Erythropoietin (EPO) is the hormonal regulator of red cell production and provided the paradigm for oxygen-regulated gene expression that led to the discovery of hypoxia-inducible factor (HIF). In this issue of the JCI, Rankin and colleagues show, using targeted gene inactivation, that induction of Epo expression in murine liver is dependent on the integrity of HIF-2α, and not HIF-1α (see the related article beginning on page 1068). These results demonstrate distinct functions for different HIF-α isoforms that could potentially be exploited in therapeutic approaches to anemia.

Erythropoietin (EPO) is a hematopoietic growth factor that, by regulating production of red cells, controls one of the key determinants of physiological oxygen homeostasis, blood oxygen-carrying capacity. In the adult, EPO is produced mainly by the kidneys but also by the liver, and recombinant EPO has been established as the mainstay of treatment of certain types of anemia, particularly anemia associated with chronic kidney disease. In response to severe hypoxia, levels of EPO mRNA and EPO protein production by subsets of cells within the kidneys and liver can increase 1,000-fold or more. This striking response was an initial focus for studies of oxygen-regulated gene expression, and hypoxia-inducible factor (HIF) was defined as the central transcriptional mediator of this process (1). When investigators first probed the molecular pathways underlying oxygen-regulated transcription of the EPO gene, an entirely unanticipated finding was that the pathways involved were widespread and not confined to cells in the kidneys and liver that respond to hypoxia by the production of EPO (2). It is now recognized that the HIF system operates in essentially all cells and can directly or indirectly regulate hundreds of genes whose patterns of response to hypoxia and whose physiological functions are quite distinct from those of EPO. These include genes involved in functionally diverse responses such as cell motility and differentiation, matrix metabolism, and angiogenesis; genes with contrasting kinetic responses to hypoxia such as the massively inducible EPO gene versus the modestly inducible EPO gene encoding glycolytic enzymes; and genes encoding proteins with apparently opposing physiological functions, such as growth factors and proapoptotic mediators (3). The unexpected pleiotropism of the HIF transcriptional cascade presumably underpins the complexity of oxygen homeostasis, but it begs a fundamental question as to how the system could orchestrate such diverse responses.

The oxygen-sensitive HIF hydroxylase pathway
HIF is an α/β heterodimer that binds hypoxia response elements (HREs) at target gene loci under hypoxic conditions (Figure 1). In the presence of oxygen, HIF is inactivated by posttranslational hydroxylation of specific amino acid residues within its α subunits. Prolyl hydroxylase promotes interaction with the von Hippel–Lindau protein (pVHL) E3 ubiquitin ligase complex and proteolytic inactivation by proteasomal degradation, while asparaginyl hydroxylation blocks coactivator recruitment. These hydroxylation steps are catalyzed by a set of non-heme Fe(II)- and 2-oxoglutarate-dependent dioxygenases whose absolute requirement for molecular oxygen confers sensitivity to hypoxia (4). So, how might such an apparently simple pathway transduce hypoxia signaling with the precision required to maintain physiological oxygen homeostasis, and how might the existence of different HIF-α isoforms contribute to this process? Answers to these questions should interest a range of medical scientists; both those seeking to understand the basic biology of oxygen homeostasis and those seeking to assess the feasibility of manipulating hypoxia signaling pathways for therapeutic purposes.

HIF-1α and HIF-2α isoforms
HIF-1α was the original HIF isoform identified by affinity purification using oligonucleotides from the EPO locus (1), while HIF-2α and HIF-3α were identified by homology searches or screens for interaction partners with HIF-1β. HIF-3α is the more distantly related isoform and, in certain splicing arrangements, encodes a polypeptide that antagonizes HRE-dependent gene expression. However, HIF-1α and HIF-2α are closely related, and both activate HRE-dependent gene transcription (3). Nevertheless, knock-out studies in mice demonstrate that HIF-1α and HIF-2α play nonredundant roles, and inactivation of each one results in a distinctly different phenotype. This may result, in part, from differences in tissue-specific and temporal patterns of induction of each isoform (5–7), but, not uncommonly, both isoforms are expressed within a given cell type, and the results of several studies, including those

Nonstandard abbreviations used: CCRC, clear cell renal carcinoma; EPO, erythropoietin; HIF, hypoxia-inducible factor; HRE, hypoxia response element; pVHL, von Hippel–Lindau protein; VHL, von Hippel-Lindau.

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HIF-1 and HIF-2: working alone or together in hypoxia?

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Erythropoietin (EPO) is the hormonal regulator of red cell production and provided the paradigm for oxygen-regulated gene expression that led to the discovery of hypoxia-inducible factor (HIF). In this issue of the JCI, Rankin and colleagues show, using targeted gene inactivation, that induction of Epo expression in murine liver is dependent on the integrity of HIF-2α, and not HIF-1α (see the related article beginning on page 1068). These results demonstrate distinct functions for different HIF-α isoforms that could potentially be exploited in therapeutic approaches to anemia.
reported in this issue by Rankin et al. (8), suggest that HIF-1α and HIF-2α may have distinct transcriptional targets. If so, might distinct transcriptional responses to HIF-1α and HIF-2α be integrated in a way that supports a particular type of physiological adaptation to hypoxia? Recent findings do suggest that this may be the case, though our current understanding is far from clear.

Thus, the transcription of genes encoding enzymes that operate in a coordinated way in the glycolytic pathway appears to be driven by HIF-1α and not HIF-2α (9). A more fundamental question concerns the role (if any) of HIF-α isoform selectivity in coordinating more complex patterns of response such as the dichotomy between pro-survival and proliferative responses to hypoxia on the one hand and apoptotic and anti-proliferative responses to hypoxia on the other. Evidence of distinct roles for HIF-1α and HIF-2α in regulating cell differentiation and in promoting the growth of certain tumors might be taken to support this (10, 11). HIF-2α has been associated with, and appears to promote, an undifferentiated phenotype in pluripotential cells (7, 12).

Distinct roles for HIF-1α versus HIF-2α in promoting tumor growth have, so far, been most clearly defined in von Hippel–Lindau (VHL) disease–associated clear cell renal carcinoma (CCRC). The tumor suppressor gene VHL, which is mutated in the majority of CCRCs, encodes the recognition component of the ubiquitin ligase that directs hydroxylated HIF-1α and HIF-2α to the ubiquitin/proteasome pathway. When VHL is inactivated, both HIF-1α and HIF-2α are stabilized and the HIF transcriptional cascade is constitutively activated (4, 10). For reasons that remain unclear, in neoplastic epithelial cells of CCRCs, the normal predominance of HIF-1α expression in nonneoplastic renal tubules (5) is altered strikingly in favor of HIF-2α expression. Furthermore, genetic manipulation in CCRC cells indicates that activation of HIF-2α but not HIF-1α promotes tumor growth (13, 14). This parallels patterns of transcriptional selectivity in CCRCs where-by HIF-2α drives the expression of genes encoding pro-survival factors such as VEGF, TGF-α, and cyclin D1, whereas HIF-1α drives the expression of genes encoding pro-apoptotic factors such as BCL2/adenovirus E1B–interacting protein 1, NIF3 (BNIP3) (14). However, these results are observed in advanced cancer and CCRC-derived cell lines and appear to be somewhat different from those observed in non–CCRC-derived cells (7, 9, 14). Thus, they may reflect events that alter HIF-α transcriptional selectivity during tumor growth and not an intrinsic pattern of HIF-α transcriptional selectivity that is manifest in tissues of the intact organism. Interestingly, other data support the existence of cell-, condition-, or even disease-specific alterations in the transcriptional activities of HIF-1α and HIF-2α. It has been reported that in mouse embryonic fibroblasts, HIF-2α is transcriptionally inactive and retained in the cytoplasm (15), while evidence has been provided for a titratable repressor that restricts the activity of nuclear HIF-2α in embryonic stem cells (16). Thus, it appears that, superimposed on target gene–specific patterns of transcriptional selectivity, are one or more additional levels of control that limit or enhance the activity of HIF-2α in certain contexts.

**Regulation of Epo by HIF-2α**

Induction of erythropoiesis via the pro-survival growth factor EPO represents a discrete physiological response to hypoxia and a credible therapeutic target. Though the results of studies of constitutive inactivation of HIF-α isoforms have suggested that, at least under some circumstances, both HIF-1α or HIF-2α contribute to the EPO-mediated response, these studies are potentially confounded by effects of systemic HIF-α inactivation and have not permitted a clear comparison of the activities of the two isoforms (17, 18). In this issue of *JCI*, Rankin and colleagues present a thorough analysis of conditional inactivation of HIF-1α and/or HIF-2α...
in the hepatocytes of mice (8). In each of four different situations associated with enhanced hepatic EPO expression (pVHL inactivation, early postnatal anemia, and chemical stimulation by hypoxia mimetics), the authors show that this response is either dominantly or exclusively dependent on the integrity of HIF-2α rather than HIF-1α. The results of this work are consistent with several other previously reported observations linking HIF-2α with Epo regulation; a previous study using siRNA to suppress the expression of HIF-2α in induction of Epo production (19). In normal kidney, the Epo gene is expressed in interstitial fibroblasts (20), a cell type that dominantly expresses HIF-2α (5). Excessive erythrocytosis (abnormally high numbers of red blood cells) is a well-recognized, though generally late, complication, though generally late, complication of a hepatic nuclear factor–4 (HNF-4)–specific responses to hypoxia are orchestrated and, potentially, how specific pharmacological manipulation of hypoxia pathways might be used to induce the production of Epo in the treatment of anemia.

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The deregulation of homeobox (HOX) genes in acute myeloid leukemia (AML) and the potential for these master regulators to perturb normal hematopoiesis is well established. To date, overexpression of HOX genes in AML has been attributed to specific chromosomal aberrations and abnormalities involving mixed-lineage leukemia (MLL), an upstream regulator of HOX genes. The finding reported in this issue of the JCI by Scholl et al. that causal-type homeobox transcription factor 2 (CDX2), which is capable of affecting HOX gene expression during embryogenesis, is overexpressed in 90% of patients with AML and induces a transplantable AML in murine models provides an alternative mechanism for HOX-induced leukemogenesis and yields important insights into the hierarchy of HOX gene regulation in AML (see the related article beginning on page 1037).

Acute myeloid leukemia (AML) is a heterogeneous disease in which hematopoietic progenitor cells acquire genetic lesions that lead to a block in differentiation, increased self-renewal, and unregulated proliferation. The emergence of leukemic blasts appears to require at least two major genetic “hits,” involving perturbations in growth factor signaling pathways and hematopoietic differentiation programs (1).

Among the receptor tyrosine kinases (RTKs), fms-like tyrosine kinase 3 (FLT3), which plays important roles in hematopoietic progenitor cell survival and proliferation, is overexpressed in a significant proportion of AMLs, and mutations resulting in the constitutive activation of FLT3 occur in approximately 33% of patients (2). Mutations leading to the constitutive activation of a related RTK, c-KIT, and of signaling intermediates such as RAS, are also frequently described in AML (3). The dysregulation of associated signaling pathways (e.g., Ras/MAPK, PI3K/ AKT, and JAK/STAT) is thought to result in growth factor–independent proliferation and clonal expansion of hematopoietic progenitors.

The second hit targets transcription factors capable of disrupting hematopoietic cell differentiation. This may occur following the dysregulation of specific gene regulators as a result of gene amplification (e.g., v-myc myelocytomatosis viral oncogene homolog [MYC], mixed-lineage leukemia [MLL], genes at the chromosome 11q23 locus (4)); point mutations in transcriptional regulators (e.g., CCAAT/enhancer–binding protein [C/EBP], runt-related transcription factor 1 [RUNX1]); and chromosomal translocations resulting in the fusion of promyelocytic leukemia (PML) and the retinoic acid receptor α (RARα) to yield the PML-RARα chimeric protein or fusion of RUNX1 and runt-related transcription factor 1, translocated to 1 (RUNX1T1), which gives rise to the RUNX1-RUNX1T1 fusion protein (1). It is becoming increasingly clear that one set of genes commonly affected by these chimerical and mutated transcriptional regulators are the homeobox (HOX) genes. Accordingly, overexpression of homebox master transcription factors, which fulfill critical roles in embryonic development, organogenesis, and normal hematopoietic differentiation, is a common feature of AML (5).

**HOX genes: from hematopoiesis to leukemia**

In mammals, HOX genes are located in two main clusters, the primordial cluster and the ParaHox cluster, which are thought to originate from the duplication of a hypothetical ProtoHox cluster of four genes early in evolution (6). The primordial HOX cluster consists of 13 paralogous groups of genes that exist as distinct, unlinked complexes on human chromosomes 7p15 (HOXA), 17q21 (HOXB), 12q13 (HOXC), and 2q31 (HOXD), and the cluster is organized such that during embryonic development, the order of expression along the anterior-posterior embryonic axis (3′ to 5′) is colinear with the alignment of genes on the chromosome (Figure 1). During hematopoiesis, HOX genes are expressed in lineage- and stage-specific combinations; however, cell commitment to myeloid or erythroid lineages is accom-

Nonstandard abbreviations used: AML, acute myeloid leukemia; ATRA, all-trans retinoic acid; CDX2, caudal-type homeobox 2 transcription factor 2; ETV6, ets variant gene 6; FLT3, fms-like tyrosine kinase 3; HOX, homeobox; MEIS1, myeloid ecotropic viral integration site 1; MLL, mixed-lineage leukemia; PML, promyelocytic leukemia; RARα, retinoic acid receptor α; RTK, receptor tyrosine kinase; RUNX1, runt-related transcription factor 1.

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