Journal of Cellular Biochemistry 104:536–544 (2008)

PROSPECT

Does Inhibition of Degradation of Hypoxia-Inducible Factor (HIF) α Always Lead to Activation of HIF? Lessons Learnt From the Effect of Proteasomal Inhibition on HIF Activity

Stefan Kaluz,* Milota Kaluzová, and Eric J. Stanbridge

Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, California 92697-4025

Abstract At the cellular level hypoxia induces transcriptional response that is mediated by the transcription factor hypoxia-inducible factor (HIF). HIF is regulated at the level of its α subunit by 2-oxoglutarate (2OG)-dependent oxygenases that hydroxylate specific prolyl and asparaginyl residues of HIF-α, affecting its stability and activity, respectively. In the presence of O_2, the α subunit is degraded in a complex process with several distinct steps. In the first step, the degradation process is initiated by prolyl hydroxylases (PHDs). In the second step, the von Hippel-Lindau (VHL)/E3 ligase complex recognizes the hydroxylated HIF-α and mediates its polyubiquitylation by the ubiquitin-conjugating enzyme E2. In the third step, the polyubiquitylated HIF-α is translocated to the proteasome where it is degraded. Degradation of HIF-α can be inhibited at any of the three levels either by various pharmacological inhibitors or due to inactivation of genes whose products regulate the HIF system. The emerging data about inactivation of HIF under conditions of proteasomal inhibition prompted us to provide an overview contrasting the outcome of inhibition at various stages of the degradative pathway for HIF activity. J. Cell. Biochem. 104: 536–544, 2008.

Key words: hypoxia-inducible factor; proteasome; proteasomal inhibitor

Cells experiencing lowered O_2 levels undergo a variety of biological responses in order to adapt to these unfavorable conditions. At a molecular level, hypoxic cells respond by increased expression of a number of gene products that will facilitate survival under these conditions. The master switch, orchestrating the cellular response to low O_2 levels, is the transcription factor hypoxia-inducible factor (HIF). The role of the HIF system in tumor development, angiogenesis, glucose/energy metabolism, and ischemic disease is widely documented; for review see [Harris, 2002; Semenza, 2003]. HIF is a heterodimer that consists of two members of the basic helix–loop–helix (bHLH) Per, ARNT, and Sim (PAS) family: the constitutively expressed HIF-β (also known as arylhydrocarbon receptor nuclear translocator) and an HIF-α (the hypoxic response factor) [Wang et al., 1995]. In humans, three different α subunits (HIF-1α, HIF-2α, and HIF-3α; each encoded by a distinct locus) have been identified [Huang and Bunn, 2003]. The structurally closely related subunits share a unique O_2-dependent degradation domain (ODDD). Central to the ODDD function are proline residues (two for HIF-1α and HIF-2α, and one for HIF-3α). The other domains in the α subunits include bHLH, PAS (mediating DNA binding and dimerization with HIF-β), and two transactivation domains: N-terminal activation domain (NAD) and C-terminal activation domain (CAD) [Pugh et al., 1997].

Hypoxia activates HIF by controlling two molecular switches that converge on α subunits (Fig. 1). The first switch controls the overall level of HIF-α in the cell. Although HIF-α is constitutively expressed, it is rapidly degraded in the presence of O_2. HIF-α is earmarked for

*Correspondence to: Dr. Stefan Kaluz, Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, CA 92697-4025.
E-mail: skaluz@uci.edu

Received 20 September 2007; Accepted 23 October 2007
DOI 10.1002/jcb.21644

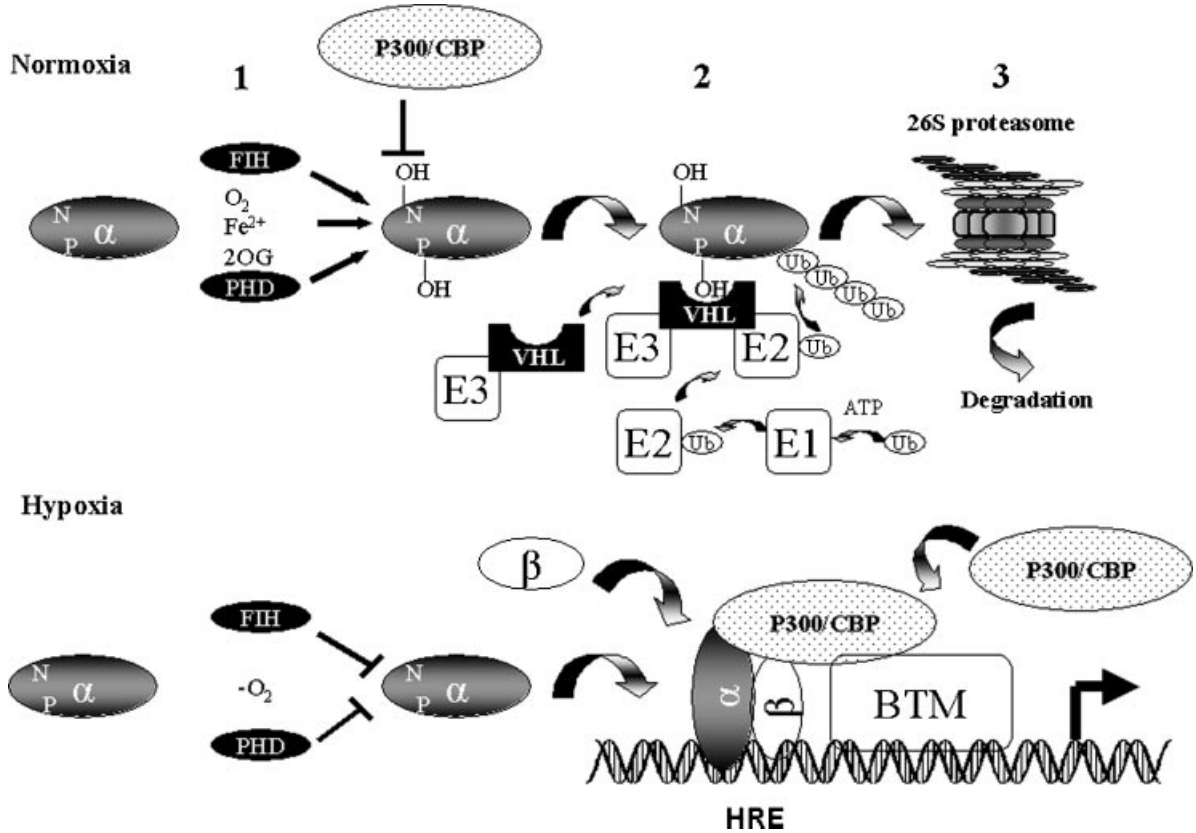

Fig. 1. The outline of regulation of stability and transcriptional activity of HIF-α. Degradation of HIF-α can be viewed as a colinear process consisting of three distinct steps: (**1**) hydroxylation of HIF-α by PHDs; (**2**) recognition of the hydroxylated HIF-α by the E3 complex and ubiquitylation by E2/E1; and (**3**) degradation in the 26S proteasome. Inhibition of the process at any level will result in accumulation of HIF-α. BTM, basic transcriptional machinery; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; FIH-1, factor inhibiting HIF-1; HRE, hypoxia-response element; 2OG, 2-oxoglutarate; PHD, prolylhydroxylase; Ub, ubiquitin; VHL, von Hippel-Lindau protein.

degradation by hydroxylation of prolines at positions 402 and 564 (in HIF-1α) in the ODDD [Ivan et al., 2001; Jaakkola et al., 2001]. Hydroxylation of these prolines is catalyzed by three closely related oxygenases, termed PHD1, PHD2, and PHD3 [Schofield and Ratcliffe, 2005]. Hydroxylated prolines enable specific recognition of HIF-α by the von Hippel-Lindau (VHL) protein [Ivan et al., 2001; Jaakkola et al., 2001] which, in a complex with elongin B, elongin C, and Cul2, functions as an E3 ubiquitin ligase for HIF-α [Kondo and Kaelin, 2001]. Polyubiquitylated HIF-α is recognized and degraded by the 26S proteasome [Maxwell et al., 1999].

The second molecular switch regulates transcriptional activity of HIF-α. For transcriptional activity, HIF-1 requires recruitment of p300/CBP, widely employed transcriptional coactivators, to the HIF-α CAD [Arany et al., 1996]. In the absence of the ODDD the CAD is stable, but its transcriptional activity is hypoxia-inducible. In normoxia, the factor inhibiting HIF-1 (FIH-1), an oxygenase distantly related to prolyl hydroxylases (PHDs), binds to the C-terminal part of HIF-α and by hydroxylating N803 [Lando et al., 2002a] diminishes interactions with p300/CBP [Freedman et al., 2002]. Reduction of oxygenase activity in hypoxia simultaneously inhibits proline and asparagine hydroxylation, allowing concomitant accumulation and transcriptional activation of HIF-α. HIF-α then translocates to the nucleus, dimerizes with HIF-β, recruits p300/CBP, and induces the expression of its transcriptional targets via binding to hypoxia-response elements (HRE) with the core sequence G/ACGTG.

For simplicity, in the preceding paragraphs we described how HIF-α is regulated by hypoxia, its major physiological inhibitor. However, the multi-stage process leading to the ultimate degradation of HIF-α can be inhibited at

multiple levels by pharmacological agents or in pathophysiological conditions. Mechanistically, we will consider inhibition at three different levels: (1) level of PHDs; (2) level of ubiquitylation; and (3) level of proteasomal degradation (Fig. 1). Next, we will review the available data about the functionality of HIF-α stabilized either by various pharmacological inhibitors or due to inactivation of genes whose products have a regulatory role in the HIF system.

INHIBITION OF PHDs

Enzymes that initiate degradation of HIF-α by hydroxylating either of the two prolines in the ODDD are the PHDs. PHD1, PHD2, and PHD3, which have closely related catalytic domains, belong to the superfamily of 2-oxoglutarate (2OG)-dependent oxygenases. PHDs use the citric acid cycle intermediate 2OG as a co-substrate, and for optimal activity, in addition to O_2, require Fe(II) and ascorbate as cofactors [Bruick and McKnight, 2001; Epstein et al., 2001]. A high rate of turnover of PHD enzymes might suggest that hydroxylation capacity for a low abundance transcription factor would not be limiting. However, there is evidence for the key regulatory role of PHD activity and it does become limiting when HIF is induced (reviewed in [Schofield and Ratcliffe, 2005]). The reaction is initiated when the enzyme-Fe(II) complex first binds 2OG, then its target substrate. Following the displacement of a water molecule on Fe(II) by the target substrate, molecular O_2 associates with the complex. This results in production of succinate, CO_2, and a highly reactive ferryl species (Fe^{IV}=O) that oxidizes the target substrate [Hausinger, 2004]. The asparaginyl-hydroxylase FIH-1 also requires O_2, Fe(II), and 2OG for activity [Lando et al., 2002b], suggesting that it will be regulated similarly to PHDs. In most of the cases listed below co-inhibition of PHDs and FIH-1 means that HIF-α accumulates in a functional form. Signaling by HIF hydroxylases has been recently reviewed [Hirota and Semenza, 2005; Schofield and Ratcliffe, 2005] and we will, therefore, briefly summarize agents known to inhibit PHD activity.

Hypoxia

By definition, PHDs and FIH-1 require O_2 for activity and hypoxia is thus the main physiological inhibitor of their activity. Here we should note two notable exemptions to the paradigm of co-inhibition of PHDs and FIH-1. The K_m of FIH-1 for O_2 is less than half that of the PHD family members, suggesting that a hypoxic window could exist in which HIF-1α would be stable due to the absence of prolyl hydroxylation and yet would be transcriptionally inactive due to hydroxylation of N803 [Koivunen et al., 2004]. On the other hand, pericellular hypoxia in dense cultures of certain transformed cells activates expression of HIF-inducible genes without appreciable accumulation of HIF-α [Kaluz et al., 2002]. Although this phenomenon is cell-type specific, it suggests an increase in HIF transcriptional activity without significant inhibition of PHDs.

Iron Chelation and Divalent Metal Cations

The dependence of PHDs and FIH-1 on Fe(II) fits well with the hypoxia-mimicking effect of iron chelators and a series of metals, including Co(II), Ni(II), and Mn(II) [Goldberg et al., 1988]. However, the underlying mechanism is not straightforward: the tight binding of Fe(II) by recombinant PHDs results in weak or no inhibition by chelators or divalent cations. This led to alternative theories, e.g., Co(II) may stabilize HIF-1 α by coordinating carboxylate residues close to P564 [Yuan et al., 2003] or that Co(II) and Ni(II) deplete intracellular ascorbate levels [Salnikow et al., 2004]. It is also possible that additional binding/transport proteins are involved in the process of removal of the catalytic Fe(II) from the PHD complex in vivo [Schofield and Ratcliffe, 2005].

2OG, 2-Oxoacids, and Citric Acid Cycle Intermediates

PHDs and FIH-1 require 2OG as co-substrate, and availability of 2OG, therefore, regulates their activity. Indeed, analogs of 2OG, like dimethyloxalylglycine, pyruvate, and oxaloacetate, act as competitive inhibitors and induce the HIF system [Dalgard et al., 2004]. Interestingly, hereditary cancer syndromes with defective enzymes in the citric acid cycle lead to reduced PHD activity and upregulation of HIF-α. Dysfunctional succinate dehydrogenase (various subunits) and fumarate hydratase cause accumulation of succinate and fumarate, respectively, and both substances act as competitive inhibitors for 2OG [Isaacs et al., 2005; Selak et al., 2005].

Ascorbate Depletion

Studies of the PHD enzymes, both in vitro and in vivo, indicate a role for ascorbate in regulation of the HIF system. Although in vitro measured values are notoriously difficult to relate to those prevailing in live cells, ascorbate provision has been shown to reduce the normoxic accumulation of HIF-α [Knowles et al., 2003]. Such effects were not seen in cells treated with 2OG analogs blocking PHD activity, or in VHL deficient cells, proving that they are the consequence of the promotion of hydroxylase activity by ascorbate [Knowles et al., 2003]. Whether there are circumstances in intact organisms when ascorbate becomes the limiting factor for activity of HIF hydroxylases remains to be determined.

Nitric Oxide (NO)

There is evidence that NO regulates the HIF system [Metzen et al., 2003], presumably because it can act, at sufficiently high concentrations, as an analog of molecular O_2 and inhibit 2OG-dependent oxygenases. However, interaction between NO and HIF system is apparently more complex as NO affects a number of other cellular processes (e.g., inhibition of the mitochondrial electron transport reduces O_2 consumption, effectively relieving cellular hypoxia [Hagen et al., 2003]). Use of NO donors can activate the HIF system under normoxia, but the same compounds may inhibit HIF under hypoxia [Sogawa et al., 1998]. The recently observed biphasic response of HIF-α and PHDs to NO treatment, with early inhibition of PHDs and stabilization of HIF-α, followed by an increase in levels of PHDs and reduction of HIF-α [Berchner-Pfannschmidt et al., 2007] is an important contribution in this area.

Reactive Oxygen Species (ROS)

Agents that affect cellular levels of ROS modulate HIF activity. Activation of HIF can be explained in terms of inactivation of 2OG-dependent oxygenases by oxidative damage through three possible mechanisms: hydroxylation of active site, fragmentation, and conversion of catalytic Fe(II) to inactive Fe(III) [Kietzmann and Gorlach, 2005]. ROS species produced by mitochondria have also been implicated in signaling hypoxia: inhibitors of mitochondrial electron transport, that reduce ROS production, also reduce HIF-α stabilization under conditions of moderate hypoxia [Chandel et al., 1998]. Again, the corresponding mechanism may not be obvious; mitochondrial inhibitors could elevate PHD activity through increased O_2 availability (due to reduction of O_2 consumption), rather than decreased ROS levels [Schofield and Ratcliffe, 2005].

INHIBITION OF UBIQUITYLATION

The majority of substrates of the ubiquitin-proteasome pathway (UPP) are marked for degradation by covalent attachment of ubiquitin, a small 8 kDa protein. Ubiquitin is initially attached through an isopeptide bond to a free amino group of a lysine residue, then a chain of ubiquitins is formed by processive addition of several ubiquitin molecules to the K48 of the preceding ubiquitin [Hershko and Ciechanover, 1998]. Ubiquitylation is carried out by a concerted action of three enzymes. First, the ubiquitin-activating enzyme E1 (UBE1) binds ubiquitin in an ATP-dependent manner. Ubiquitin is then transferred to a ubiquitin-conjugating enzyme E2. In the third step, the substrate recognition component of the ubiquitin ligase E3 binds the target, associates with E2, which then carries out several rounds of ubiquitylation of the target [Hershko and Ciechanover, 1998]. In a typical organism, there is only one E1 gene, more than 25 E2s, and hundreds of E3s. For HIF-α the E2 enzyme is UbcH5 [Iwai et al., 1999] and the E3 complex consists of the substrate recognition component VHL, elongin B, elongin C, Cul2, and Rbx1 (also called Roc1) [Kondo and Kaelin, 2001].

Inhibition of Binding of Hydroxylated HIF-α by E3 Ligase

Hydroxylation of either of the two conserved prolines by PHDs, as discussed above, enables recognition of HIF-α by VHL that is followed by rapid polyubiquitylation. Because VHL contains a single, conserved hydroxyproline-binding pocket for HIF-α, independent recognition of P402, and P564 constitutes a functional redundancy within the ODDD for mediating proteolysis. Mutation of either P alone only partially stabilizes HIF-α, whereas mutation of both markedly increases its stability and activity [Masson et al., 2001]. The critical role of

the tumor suppressor VHL in regulation of the HIF system, along with the significance of this regulation in cancer progression, is now generally accepted (for review see [Kondo and Kaelin, 2001]). VHL has no intrinsic catalytic activity and functionally it can be separated into two subdomains. Subdomain a binds directly to elongin C and subdomain b binds directly to HIF-α [Ohh et al., 2000]. Both domains are hotspots for mutations and every VHL mutant associated with classical VHL disease tested to date has been defective in either binding to elongin C or HIF-α [Kondo and Kaelin, 2001].

Several lines of evidence support the full functionality of the HIF system in the presence of defective VHL. VHL-associated tumors are highly vascularized, displaying overproduction of angiogenic factors, such as VEGF. In addition, VHL-defective cells express disproportionately high levels of other hypoxia-inducible transcripts, even under normoxic conditions. Introduction of the wild-type VHL restores the O_2-dependent regulation of HIF-α and accordingly downregulates the expression of hypoxia-inducible genes [Ivan et al., 2001; Jaakkola et al., 2001]. VHL also plays a role in FIH-1 function [Mahon et al., 2001] and the functionality of HIF-α in the presence of defective VHL can be accounted for by repression of FIH-1.

Inhibition of Ubiquitylation

The ubiquitin-conjugating enzyme UbcH5 requires K532, K538, and K547 ubiquitin acceptors for the VHL-mediated ubiquitylation HIF-1α. Ubiquitylation of the HIF-1α KKK/RRR mutant was inhibited in a manner similar to the P402, P564 mutant [Paltoglou and Roberts, 2007], leading to its considerable stabilization. While there is a plethora of evidence for the VHL function in HIF regulation, virtually no data are available on the functionality of the HIF system when E1 or E2 are eliminated/downregulated. To our knowledge, there is only one report in which the involvement of the UBE1 in regulation of HIF-1α was studied. Exposure of murine ts20TGR cells, which contain a temperature-sensitive mutant of E1, to the non-permissive temperature induced a rapid accumulation of HIF-1α under normoxic conditions [Salceda and Caro, 1997]. The authors did not elaborate on the functionality of HIF-1 in ts20GR cells under normoxic or hypoxic conditions.

INHIBITION OF PROTEASOMAL DEGRADATION

Polyubiquitylated proteins are targeted to and subsequently degraded in the 26S proteasome, a massive 2,500 kDa multi-subunit complex. The complex consists of a cylindrical 20S core catalytic component with a 19S regulatory component attached to one or both ends [Adams, 2003]. The function of the 19S regulatory component is to recognize and bind the polyubiquitylated protein, and then to cleave the ubiquitin chain off the protein substrate. In the 20S component two outer (α) rings surround two internal (β) rings that carry out the proteolysis of unfolded proteins. Each β ring consists of seven subunits containing three active enzymatic sites with trypsin-like, chymotrypsin-like, and post-glutamyl peptide hydrolase-like (caspase-like) activities [Kisselev and Goldberg, 2001; Adams, 2003].

Proteasomal inhibitors (PIs) inactivate the proteasome by forming covalent bonds with the N-terminal threonine (the catalytic nucleophile) of the β unit [Kisselev and Goldberg, 2001]. Almost all of the synthetic and natural inhibitors of the proteasome act predominantly on the chymotrypsin-like activity, but also have some much weaker effects on the other two activities [Kisselev and Goldberg, 2001]. Numerous regulators of pathways that are deregulated in malignant progression, e.g., cyclins and inhibitors of cyclin-dependent kinases, wild type p53, and IκB are substrates of the proteasome (for review see [Adams, 2004]). Therefore, it was reasoned that inhibition of the proteasome could arrest or retard cell growth by stabilizing these regulators and if specific to, or preferentially targeting neoplastic cells, this could be clinically relevant. Actively proliferating malignant cells were indeed found to be more sensitive to proteasome blockade than non-cancerous cells, although the mechanisms responsible for this increased susceptibility are not conclusively understood. Antineoplastic efficiency of the dipeptidyl boronic acid derivative bortezomib (PS-341, velcade) against advanced solid tumors, refractory hematological malignancies, and relapsed and refractory multiple myeloma exemplifies the clinical utility of PIs [Adams, 2004].

Due to the large number of proteins that are degraded by the proteasome, inhibition of the proteasome in the cellular context invariably

elicits a pleiotropic response. This makes it difficult to understand the relative contribution of inhibition of a particular pathway to the therapeutic effect; moreover, not all of the relevant targets may have been identified [Adams, 2003]. Although no systematic analysis has been performed to date, at least some of the proteins that accumulate, following inhibition of the proteasome, remain functional [Williams and McConkey, 2003].

HIF-α is one of the prototypical targets of the proteasome and, given the generally accepted importance of the HIF system in tumor progression/angiogenesis, how PI affects HIF-1 function has received remarkably little attention. It has been reported that PI does not activate HRE-driven reporter constructs in normoxic cells [Salceda and Caro, 1997; Kallio et al., 1999; Mabjeesh et al., 2002]. Proteasomal inhibition also downregulated expression of the hypoxia-markers CAIX [Mackay et al., 2005] and VEGF [Rocarro et al., 2006]. Recently, three more detailed studies on the effects of PI on HIF-dependent transcription have been published [Kaluz et al., 2006; Birle and Hedley, 2007; Shin et al., 2007]. The conclusion of these studies is that proteasomal inhibition, despite having a positive effect on HIF-1α stability, not only does not activate HIF under normoxia but it considerably interferes with hypoxia-induced HIF-1 activity. This provides grounds for the counter-intuitive hypothesis that blocking of HIF-1 function could be responsible for some of the antiangiogenic/antitumor effects of proteasomal inhibition. The mechanism by which PI inactivates HIF is not known at present, although some theories have been put forward. In the following part, we will discuss some of these theories.

Non-specific Cytotoxic Effect

Initially, the non-functionality of HIF-1 in the presence of PIs was explained in terms of a non-specific toxic effect of PI [Salceda and Caro, 1997; Mabjeesh et al., 2002]. However, this proposition was not upheld in other studies [Kaluz et al., 2006; Birle and Hedley, 2007]; moreover, before being clinically approved, bortezomib had been extensively tested for toxicity [Adams, 2004]. A non-specific inhibitory effect on transcription in general is also not supported by the data [Kaluz et al., 2006; Birle and Hedley, 2007].

Lack of HIF-1α Activation

Later, it was proposed that HIF-1α stabilized in the presence of PI lacks some modifications required for induction of its transcriptional activity [Kallio et al., 1999]. While this theory correctly anticipated the negative regulation of HIF-α transcriptional activity by FIH-1 [Lando et al., 2002a], it fails to account for inactivation of HIF-1 in hypoxic cells (in hypoxia, FIH itself is inactivated). Furthermore, site-directed mutagenesis of the HIF-1α CAD proved that modifications, such as hydroxylation or phosphorylation, are not involved in the inhibitory effect of proteasomal inhibition [Kaluz et al., 2006].

Inhibitory Effect of Polyubiquitylation

Polyubiquitylation of HIF-1α could impede the ability of HIF-1 to mediate hypoxic signal transduction and/or nuclear transport [Kallio et al., 1999]. However, the fact that the bulk of HIF-1α accumulated in the presence of PI is non-ubiquitylated is at odds with this model [Kaluz et al., 2006; Birle and Hedley, 2007]. Also, the lack of lysines in the HIF-1α CAD, the minimal fragment inhibited by PI, argues further against the regulatory role of polyubiquitylation [Kaluz et al., 2006]. According to the "activation by destruction" theory, sustained transcription mediated by certain transcription factors requires proteasomal activity to remove "spent" activators and to reset the promoter [Lipford et al., 2005]. By the same token as above, this theory cannot satisfactorily explain inhibition of the HIF-1α CAD by PI. The proposal suggesting impaired translocation of HIF-1α to the nucleus in PI-treated cells [Kallio et al., 1999] was also not sustained. Electromobility shift assays demonstrated that HIF-1 from control and PI-treated cells binds HRE equally efficiently [Salceda and Caro, 1997]. This suggests that the interaction between HIF-1α and HIF-1β, and the DNA binding affinity of HIF-1 per se is not compromised in the presence of PI.

Competition for p300/CBP Coactivators

Because coactivators p300/CBP interact with a large number of transcription factors, their amounts could become limiting, e.g., activated p53 sequesters p300/CBP away from HIF-1 [Blagosklonny et al., 1998; Schmid et al., 2004]. Although bortezomib stabilizes transcriptionally active p53 [Williams and McConkey, 2003],

competition from p53 does not explain inhibition of HIF-1 by PI as it is also observed in p53 null Saos-2 cells [Kaluz et al., 2006]. CBP/p300 interacting transactivator with ED-rich tail 2 (CITED2, previously p35srj/Mrg1) binds p300/CBP CH1 with high affinity and competitively inhibits other p300/CBP CH1-dependent transcription factors [Bhattacharya et al., 1999]. Findings that siRNA against CITED2 restored some of the PI-inhibited HIF activity led to the proposition that CITED2, stabilized in the presence of PI, mediates repression of HIF [Shin et al., 2007]. Although plausible, this proposition has an important caveat: CITED2, in a fashion similar to chetomin (a small-molecule inhibitor functioning as a general disrupter of p300/CBP CH1 interactions), has been shown to inhibit hypoxia-dependent, and other p300 CH1-dependent transcription (e.g., STAT-2) [Bhattacharya et al., 1999; Kung et al., 2004]. Whereas Shin et al. failed to investigate how PI affects the activity of other p300 CH1-dependent transcription factors, Kaluz et al. [2006] reported the opposite regulation of the HIF-1α CAD (downregulated) and STAT-2 (upregulated) Gal4 constructs by PI. Moreover, dramatic activation of p300/CBP by PI [Lonard et al., 2000; Kaluz et al., 2006] would suggest that even if transcription factors do experience decreased availability of these coactivators, this would be offset by their increased activity, as observed with STAT-2 or p53. Along these lines, the stimulatory effect of siRNA against CITED2 on HIF activity in PI-treated cells would be expected, as the pool of highly active p300/CBP available for other transcription factors increases. Another intriguing aspect of the studies of Shin et al. [2007] is that hypoxia suppressed CITED2 expression in two different cell lines, which is contrary to the previously published strong induction of CITED2 by hypoxia, regulated via an HRE in its promoter [Bhattacharya et al., 1999]. In summary, we believe that the differential effect of PI on p300/CBP CH1-dependent transcription factors (inhibition of HIF on the one hand and activation of STAT-2 and p53 on the other) is at variance with the notion that decreased availability of p300/CBP, due to increased competition from some other factor(s), is responsible for the inhibitory effect of PIs on HIF.

In conclusion, none of the theories that have been proposed so far for inactivation of HIF-1 in the presence of PI can accommodate all of the observed findings. Instead, the data seem to suggest that PI mediates a rather selective transcriptional inactivation of HIF. This transcriptional inactivation could be brought

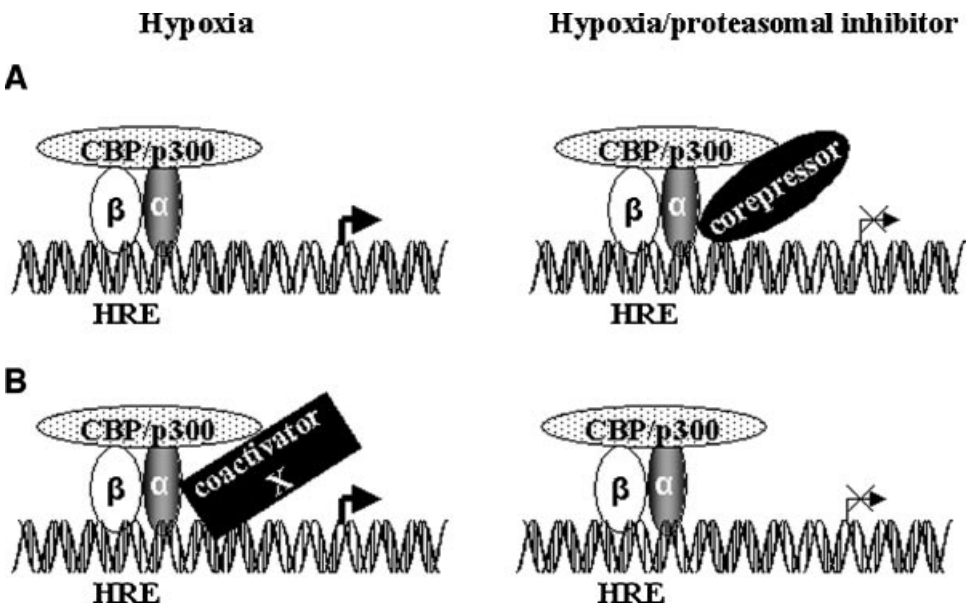

Fig. 2. The outline of the two potential mechanisms by which PI could mediate inhibition of HIF-1: induction of a corepressor (**A**) and a loss of a coactivator (**B**). The available data suggest that PI inhibits HIF at the level of the HIF transcriptional complex. In the first mechanism, the HIF-1–p300/CBP complex assembles on the HRE, but transcription in the presence of PI does not commence due to binding of a PI-induced corepressor (A). In the second mechanism, PI inhibits binding of one of the critical coactivators, leading to assembly of an incomplete, transcriptionally defective HIF complex on the HRE (B).

about by two possible mechanisms, outlined in Figure 2. In the first, the HIF-1–p300/CBP complex binds HRE, but transcription in the presence of PI does not commence due to binding of a PI-induced corepressor. Alternatively, PI could induce the loss of one of the critical coactivators from the HIF complex. Future research into this area is needed to identify the corresponding mechanism.

The review of available data confirms that, as expected, the inhibition of degradation at any level leads to stabilization and accumulation of HIF-α. In most cases, the stabilized HIF-α is functional and transactivates hypoxia-inducible genes. A notable exception to this is inhibition of the proteasome. PIs not only stabilize HIF-α in an inactive form under normoxia, but considerably inhibit activity of the hypoxia-stabilized HIF-α. Despite dramatic increase in our understanding of HIF-α regulation in the last few decades, the counter-intuitive effect of proteasomal inhibition proves that some aspects of this regulation still await explanation.

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