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HYPOXIA

# The HIF-1 $\alpha$ -C/EBP $\alpha$ Axis

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**The hypoxia inducible factors (HIFs) and CCAAT/enhancer binding protein alpha (C/EBP $\alpha$ ) are transcription factors that mediate adaptive responses to hypoxia and control aspects of energy metabolism, respectively. New evidence suggests that when HIF-1 $\alpha$  and C/EBP $\alpha$  interact, they bring about reciprocal functional changes, so that the activity of HIF-1 $\alpha$  is decreased and that of C/EBP $\alpha$  is restricted or increased in a tissue-specific manner. This Journal Club article highlights research depicting interactions between HIF-1 $\alpha$  and C/EBP $\alpha$  and discusses conditions and tissues in which this interaction might occur.**

Life in an aerobic environment is far more complex than life in an anaerobic environment, a circumstance that has probably spurred the evolution of complex life forms (1). Oxygen dependence necessitated the development of new biochemical pathways and signaling mechanisms, not only to counter toxic oxygen radicals but also to adapt to transient hypoxic conditions. Multicellular organisms ranging from the nematode *Caenorhabditis elegans* to *Homo sapiens* have evolved a highly conserved family of basic-helix-loop-helix transcription factors—the hypoxia-inducible transcription factors (HIFs)—to cope with the latter circumstance (2). These transcription factors consist of heterodimers containing a constitutively expressed  $\beta$  subunit (HIF-1 $\beta$ ) and a regulated  $\alpha$  subunit (HIF-1 $\alpha$ , -2 $\alpha$ , or -3 $\alpha$ ). The HIF $\alpha$  subunits are stabilized under conditions of acute hypoxia and translocate to the nucleus to dimerize with the  $\beta$  subunit, enabling interaction of the heterodimer with consensus HRE (hypoxia response element) sequences. This brings about alterations directly or indirectly in the expression of more than 200 genes that participate in the cellular adaptation to hypoxia, which encompasses changes in cell metabolism, cell growth, and apoptosis, and the stimulation of erythropoiesis and angiogenesis, among other responses (3).

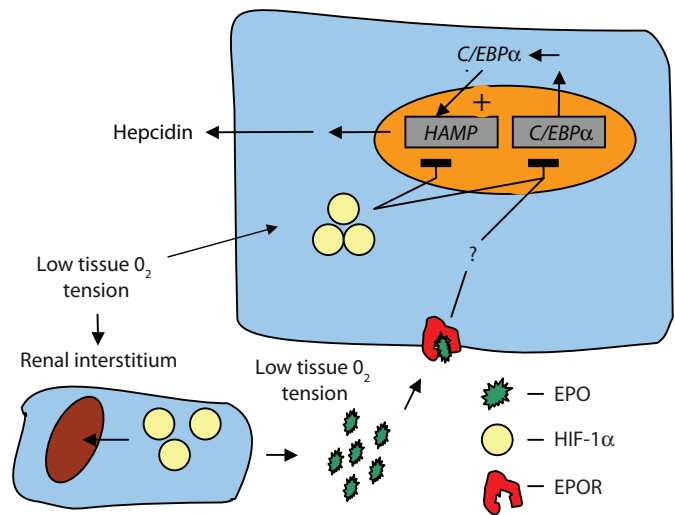
The discovery of HIF was made in relation to its ability to effect a severalfold increase in the transcription of the gene encoding erythropoietin (Epo), a peptide hormone secreted chiefly from adult renal tissue and fetal liver parenchyma under hypoxic conditions in mammalian organisms (4). Erythropoietin stimulates an

increase in red blood cell production and also indirectly increases the absorption of iron from the intestine by acting as a negative regulator of the transcription of the gene encoding hepcidin, a peptide that limits the entry of iron from the duodenal mucosa into blood plasma (5). The mechanistic basis of the down-regulation of hepcidin gene (*HAMP*) transcription by Epo is not clear. A recent publication by Pinto and colleagues (6) described the importance of down-regulating transcription of the gene encoding C/EBP $\alpha$ , a basic leucine zipper transcription factor, to Epo-mediated hepcidin suppression. C/EBP $\alpha$ , which was discovered in the mid 1980s, is widely thought to act as a key regulator of energy homeostasis as evidenced by the fact that C/EBP $\alpha$  null mice show abnormalities in synthesis and storage of glycogen and lipids (7). Pinto *et al.* found a dose-dependent decrease in the transcription of hepcidin in response to stimulation of mouse hepatocytes and HepG2 human hepatoma cell lines by recombinant Epo (rEpo). The concentrations of rEpo used in the experiments were supraphysiological and are comparable to those seen in severe hypoxic states. They further showed

that blocking Epo receptors (EPOR) using an antibody directed against the Epo receptor abrogated the effects of Epo on the transcription of *HAMP* and *C/EBP $\alpha$* . Moreover, consistent with the earlier demonstrated role of C/EBP $\alpha$  acting as a positive regulator of hepcidin gene transcription (8), Pinto *et al.* found that there was substantially decreased binding of C/EBP $\alpha$  to the hepcidin gene promoter, and this may have been the reason for the down-regulation of hepcidin seen here. Although this work suggests a possible route through which Epo could suppress hepcidin, the mechanistic basis for the reduction in C/EBP $\alpha$  remains to be determined.

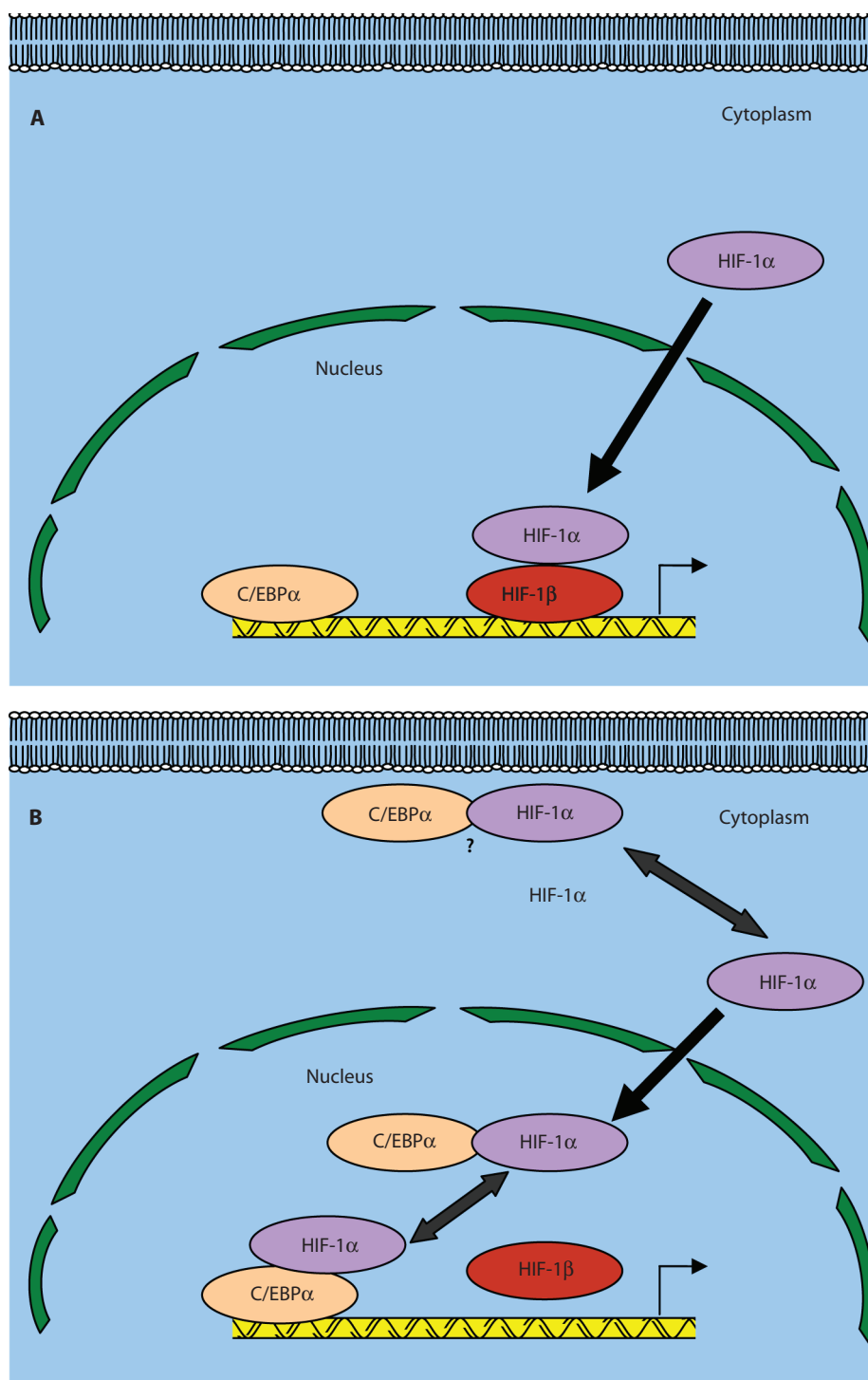
In this context, it is interesting to emphasize that Epo secretion, as stated earlier, is substantially increased over basal secretion (through the stabilization of HIF) under conditions of hypoxia. Moreover, HIF-1 suppresses the transcription of *HAMP* (9) and *C/EBP $\alpha$*  (10) by directly binding to HREs in their respective promoters.

Pinto *et al.* investigated the response of mouse hepatocytes and HepG2 cells to Epo under normoxic conditions. Their results suggested that C/EBP $\alpha$  down-regulation brought on by Epo-EPOR signaling may suppress hepcidin independent of HIF-1. Given that Epo secretion is increased only under hypoxic conditions, it seems possible



**Fig. 1.** Mechanisms by which HIF-1 modulates hepcidin gene (*HAMP*) transcription. Under hypoxic conditions, HIF-1 $\alpha$  is stabilized and translocates to the nucleus, where upon binding to HIF-1 $\beta$  it effects the transcription of various genes. HIF-1 represses transcription of *C/EBP $\alpha$*  and *HAMP* and stimulates *Epo* transcription. *Epo* independently via EPOR represses the transcription of *C/EBP $\alpha$* . The consequent decrease in C/EBP $\alpha$  further represses the transcription of *HAMP*.

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**Fig. 2.** Interaction between HIF-1 $\alpha$  and C/EBP $\alpha$ . **(A)** This panel describes the canonical pathway for HIF whereby under conditions of hypoxia, HIF-1 $\alpha$  is stabilized in the cytosol and translocates to the nucleus to bind to HIF-1 $\beta$ . **(B)** New evidence suggests that the interaction of HIF-1 $\alpha$  with its heterodimeric partner HIF-1 $\beta$  is competitively inhibited by the interaction between HIF-1 $\alpha$  and C/EBP $\alpha$  (12). The activity of C/EBP $\alpha$  may, however, be increased (13) as a result of direct protein interaction with HIF-1 $\alpha$  or transcriptionally repressed by the action of HIF-1 on the promoter of C/EBP $\alpha$  (10). Whether the interaction between HIF-1 $\alpha$  and C/EBP $\alpha$  also occurs in the cytosol is unclear.

that, during hypoxia, Epo and HIF may act synergistically to mediate the down-regulation of C/EBP $\alpha$  and consequently hepcidin. In this scenario, when extrapolated to in vivo conditions, HIF-1 stabilized by low oxygen tension would bind to HREs and in the process suppress the transcription of *HAMP* and C/EBP $\alpha$  and also increase the concentration of circulating Epo. Epo further binds to its receptor EPOR and effects a decrease in C/EBP $\alpha$  transcription. The loss of stimulatory input from C/EBP $\alpha$  leads to a further drop in the transcription of the gene encoding hepcidin (Fig. 1). As described, this scenario involves indirect cooperation between HIF-1 $\alpha$  and C/EBP $\alpha$  at the transcriptional level, through HIF-1 suppressing the transcription of C/EBP $\alpha$ , but it is also possible that hepcidin regulation by these two factors could involve a direct protein-protein interaction.

The possibility of a direct protein interaction between HIF-1 $\alpha$  and C/EBP $\alpha$  is suggested by studies done in myeloid leukemic cell lines. Chen and colleagues, who were investigating the mechanism of hypoxia-mediated differentiation of U937 myeloid leukemia cells, used experiments employing shRNA against HIF-1 $\beta$  to show that myeloid differentiation is brought about by the stable expression of HIF-1 $\alpha$  and does not require its heterodimeric partner HIF-1 $\beta$  (11). They further established that this effect instead depends on a physical interaction between C/EBP $\alpha$  and HIF-1 $\alpha$ . The interaction, which was mapped, involved the bHLH domain of HIF-1 $\alpha$  and the transactivation domain of C/EBP $\alpha$  (12). This interaction prevented the binding of the HIF-1 $\alpha$  subunit to the  $\beta$  subunit in a competitive manner; however, the effects of the interaction on the activity of C/EBP $\alpha$  were not investigated (Fig. 2). These studies (11, 12) also did not address the effect of hypoxia on the transcription of C/EBP $\alpha$ , although C/EBP $\alpha$  protein levels were decreased in whole-cell protein extracts obtained from U937 cells that stably overexpressed HIF-1 $\alpha$  (11). However, endogenous expression of C/EBP $\alpha$  protein was not altered in U937 cells that were exposed to hypoxia-mimetic CoCl<sub>2</sub> (12).

An earlier study by Jiang *et al.* showed, using the luciferase reporter construct assay, that the transcriptional activity of C/EBP $\alpha$  was increased when both HIF-1 $\alpha$  and C/EBP $\alpha$  were coexpressed after transfection in nonhematopoietic COS7 cells and 293T cells compared to when C/EBP $\alpha$  was expressed alone (13). Physical inter-

action between HIF-1 $\alpha$  and C/EBP $\alpha$  was demonstrated using coimmunoprecipitation assay in nuclear protein extracts of CoCl<sub>2</sub>-treated U937 cells (12) and whole-cell protein extracts from the above-mentioned nonhematopoietic cell lines coexpressed with HIF-1 $\alpha$  and C/EBP $\alpha$  (13). Whether the interaction between HIF-1 $\alpha$  and C/EBP $\alpha$  takes place only in the nucleus or in both cytoplasm and nucleus remains to be determined.

HIF has also been shown to down-regulate the transcription of C/EBP $\alpha$  in T-47D breast cancer cell lines and, more interestingly, C/EBP $\alpha$  was found to relocalize from the nucleus to the cytoplasm when T-47D cells were subjected to hypoxia (1% O<sub>2</sub>) compared to normoxia (21% O<sub>2</sub>) (10). Furthermore, hypoxia decreased the stability of C/EBP $\alpha$  mRNA in these cells. Although the results of Chen *et al.* and Seiffeddine *et al.* may sound contradictory, it may just be a reflection of differences in the tissues used for the studies. Neither study has sufficiently probed the interaction between HIF-1 $\alpha$  and C/EBP $\alpha$  in its entirety. Future studies addressing the effects of hypoxia on the endogenous expression of C/EBP $\alpha$ , the subcellular localization of both HIF and C/EBP $\alpha$  in hypoxia, and quantification of the changes in the activities of both the transcription factors simultaneously may be more informative.

Further studies are needed to understand this interaction better and to characterize the interactions between HIF-1 $\alpha$  and C/EBP $\alpha$  spatiotemporally in different tissues in both normoxic and hypoxic states. Using Western blot analysis of different

subcellular fractions to study the relative amounts of both these factors within cells subjected to hypoxia may help us understand better the reciprocal regulation brought about by the HIF-1 $\alpha$  and C/EBP $\alpha$  interaction. Furthermore, the intrinsic relation between oxygen availability and energy homeostasis makes the interaction between these two transcription factors all the more interesting. Understanding the nature of this interaction may hold potential therapeutic strategies in disease conditions characterized by hypoxia, including myocardial ischemia, stroke, and cancers as, for example, the overexpression of C/EBP $\alpha$  may inhibit the growth of tumors by inhibiting cell proliferation mediated by HIF in hypoxic tumor tissue (12).

References and Notes

1. J. Raymond, D. Segre, The effect of oxygen on biochemical networks and the evolution of complex life. *Science* **311**, 1764–1767 (2006).
2. D. Hoogewijs, N. B. Terwilliger, K. A. Webster, J. A. Powell-Coffman, S. Tokishita, H. Yamagata, T. Hankeln, T. Burmester, K. T. Rytönen, M. Nikinmaa, D. Abele, K. Heise, M. Lucassen, J. Fandrey, P. H. Maxwell, S. Pahlman, T. A. Gorr, From critters to cancers: Bridging comparative and clinical research on oxygen sensing, HIF signaling, and adaptations towards hypoxia. *Integr. Comp. Biol.* **47**, 552–577 (2007).
3. R. H. Wenger, D. P. Stiehl, G. Camenisch, Integration of oxygen signaling at the consensus HRE. *Sci. STKE* **2005**, re12 (2005).
4. B. L. Ebert, H. F. Bunn, Regulation of erythropoietin gene. *Blood* **94**, 1864–1877 (1999).
5. E. Fein, U. Merle, R. Eehalt, T. Herrmann, H. Kulaksiz, Regulation of hepcidin in HepG2 and RINm5F cells. *Peptides* **28**, 951–957 (2007).
6. J. P. Pinto, S. Ribeiro, H. Pontes, S. Thowfeequ, D. Tosh, F. Carvalho, G. Porto, Erythropoietin mediates hepcidin expression in hepatocytes through EPOR signaling and regulation of C/EBP $\alpha$ . *Blood* **111**, 5727–5733 (2008).
7. W. C. Yeh, S. L. McKnight, Regulation of adipose maturation and energy homeostasis. *Curr. Opin. Cell Biol.* **7**, 885–890 (1995).
8. B. Courselaud, C. Pigeon, Y. Inoue, J. Inoue, F. J. Gonzalez, P. Leroyer, D. Gilot, K. Boudjema, C. Guguen-Guillouzo, P. Brissot, O. Loréal, G. Ilyin, C/EBP $\alpha$  regulates hepatic transcription of hepcidin, an antimicrobial peptide and regulator of iron metabolism. *J. Biol. Chem.* **277**, 41163–41170 (2002).
9. C. Peyssonnaud, A. S. Zinkernagel, R. A. Schuepbach, E. Rankin, S. Vaulont, V. H. Haase, V. Nizet, R. S. Johnson, Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). *J. Clin. Invest.* **117**, 1926–1932 (2007).
10. R. Seiffeddine, A. Dreiem, E. Blanc, M. C. Fulchignoni-Lataud, M. A. Le Frere Belda, F. Lecuru, T. H. Mayi, N. Mazure, V. Favaudon, C. Massaad, R. Barouki, L. Massaad-Massade, Hypoxia down-regulates CCAAT/enhancer binding protein- $\alpha$  expression in breast cancer cells. *Cancer Res.* **68**, 2158–2165 (2008).
11. L. P. Song, J. Zhang, S. F. Wu, Y. Huang, Q. Zhao, J. P. Cao, Y. L. Wu, L. S. Wang, G. Q. Chen, Hypoxia-inducible factor-1 $\alpha$ -induced differentiation of myeloid leukemic cells is its transcriptional activity independent. *Oncogene* **27**, 519–527 (2008).
12. L. Yang, Y. Jiang, S. F. Wu, M. Y. Zhou, Y. L. Wu, G. Q. Chen, CCAAT/enhancer-binding protein  $\alpha$  antagonizes transcriptional activity of hypoxia-inducible factor 1  $\alpha$  with direct protein-protein interaction. *Carcinogenesis* **29**, 291–298 (2008).
13. Y. Jiang, Z.-H. Xue, W.-Z. Shen, K.-M. Du, H. Yan, Y. Yu, Z.-G. Peng, M.-G. Song, J.-H. Tong, Z. Chen, Y. Huang, M. Lübbert, G.-Q. Chen, Desferrioxamine induces leukemic cell differentiation potentially by hypoxia-inducible factor-1 $\alpha$  that augments transcriptional activity of CCAAT/enhancer-binding protein- $\alpha$ . *Leukemia* **19**, 1239–1247 (2005).
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