoxia-inducible factor-1alpha (HIF-1alpha) under in vivo hypoxia in rat brainstem. Eur J Neurosci 2001;14:1981–91.
[21] Heidbreder M, Frohlich F, Johren O, Dendorfer A, Qadri F, Dominiak P. Hypoxia rapidly activates HIF-3alpha mRNA expression. FASEB J 2003;17:1541–3.
[22] Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 1998;95:7987–9792.
[23] Pugh CW, O'Rourke JF, Nagao M, Gleadle JM, Ratcliffe PJ. Activation of hypoxia-inducible factor-1: definition of regulatory domains within the alpha subunit. J Biol Chem 1997;272:11205–14.
[24] Jiang BH, Zheng JZ, Leung SW, Roe R, Semenza GL. Transactivation and inhibitory domains of hypoxia-inducible factor 1alpha. Modulation of transcriptional activity by oxygen tension. J Biol Chem 1997;272:19253–60.
[25] Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 2001;292:468–72.
[26] Masson N, Willam C, Maxwell PH, Pugh CW, Ratcliffe PJ. Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J 2001;20:5197–206.
[27] Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999;399:271–5.
[28] Tanimoto K, Makino Y, Pereira T, Poellinger L. Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. EMBO J 2000;19:4298–309.
[29] Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, et al. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. Nat Cell Biol 2000;2:423–7.
[30] Min JH, Yang H, Ivan M, Gertler F, Kaelin Jr WG, Pavletich NP. Structure of an HIF-1alpha–pVHL complex: hydroxyproline recognition in signaling. Science 2002;296:1886–9.
[31] Salceda S, Caro J. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 1997;272:22642–7.
[32] Kaelin WGJ, Maher ER. The VHL tumour-suppressor gene paradigm. Trends Genet 1998;14:423–6.
[33] Kamura T, Sato S, Haque D, Liu L, Kaelin WGJ, Conaway RC, et al. The elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. Genes Dev 1998;12:3872–81.
[34] Kallio PJ, Wilson WJ, O'Brien S, Makino Y, Poellinger L. Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway. J Biol Chem 1999;274:6519–25.
[35] Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 2001;292:464–8.
[36] Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science 2001;294:1337–40.
[37] Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, et al. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001;107:43–54.
[38] Ivan M, Haberberger T, Gervasi DC, Michelson KS, Gunzler V, Kondo K, et al. Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. Proc Natl Acad Sci USA 2002;99:13459–64.
[39] Oehme F, Ellinghaus P, Kolkhof P, Smith TJ, Ramakrishnan S, Hutter J, et al. Overexpression of PH-4, a novel putative proline 4-hydroxylase, modulates activity of hypoxia-inducible transcription factors. Biochem Biophys Res Commun 2002;296:343–9.
[40] Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev 2001;15:2675–86.
[41] Hewitson KS, McNeill LA, Riordan MV, Tian YM, Bullock AN, Welford RW, et al. Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. J Biol Chem 2002;277:26351–5.

[42] Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 2002;16:1466–71.
[43] Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science 2002;295:858–61.
[44] Huang LE, Arany Z, Livingston DM, Bunn HF. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 1996;271:32253–9.
[45] Ema M, Hirota K, Mimura J, Abe H, Yodoi J, Sogawa K, et al. Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. EMBO J 1999;18:1905–14.
[46] Carrero P, Okamoto K, Coumailleau P, O'Brien S, Tanaka H, Poellinger L. Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha. Mol Cell Biol 2000;20:402–15.
[47] Zelzer E, Levy Y, Kahana C, Shilo BZ, Rubinstein M, Cohen B. Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. EMBO J 1998;17:5085–94.
[48] Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E. Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway. J Biol Chem 2002;277:27975–81.
[49] Kietzmann T, Samoylenko A, Roth U, Jungermann K. Hypoxia-inducible factor-1 and hypoxia response elements mediate the induction of plasminogen activator inhibitor-1 gene expression by insulin in primary rat hepatocytes. Blood 2003;101:907–14.
[50] Görlach A, Diebold I, Schini-Kerth VB, Berchner-Pfannschmidt U, Roth U, Brandes RP, et al. Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase. Circ Res 2001;89:47–54.
[51] Richard DE, Berra E, Pouyssegur J. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. J Biol Chem 2000;275:26765–71.
[52] Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 2002;277:38205–11.
[53] Stiehl DP, Jelkmann W, Wenger RH, Hellwig-Burgel T. Normoxic induction of the hypoxia-inducible factor 1alpha by insulin and interleukin-1beta involves the phosphatidylinositol 3-kinase pathway. FEBS Lett 2002;512:157–62.
[54] Hirota K, Fukuda R, Takabuchi S, Kizaka-Kondoh S, Adachi T, Fukuda K, et al. Induction of hypoxia-inducible factor 1 activity by muscarinic acetylcholine receptor signaling. J Biol Chem 2004;279:41521–8.
[55] Duyndam MC, Hulscher TM, Fontijn D, Pinedo HM, Boven E. Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1alpha protein by the oxidative stressor arsenite. J Biol Chem 2001;276:48066–76.
[56] Gao N, Jiang BH, Leonard SS, Corum L, Zhang Z, Roberts JR, et al. p38 signaling-mediated hypoxia-inducible factor 1alpha and vascular endothelial growth factor induction by Cr(VI) in DU145 human prostate carcinoma cells. J Biol Chem 2002;277:45041–8.
[57] Kim CH, Cho YS, Chun YS, Park JW, Kim MS. Early expression of myocardial HIF-1alpha in response to mechanical stresses: regulation by stretch-activated channels and the phosphatidylinositol 3-kinase signaling pathway. Circ Res 2002;90:E25–33.
[58] Finkel T. Reactive oxygen species and signal transduction. IUBMB Life 2001;52:3–6.
[59] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases—the role of oxidant stress. Circ Res 2000;87:840–4.
[60] Fre BA, Crapo JD. Free-radicals and tissue-injury. Lab Invest 1982;47:412–26.
[61] Brigelius-Flohe R, Banning A, Schnurr K. Selenium-dependent enzymes in endothelial cell function. Antioxid Redox Signal 2003;5:205–15.
[62] Cadenas E, Sies H. Oxidative stress—excited oxygen species and enzyme-activity. Adv Enzyme Regul 1984;23:217–37.
[63] Bokoch GM, Knaus UG. NADPH oxidases: not just for leukocytes anymore! Trends Biochem Sci 2003;28:502–8.
[64] Görlach A, Kietzmann T, Hess J. Redox signaling through NADPH oxidases: involvement in vascular proliferation and coagulation. Ann N Y Acad Sci 2002;973:505–7.
[65] Babior BM. NADPH oxidase. Curr Opin Immunol 2004;16:42–7.
[66] Griendling KK. Novel NAD(P)H oxidases in the cardiovascular system. Heart 2004;90:491–3.
[67] Lambeth JD. NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 2004;4:181–9.
[68] Görlach A, Holtermann G, Jelkmann W, Hancock JT, Jones SA, Jones OT, et al. Photometric characteristics of haem proteins in erythropoietin-producing hepatoma cells (HepG2). Biochem J 1993;290:771–6.
[69] Görlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ Res 2000;87:26–32.
[70] Walling C. Fenton's reagent revisited. Acc Chem Res 1975;8:125–31.
[71] Mohazzab H, Kaminski PM, Wolin MS. Lactate and pO_2 modulate superoxide anion production in bovine cardiac myocytes: potential role of NADH oxidase. Circulation 1997;96:614–20.
[72] Zhu H, Qiu H, Yoon HW, Huang S, Bunn HF. Identification of a cytochrome b-type NAD(P)H oxidoreductase ubiquitously expressed in human cells. Proc Natl Acad Sci USA 1999;96:14742–7.
[73] Goldberg MA, Dunning SP, Bunn HF. Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science 1988;242:1412–5.
[74] Kietzmann T, Schmidt H, Probst I, Jungermann K. Modulation of the glucagon-dependent activation of the phosphoenolpyruvate carboxykinase gene by oxygen in rat hepatocyte cultures. Evidence for a heme protein as oxygen sensor. FEBS Lett 1992;311:251–5.
[75] Kietzmann T, Schmidt H, Unthan FK, Probst I, Jungermann K. A ferro-heme protein senses oxygen levels, which modulate the glucagon-dependent activation of the phosphoenolpyruvate carboxykinase gene in rat hepatocyte cultures. Biochem Biophys Res Commun 1993;195:792–8.
[76] Fandrey J, Frede S, Jelkmann W. Role of hydrogen peroxide in hypoxia-induced erythropoietin production. Biochem J 1994;303:507–10.
[77] Kietzmann T, Freimann S, Bratke J, Jungermann K. Regulation of the gluconeogenic phosphoenolpyruvate carboxykinase and glycolytic aldolase A gene expression by O_2 in rat hepatocyte cultures. Involvement of hydrogen peroxide as mediator in the response to O_2. FEBS Lett 1996;388:228–32.
[78] Kietzmann T, Roth U, Freimann S, Jungermann K. Arterial oxygen partial pressures reduce the insulin-dependent induction of the perivenously located glucokinase in rat hepatocyte cultures: mimicry of arterial oxygen pressures by H_2O_2. Biochem J 1997;321:17–20.
[79] Kroll SL, Czyzyk KM. Role of H_2O_2 and heme-containing O_2 sensors in hypoxic regulation of tyrosine hydroxylase gene expression. Am J Physiol 1998;274:C167–74.
[80] Wang GL, Jiang BH, Semenza GL. Effect of altered redox states on expression and DNA-binding activity of hypoxia-inducible factor 1. Biochem Biophys Res Commun 1995;212:550–6.

[81] Welsh SJ, Bellamy WT, Briehl MM, Powis G. The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1alpha protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis. Cancer Res 2002;62:5089–95.
[82] Liu Q, Berchner-Pfannschmidt U, Moller U, Brecht M, Wotzlaw C, Acker H, et al. A Fenton reaction at the endoplasmic reticulum is involved in the redox control of hypoxia-inducible gene expression. Proc Natl Acad Sci USA 2004;101:4302–7.
[83] Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, et al. Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. Blood 1998;92:2260–8.
[84] Yang ZZ, Zhang AY, Yi FX, Li PL, Zou AP. Redox regulation of HIF-1alpha levels and HO-1 expression in renal medullary interstitial cells. Am J Physiol Renal Physiol 2003;284:F1207–15.
[85] Nikinmaa M, Pursiheimo S, Soitamo AJ. Redox state regulates HIF-1alpha and its DNA binding and phosphorylation in salmonid cells. J Cell Sci 2004;117:3201–6.
[86] Fu XW, Wang D, Nurse CA, Dinauer MC, Cutz E. NADPH oxidase is an O_2 sensor in airway chemoreceptors: evidence from K^+ current modulation in wild-type and oxidase-deficient mice. Proc Natl Acad Sci USA 2000;97:4374–9.
[87] Archer SL, Reeve HL, Michelakis E, Puttagunta L, Waite R, Nelson DP, et al. O_2 sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. Proc Natl Acad Sci USA 1999;96:7944–9.
[88] Sanders KA, Sundar KM, He L, Dinger B, Fidone S, Hoidal JR. Role of components of the phagocytic NADPH oxidase in oxygen sensing. J Appl Physiol 2002;93:1357–64.
[89] Wenger RH, Marti HH, Schuerer MC, Kvietikova I, Bauer C, Gassmann M, et al. Hypoxic induction of gene expression in chronic granulomatous disease-derived B-cell lines: oxygen sensing is independent of the cytochrome b558-containing nicotinamide adenine dinucleotide phosphate oxidase. Blood 1996;87:756–61.
[90] Goyal P, Weissmann N, Grimminger F, Hegel C, Bader L, Rose F, et al. Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. Free Radic Biol Med 2004;36:1279–88.
[91] Hirota K, Semenza GL. Rac1 activity is required for the activation of hypoxia-inducible factor 1. J Biol Chem 2001;276:21166–72.
[92] Görlach A, Berchner-Pfannschmidt U, Wotzlaw C, Cool RH, Fandrey J, Acker H, et al. Reactive oxygen species modulate HIF-1 mediated PAI-1 expression: Involvement of the GTPase Rac1. Thromb Haemost 2003;89:927–35.
[93] Mills E, Jobsis FF. Mitochondrial respiratory chain of carotid body and chemoreceptor response to changes in oxygen tension. J Neurophysiol 1972;35:405–28.
[94] Duchen MR, Biscoe TJ. Mitochondrial function in type I cells isolated from rabbit arterial chemoreceptors. J Physiol 1992;450:13–31.
[95] Wyatt CN, Buckler KJ. The effect of mitochondrial inhibitors on membrane currents in isolated neonatal rat carotid body type I cells. J Physiol 2004;556:175–91.
[96] Archer SL, Huang J, Henry T, Peterson D, Weir EK. A redox-based O_2 sensor in rat pulmonary vasculature. Circ Res 1993;73:1100–12.
[97] Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc Natl Acad Sci USA 1998;95:11715–20.
[98] Michelakis ED, Hampl V, Nsair A, Wu X, Harry G, Haromy A, et al. Diversity in mitochondrial function explains differences in vascular oxygen sensing. Circ Res 2002;90:1307–15.
[99] Paky A, Michael JR, Burke-Wolin TM, Wolin MS, Gurtner GH. Endogenous production of superoxide by rabbit lungs: effects of hypoxia or metabolic inhibitors. J Appl Physiol 1993;74:2868–74.
[100] Hool LC, Arthur PG. Decreasing cellular hydrogen peroxide with catalase mimics the effects of hypoxia on the sensitivity of the L-type Ca^{2+} channel to beta-adrenergic receptor stimulation in cardiac myocytes. Circ Res 2002;91:601–9.
[101] Marshall C, Mamary AJ, Verhoeven AJ, Marshall BE. Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction. Am J Respir Cell Mol Biol 1996;15:633–44.
[102] Enomoto N, Koshikawa N, Gassmann M, Hayashi J, Takenaga K. Hypoxic induction of hypoxia-inducible factor-1alpha and oxygen-regulated gene expression in mitochondrial DNA-depleted HeLa cells. Biochem Biophys Res Commun 2002;297:346–52.
[103] Carriere A, Fernandez Y, Rigoulet M, Penicaud L, Casteilla L. Inhibition of preadipocyte proliferation by mitochondrial reactive oxygen species. FEBS Lett 2003;550:163–7.
[104] Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, et al. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O_2 sensing. J Biol Chem 2000;275:25130–8.
[105] Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. J Biol Chem 1998;273:11619–24.
[106] Rota C, Chignell CF, Mason RP. Evidence for free radical formation during the oxidation of 2′,7′-dichlorofluorescin to the fluorescent dye 2′,7′-dichlorofluorescein by horseradish peroxidase: possible implications for oxidative stress measurements. Free Radic Biol Med 1999;27:873–81.
[107] Rota C, Fann YC, Mason RP. Phenoxyl free radical formation during the oxidation of the fluorescent dye 2′,7′-dichlorofluorescein by horseradish peroxidase. Possible consequences for oxidative stress measurements. J Biol Chem 1999;274:28161–8.
[108] Ohashi T, Mizutani A, Murakami A, Kojo S, Ishii T, Taketani S. Rapid oxidation of dichlorodihydrofluorescin with heme and hemoproteins: formation of the fluorescein is independent of the generation of reactive oxygen species. FEBS Lett 2002;511:21–7.
[109] Burkitt MJ, Wardman P. Cytochrome c is a potent catalyst of dichlorofluorescin oxidation: implications for the role of reactive oxygen species in apoptosis. Biochem Biophys Res Commun 2001;282:329–33.
[110] Hirsch-Ernst KI, Kietzmann T, Ziemann C, Jungermann K, Kahl GF. Physiological oxygen tensions modulate expression of the mdr1b multidrug-resistance gene in primary rat hepatocyte cultures. Biochem J 2000;350(Part 2):443–51.
[111] Chandel NS, Schumacker PT, Arch RH. Reactive oxygen species are downstream products of TRAF-mediated signal transduction. J Biol Chem 2001;276:42728–36.
[112] Hagen T, Taylor CT, Lam F, Moncada S. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha. Science 2003;302:1975–8.
[113] Searle GJ, Hartness ME, Hoareau R, Peers C, Kemp PJ. Lack of contribution of mitochondrial electron transport to acute O(2) sensing in model airway chemoreceptors. Biochem Biophys Res Commun 2002;291:332–7.
[114] Vaux EC, Metzen E, Yeates KM, Ratcliffe PJ. Regulation of hypoxia-inducible factor is preserved in the absence of a functioning mitochondrial respiratory chain. Blood 2001;98:296–302.
[115] Srinivas V, Leshchinsky I, Sang N, King MP, Minchenko A, Caro J. Oxygen sensing and HIF-1 activation does not require an active mitochondrial respiratory chain electron-transfer pathway. J Biol Chem 2001;276:21995–8.
[116] Ebert BL, Firth JD, Ratcliffe PJ. Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter-1 via distinct *cis*-acting sequences. J Biol Chem 1995;270:29083–9.
[117] Park JH, Kim TY, Jong HS, Kim TY, Chun YS, Park JW, et al. Gastric epithelial reactive oxygen species prevent normoxic degradation of hypoxia-inducible factor-1alpha in gastric cancer cells. Clin Cancer Res 2003;9:433–40.

[118] BelAiba RS, Djordjevic T, Bonello S, Flugel D, Hess J, Kietzmann T, et al. Redox-sensitive regulation of the HIF pathway under non-hypoxic conditions in pulmonary artery smooth muscle cells. Biol Chem 2004;385:249–57.
[119] Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ. Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. Cancer Res 2003;63:1764–8.
[120] Zhu XY, Rodriguez-Porcel M, Bentley MD, Chade AR, Sica V, Napoli C, et al. Antioxidant intervention attenuates myocardial neovascularization in hypercholesterolemia. Circulation 2004;109:2109–15.
[121] Kim HH, Lee SE, Chung WJ, Choi Y, Kwack K, Kim SW, et al. Stabilization of hypoxia-inducible factor-1alpha is involved in the hypoxic stimuli-induced expression of vascular endothelial growth factor in osteoblastic cells. Cytokine 2002;17:14–27.
[122] Shatrov VA, Sumbayev VV, Zhou J, Brune B. Oxidized low-density lipoprotein (oxLDL) triggers hypoxia-inducible factor-1alpha (HIF-1alpha) accumulation via redox-dependent mechanisms. Blood 2003;101:4847–9.
[123] Tacchini L, Dansi P, Matteucci E, Desiderio MA. Hepatocyte growth factor signalling stimulates hypoxia inducible factor-1 (HIF-1) activity in HepG2 hepatoma cells. Carcinogenesis 2001;22:1363–71.
[124] Haddad JJ, Olver RE, Land SC. Antioxidant/pro-oxidant equilibrium regulates HIF-1alpha and NF-kappa B redox sensitivity. Evidence for inhibition by glutathione oxidation in alveolar epithelial cells. J Biol Chem 2000;275:21130–9.
[125] Khomenko T, Deng X, Sandor Z, Tarnawski AS, Szabo S. Cysteamine alters redox state. HIF-1alpha transcriptional interactions and reduces duodenal mucosal oxygenation: novel insight into the mechanisms of duodenal ulceration. Biochem Biophys Res Commun 2004;317:121–7.
[126] Wakisaka N, Kondo S, Yoshizaki T, Murono S, Furukawa M, Pagano JS. Epstein-Barr virus latent membrane protein 1 induces synthesis of hypoxia-inducible factor 1 alpha. Mol Cell Biol 2004;24:5223–34.
[127] Wellman TL, Jenkins J, Penar PL, Tranmer B, Zahr R, Lounsbury KM. Nitric oxide and reactive oxygen species exert opposing effects on the stability of hypoxia-inducible factor-1alpha (HIF-1alpha) in explants of human pial arteries. FASEB J 2004;18:379–81.
[128] Zelko IN, Folz RJ. Extracellular superoxide dismutase functions as a major repressor of hypoxia-induced erythropoietin gene expression. Endocrinology 2005;146:332–40.
[129] Jung Y, Isaacs JS, Lee S, Trepel J, Liu ZG, Neckers L. Hypoxia-inducible factor induction by tumor necrosis factor in normoxic cells requires receptor-interacting protein-dependent nuclear factor kappa beta activation. Biochem J 2003;370:1011–7.
[130] Khatri JJ, Johnson C, Magid R, Lessner SM, Laude KM, Dikalov SI, et al. Vascular oxidant stress enhances progression and angiogenesis of experimental atheroma. Circulation 2004;109:520–5.
[131] Lee C, Kim SJ, Jeong DG, Lee SM, Ryu SE. Structure of human FIH-1 reveals a unique active site pocket and interaction sites for HIF-1 and VHL. J Biol Chem 2003;278:7558–63.
[132] McNeill LA, Hewitson KS, Claridge TD, Seibel JF, Horsfall LE, Schofield CJ. Hypoxia-inducible factor asparaginyl hydroxylase (FIH-1) catalyses hydroxylation at the beta-carbon of asparagine-803. Biochem J 2002;367:571–5.
[133] Hirsila M, Koivunen P, Gunzler V, Kivirikko KI, Myllyharju J. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. J Biol Chem 2003;278:30772–80.
[134] Yu F, White SB, Zhao Q, Lee FS. HIF-1alpha binding to VHL is regulated by stimulus-sensitive proline hydroxylation. Proc Natl Acad Sci USA 2001;98:9630–5.
[135] Schoenfeld AR, Davidowitz EJ, Burk RD. Endoplasmic reticulum/cytosolic localization of von Hippel-Lindau gene products is mediated by a 64-amino acid region. Int J Cancer 2001;91:457–67.
[136] Metzen E, Berchner-Pfannschmidt U, Stengel P, Marxsen JH, Stolze I, Klinger M, et al. Intracellular localisation of human HIF-1 alpha hydroxylases: implications for oxygen sensing. J Cell Sci 2003;116:1319–26.
[137] Wang GL, Jiang BH, Semenza GL. Effect of protein kinase and phosphatase inhibitors on expression of hypoxia-inducible factor 1. Biochem Biophys Res Commun 1995;216:669–75.
[138] Mazure NM, Chen EY, Laderoute KR, Giaccia AJ. Induction of vascular endothelial growth factor by hypoxia is modulated by a phosphatidylinositol 3-kinase/Akt signaling pathway in Ha-ras-transformed cells through a hypoxia inducible factor-1 transcriptional element. Blood 1997;90:3322–31.
[139] Richard DE, Berra E, Gothie E, Roux D. Pouyssegur J. p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. J Biol Chem 1999;274:32631–7.
[140] Sodhi A, Montaner S, Miyazaki H, Gutkind JS. MAPK and Akt act cooperatively but independently on hypoxia inducible factor-1alpha in rasV12 upregulation of VEGF. Biochem Biophys Res Commun 2001;287:292–300.
[141] Lee E, Yim S, Lee SK, Park H. Two transactivation domains of hypoxia-inducible factor-1alpha regulated by the MEK-1/p42/p44 MAPK pathway. Mol Cells 2002;14:9–15.
[142] Zundel W, Schindler C, Haas KD, Koong A, Kaper F, Chen E, et al. Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev 2000;14:391–6.
[143] Jiang BH, Jiang G, Zheng JZ, Lu Z, Hunter T, Vogt PK. Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth Differ 2001;12:363–9.
[144] Gao N, Shen L, Zhang Z, Leonard SS, He H, Zhang XG, et al. Arsenite induces HIF-1alpha and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. Mol Cell Biochem 2004;255:33–45.
[145] Gao N, Ding M, Zheng JZ, Zhang Z, Leonard SS, Liu KJ, et al. Vanadate-induced expression of hypoxia-inducible factor 1 alpha and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. J Biol Chem 2002;277:31963–71.
[146] Djordjevic T, Pogrebniak A, BelAiba RS, Bonello S, Wotzlaw C, Acker H, et al. The expression of the NADPH oxidase subunit p22phox is regulated by a redox-sensitive pathway in endothelial cells. Free Radic Biol Med 2005;38:616–30.
[147] Chan DA, Sutphin PD, Denko NC, Giaccia AJ. Role of prolyl hydroxylation in oncogenically stabilized hypoxia-inducible factor-1alpha. J Biol Chem 2002;277:40112–7.
[148] Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001;21:3995–4004.
[149] Roth U, Curth K, Unterman TG, Kietzmann T. The transcription factors HIF-1 and HNF-4 and the coactivator p300 are involved in insulin-regulated glucokinase gene expression via the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem 2004;279:2623–31.
[150] Bardos JI, Chau NM, Ashcroft M. Growth factor-mediated induction of HDM2 positively regulates hypoxia-inducible factor 1alpha expression. Mol Cell Biol 2004;24:2905–14.
[151] Mottet D, Dumont V, Deccache Y, Demazy C, Ninane N, Raes M, et al. Regulation of hypoxia-inducible factor-1alpha protein level during hypoxic conditions by the phosphatidylinositol 3-kinase/Akt/glycogen synthase kinase 3beta pathway in HepG2 cells. J Biol Chem 2003;278:31277–85.
[152] Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, et al. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol 2002;22:7004–14.

[153] Tang TT, Lasky LA. The forkhead transcription factor FOXO4 induces the down-regulation of hypoxia-inducible factor 1 alpha by a von Hippel-Lindau protein-independent mechanism. J Biol Chem 2003;278:30125–35.
[154] Minet E, Arnould T, Michel G, Roland I, Mottet D, Raes M, et al. ERK activation upon hypoxia: involvement in HIF-1 activation. FEBS Lett 2000;468:53–8.
[155] Liu Q, Moller U, Flugel D, Kietzmann T. Induction of plasminogen activator inhibitor I gene expression by intracellular calcium via hypoxia-inducible factor-1. Blood 2004;104:3993–4001.
[156] Qian D, Lin HY, Wang HM, Zhang X, Liu DL, Li QL, et al. Normoxic induction of the hypoxic-inducible factor-1 alpha by interleukin-1 beta involves the extracellular signal-regulated kinase 1/2 pathway in normal human cytotrophoblast cells. Biol Reprod 2004;70:1822–7.
[157] Fukuda R, Kelly B, Semenza GL. Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. Cancer Res 2003;63:2330–4.
[158] Wang FS, Wang CJ, Chen YJ, Chang PR, Huang YT, Sun YC, et al. Ras induction of superoxide activates ERK-dependent angiogenic transcription factor HIF-1alpha and VEGF-A expression in shock wave-stimulated osteoblasts. J Biol Chem 2004;279:10331–7.
[159] Kietzmann T, Jungermann K, Görlach A. Regulation of the hypoxia-dependent plasminogen activator inhibitor 1 expression by MAP kinases in HepG2 cells. Thromb Haemost 2003;89:666–74.
[160] Conrad PW, Rust RT, Han J, Millhorn DE, Beitner-Johnson D. Selective activation of p38alpha and p38gamma by hypoxia. Role in regulation of cyclin D1 by hypoxia in PC12 cells. J Biol Chem 1999;274:23570–6.
[161] Sodhi A, Montaner S, Patel V, Zohar M, Bais C, Mesri EA, et al. The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1alpha. Cancer Res 2000;60:4873–80.
[162] Mishra OP, Delivoria-Papadopoulos M. Effect of hypoxia on the expression and activity of mitogen-activated protein (MAP) kinase-phosphatase-1 (MKP-1) and MKP-3 in neuronal nuclei of newborn piglets: the role of nitric oxide. Neuroscience 2004;129:665–73.
[163] Tonks NK. PTP1B: from the sidelines to the front lines! FEBS Lett 2003;546:140–8.
[164] Meng TC, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. Mol Cell 2002;9:387–99.
[165] Furst R, Brueckl C, Kuebler WM, Zahler S, Krotz F, Görlach A, et al. Atrial natriuretic peptide induces mitogen-activated protein kinase phosphatase-1 in human endothelial cells via Rac1 and NAD(P)H oxidase/Nox2-activation. Circ Res 2005;96:43–53.
[166] Seta KA, Kim R, Kim HW, Millhorn DE, Beitner-Johnson D. Hypoxia-induced regulation of MKP-1: Identification by subtractive suppression hybridization and cDNA microarrays. J Biol Chem 2001;276:44405–12.
[167] Liu C, Shi Y, Han Z, Pan Y, Liu N, Han S, et al. Suppression of the dual-specificity phosphatase MKP-1 enhances HIF-1 transactivation and increases expression of EPO. Biochem Biophys Res Commun 2003;312:780–6.
[168] Marchetti S, Gimond C, Roux D, Gothie E, Pouyssegur J, Pages G. Inducible expression of a MAP kinase phosphatase-3-GFP chimera specifically blunts fibroblast growth and ras-dependent tumor formation in nude mice. J Cell Physiol 2004;199:441–50.