THE REGULATION OF ERYTHROPOIETIN AND OTHER GENES BY HYPOXIA: THE SEARCH FOR UNIVERSAL MECHANISMS

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RESUMEN: La Regulación de la Eritropoyetina y Otros Genes por la Hipoxia: la Búsqueda de Mecanismos Universales

En los mamíferos, la eritropoyesis es controlada por la hormona eritropoyetina, cuyos niveles son dictados por el aporte tisular de oxígeno. El control de la producción de eritropoyetina en células especializadas es ejercida principalmente a través de una secuencia reforzadora crítica y la transcripción del factor-1 inducible por hipoxia (HIF1). El hallazgo de que el reforzador de la eritropoyetina era inducible por hipoxia en la mayor parte de células sino en todas, llevó a la búsqueda de otros genes regulados por este mecanismo. La evidencia de tal regulación proviene de un examen de la expresión de genes en cultivo celular y ha utilizado tres enfoques diferentes. Primero, que como para la eritropoyetina había una inducción de expresión genética por la hipoxia, por ciertos metales de transición y por quelantes de hierro. Segundo, las secuencias de acción-cis que conferían inducción hipóxica a estos genes tenían marcada similaridad con la secuencia crítica en el reforzador de eritropoyetina y podían unirse al complejo transcripcional HIF-1. Tercero, había inducción reducida o ausente de la expresión de genes particulares en las células deficientes en HIF-1. Tal evidencia ha sido obtenida para la regulación hipóxica de muchos genes, incluyendo eritropoyetina, factores de crecimiento angiogénicos, enzimas glicolíticas, transportadores de glucosa, tirosina hidroxilasa, endotelina y sintetasa inducible de óxido nítrico. Se están realizando investigaciones adicionales sobre el mecanismo sensor de oxígeno subyacente y sobre el rol de este sistema de respuesta in vivo.

Palabras claves: Hipoxia, Oxígeno, Eritropoyetina, Angiogénesis

RÉSUMÉ: La régulation de l'érythropoïétine et autres gènes par hypoxie : recherche de mécanismes universels.

Chez les mammifères, l'érythropoïèse est contrôlée par l'hormone érythropoïétine dont les niveaux sont fonction de l'apport tissulaire d'oxygène. Le contrôle de la production d'érythropoïétine dans des cellules spécialisées s'exerce principalement par l'intermédiaire d'une séquence critique de renfort et la transcription du facteur -1 inductible par hypoxie (HIF-1). La découverte du fait que le renforçateur de l'érythropoïétine était inductible par hypoxie, dans la plus grande partie des cellules si ce n'est dans toutes, a conduit à rechercher d'autres gènes régulés par ce mécanisme. L'évidence d'une telle régulation dérive d'un examen de l'expression de gènes dans une culture de cellules, utilisant trois approches différentes. On a observé, premièrement, que comme pour l'érythropoïétine il y avait une induction de l'expression génétique par hypoxie, certains métaux de transition et par chélateurs du fer; deuxièmement, que les séquences d'action *cis* permettant l'induction hypoxique de ces gènes montraient une nette similitude avec la séquence critique du renforçateur de l'érythropoïétine et pouvaient s'unir au complexe transcriptionnel HIF-1; troisièmement, que l'induction était réduite ou absente de l'expression des gènes particuliers dans les cellules déficientes en HIF-1. Une telle évidence a été obtenue pour la régulation hypoxique de nombreux gènes, y compris l'érythropoïétine, les facteurs de croissance angiogéniques, les enzymes glycolytiques, les transporteurs de glucose, la tyroxine hydroxylase, l'endothéline et la synthétase inductible d'oxyde nitrique. Des investigations supplémentaires sont en cours concernant le mécanisme senseur d'oxygène qui sous-tend cette réponse, ainsi que sur le rôle de ce système *in vivo*.

Mots-clés : Hypoxie, Oxygène, Erythropoïétine, Angiogenèse.

SUMMARY: The regulation of erytropoietin and other genes by hypoxia: the search for universal mechanisms

Oxygen is of fundamental importance for living organisms. In higher organisms it plays a vital role in energy provision by acting as the terminal acceptor in the electron transport chain. It also has an essential role in many critical biosynthetic reactions. Despite performing these vital functions, oxygen can exert toxic effects because of the formation of reactive oxygen species. Achieving the appropriate delivery of oxygen to tissues and cells, adjusting metabolism accordingly and minimising the toxicity of oxygen is crucial for living organisms. In order for this to occur, organisms must be able to sense oxygen and respond to changes in oxygen tension by altering the expression of particular genes.

In single cellular organisms, oxygen is obtained from the environment by simple diffusion. In larger, multicellular organisms, complex organs are necessary to supply oxygen to tissues. In mammals oxygen is absorbed by the lungs into the blood. Oxygen has a relatively poor aqueous solubility. To achieve sufficient oxygen supply to the tissues, oxygen carriage in the blood is considerably enhanced by the transport of oxygen liganded to haemoglobin, packaged within red blood cells. A complex vasculature enables the delivery of this oxygenated blood to the tissues. In order for this supply to be optimally maintained, precise distribution of the vasculature to the lungs and other tissues is essential, as is the control of ventilation and red blood cell production.

Key words : Hypoxia, Erytropoietin, Angrogenesis

The regulation of erythropoiein by oxygen

Erythropoiesis is controlled primarily by the hormone erythropoietin whose levels are dictated by tissue oxygen supply. The levels of erythropoietin are altered primarily by changes in gene transcription. Studies of the mechanism of this regulation have been considerably advanced by the demonstration of erythropoietin production in cell culture by the hepatoblastomacell lines, Hep3B and HepG2 (Goldberg et al., 1987). Such experiments demonstrated the capacity for erythropoietin production and altered gene transcription in response to hypoxia in a single cell type. The use of such cells has also permitted an analysis of the cis-acting DNA elements which confer hypoxic regulation to the erythropoietin gene. Several groups independently established the critical role of an enhancer element in the 3'-

flanking region of the erythropoietin gene (Beck et al., 1991; Pugh et al., 1991; Semenza et al., 1991). A 26 base pair sequence has been isolated which could convey hypoxically inducible expression when placed near to heterologous promoters and when re-iterated.

The oxygen sensor

The nature of the oxygen sensor which mediates the regulation of erythropoietin is of great interest. Metabolic inhibitors such as cyanide do not mimic the effect of hypoxia (Tan and Ratcliffe, 1991). These experiments have led to the hypothesis that it is oxygen itself which is being sensed rather than some consequence of hypoxic metabolic compromise such as altered phosphorylation potential, or a non-specific cellular stress response.

A very interesting observation in erythropoietin production is the stimulation of erythropoietin levels by cobaltous ions. This has been observed in humans, experimental animals and in the isolated kidney (Goldwasser et al., 1958; Fisher and Birdwell, 1961; Goldbergetal., 1988). Goldberg and colleagues have analysed these observations in more detail utilising Hep3B cells. They showed that cobalt, nickel and manganese ions could all stimulate the production of erythropoietin by normoxic cells in a dose dependent manner but that other transition metals were without effect (Goldberg et al., 1988). This stimulation was of a similar magnitude to that achieved by exposure to 1% hypoxia and was not additive to it. This led them to hypothesise that the oxygen sensor was a haemoprotein which, analogous to haemoglobin, formed a 'deoxy' confomation in the absence of oxygen. They proposed that the transition metal ions cobalt, nickel and manganese could substitute for ferrous ion in the porphyrin ring of haem. These metalloporphyrins would be unable to bind oxygen and the sensing haemoprotein would be locked in the 'deoxy' conformation.

They provided further evidence for the involvement of a haemoprotein in the sensing process. Carbon monoxide is relatively inert chemically but has a particular affinity for haem. The toxicity of carbon monoxide relates in large part to high affinity binding to haemoglobin 'locking' it in its 'oxy' conformation, thereby,decreasing oxygen affinity and reducing tissue oxygen delivery. The exposure of Hep3B cells to 10% carbon monoxide inhibited the hypoxia-induced increase in erythropoietin. Although these results provide strong circumstantial evidence for a haemoprotein sensor, alternative explanations can be provided for the experimental observations. The inhibitory effects of compounds may not be specific. There are many essential haemoproteins in cells, some of which may play a critical role in oxygen sensing but not via the liganding of molecular oxygen.

Semenza and colleagues have demonstrated an increase in erythropoietin mRNA following exposure of Hep3B cells to the iron chelator desferrioxamine (Wang and Semenza, 1993a). It is more difficult to accommodate this important observation in a simple model of a haem based sensing molecule. Haem iron is not chelatable but examples exist among non-haem ferroprotein sensors of labile iron atoms being susceptible to chelation such as FNR and the iron response element binding protein. Whatever the explanation for the action of these agents on erythropoietin expression, they provide an important set of characteristic pharmacological responses. This pattern of response can be used to examine the existence of the same or similar mechanisms of sensing and signal transduction in the regulation of other genes by oxygen.

Hypoxia-inducible factor-1

The nature of the transcription factor (s) which mediate this transcriptional response to hypoxia has been elucidated by Semenza and Wang. Initially they demonstrated that nuclear extracts from Hep3B cells contained DNA binding activity induced by hypoxia which bound specifically to the cis-acting 3' hypoxia inducible enhancer element from the erythropoietin gene (Semenza et al., 1991). They termed this DNA-bindingactivity hypoxia-inducible factor-1 (HIF-1). Other stimuli such as cobaltous ions, known to mimic the hypoxic induction of erythropoietin mRNA, also produced an inducible DNA-binding activity. Recently, Wang and Semenza have purified this DNA-bindingactivity and shown that HIF- 1 exists in a heterodimeric form of the 120 kDa HIF-l α factor complexed with a 91-94 kDa HIF-1β subunit. The HIF lα cDNA encoded a predicted 826 amino acid protein of 93 kDa which possessed substantial homology to the Drosophila melanogaster basic-helix-loop-helix transcription factors period (Per) and single-minded (Sim) and to the mammalian aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) which all possess a 150 amino acid domain known as the PAS domain (PerARNT--AHR-Sim). The HIF-1β cDNA encoded a 789 amino acid protein with a calculated mass of 87 kDa. The sequence was identical to the previously described aryl hydrocarbon receptor nuclear

translocator (ARNT). This molecule is essential for the transcriptional response to certain aryl hydrocarbons which has been termed the xenobiotic response (for review see (Hankinson, 1995)).

The widespread nature of the system controlling erythropoietin

It had been believed that this mechanism of transcriptional control of the erythropoietin gene by hypoxia would be restricted to those specialised cells which produced erythropoietin. However, Maxwell and colleagues demonstrated that reporter genes containing the hypoxically responsive cis-acting element from the erythropoietin gene could be induced by hypoxia in a variety of different ce]l types (Maxwell et al., 1993). Cells which do not produceerythropoietin and which are not derived from liver or kidney were all able to show hypoxic induction of these transiently transfected reporter constructs. Again, the characteristic responses of the erythropoietin gene to treatment with cobalt was preserved. No induction was seen with metabolic inhibitors such as cyanide (Maxwell et al., 1993). Heat shock induction is also known to operate widely in cells when exposed to noxious stimuli, including anoxia. This generalised mechanism of hypoxic sensing did not appear to be a manifestation of the heat shock response; heat shock did not activatethe erythropoietin enhancer, the 1% hypoxia used did not activate heat shock proteins and cyclohexirnide blocked the erythropoietin enhancer mediated response in contrast to its known inducing effect upon the heat shock response. These observations suggested that many, perhaps all, cells shared similar mechanisms for oxygen sensing, signal transduction and transcriptional activation. Furthermore, it appeared likely that other genes would be regulated by hypoxia via this system.

It was demonstrated subsequently that the HIF- 1 DNA-bindingactivity was also present in a variety of non-erythropoietin producing cells, indeed in all cell types tested (Wang and Semenza, 1993b). These observations, also described by Beck and colleagues (Beck et al., 1993), supported the concept that this system of hypoxic gene regulation was widely present and might regulate the expression of other hypoxically inducible genes.

The regulation of other genes by hypoxia

GIycolytic enzymes

The critical role of oxygen as the terminal electron acceptor in the electron transport chain means that energy provision is closely linked to oxygen supply. In hypoxic conditions reduction in aerobic respiration is associated with an increase in glycolytic flux to provide an increase energy supply. In the short term, increases in glycolysis are mediated by changes in glycolytic flux via alterations in the concentrations of allosteric modulators or enzyme phosphorylation. However, over longer time periods changes in the levels of glycolytic enzymes may be achieved by changes in gene expression mediated by hormonal or other influences (for review see (Pilkis and Granner, 1992)).

Evidence for the regulation of glycolytic enzyme expression by hypoxia has been provided by Webster and colleagues (Webster, 1987; Webster et al., 1990). They observed an increase in gene transcription in response to 2% hypoxia in skeletal myoblasts. The enzymes lactate dehydrogenase, pyruvate kinase, triosephosphate dehydrogenase and aldolase all showed an increase in gene transcription in nuclear run on assays. Furthermore, a reciprocal dcrease was seen in the abundance of mitochondriatly encoded respiratory enzymes. These observations supported the existence of a mechanism for the co-ordinated regulation of glycolytic enzyme expression by oxygen availability, perhaps via common trans-acting factors and similar cis-elements.

The widespread operation of the system controlling erythropoietin regulation raised the question of the role of this system in non-erythropoietin producing cells. The hypoxic regulation of glycolytic enzymes was thus an attractive candidate. The serendipitous observation that a neomycin resistance marker under the control of the phosphoglycerate kinase (PGK) promoter showed hypoxic inducibility led to an examination of the hypoxic regulation of PGK expression. Hypoxic induction of PGK and LDH-A by hypoxia was demonstrated in several tissue culture lines (Firth et al., 1994). This regulation shared several important sirnilarities with the regulation of erythropoietin. The induction could be mimicked by cobaltous ions and by the iron chelator desferrioxamine but cyanide had no effect. Similar observations were made with lactatedehydrogenaseA (Firth et al., 1994; Firth et al., 1995)and subsequently with Aldolase A, Pyruvate kinase M and phospofructokinase (Semenza et al., 1994; Ebert et al., 1995b). The analysis of the PGK and LDH-A promoters utilising the transient transfection of deleted promoter-reporter constructs defined short cis-acting sequences which conveyed hypoxic inducibility. Furthernore, the critical 18 base pair

element in the PGK enhancer/promoter shared substantial homology with the erythropoietin 3'-enhancer including a 9 bp region which was identicalexcept for a G to C substitution (Firth et al., 1994). Electrophoretic mobility shift assays utilising oligonucleotides from these promoters produced hypoxically inducible protein binding with similar characteristics to the HIF-1 complex and which could cross compete with oligonucleotides derived from the erythropoietin 3'-enhancer (Firth et al., 1994; Semenza et al., 1994).

Glucose transporters

In order to respond to an increased rate of glycolysis cells require an increased provision of glucose. Glucose is transported across cell membranes by the process of facilitated diffusion mediated by the glucose transporter farnily. Indeed for one of these transporters, Glut-l, there is evidence of increased expression in response to hypoxia (Loike et al., 1992). However, mitochondrial inhibitors can also increase Glut-1 mRNA (Shetty et al., 1992) perhaps suggesting that induction by hypoxia is due to the hypoxic compromise of metabolism and is distinct from the mechanism underlying erythropoietin regulation. Recently however, the demonstration of the induction of Glut- 1 and Glut-3 mRNA by hypoxia was shown to share similarities with erythropoietin regulation. The induction could be mimicked by cobalt and by iron chelators (Ebert et al., 1995b). An enhancer lying 5' to the mouse Glut-1 gene was found to convey responses both to hypoxia and to mitochondrial inhibitors. The response to hypoxia was conveyed by sequence containing a HIF-1-like binding site whilst response to mitochondrial inhibitors was mediated by a serum response element lying 100 bp 5' (Ebert et al., 1995a). Thus it appears that rather than demonstrating a different mechanism of oxygen sensing, the regulation of Glut-1 by hypoxia and mitochondrial inhibitors is mediated by two different sensing systems, one of which resembles that involved in erythropoietin regulation.

Gluconeogenic enzymes

In the liver there is metabolic zonation of enzymes, notably a preferential periportal distribution of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and increased expression of enzymes favouring glycolysis such as glucokinase in the perivenous zone (Jungermann, 1995). There is evidence to suggest that this zonation may be mediated, at least in part, by oxygenation. Increased oxygenation results in the stimulation of gluconeogenesis by hepatocytes whilst hypoxia favours enhanced glycolysis. The glucagon stimulated expression of one critical gluconeogenicenzyme PEPCK is increased when hepatocytes are exposed to arterial oxygen tensions (Hellkamp et al., 1991). This modulation by oxygen is lost if the cells are pretreated with cobaltous ions (Kietzmann et al., 1992) and carbon monoxide blocks the effect of hypoxia (Kietzmann et al., 1993). This supports the involvement of a similar oxygen sensing system to that which regulates erythropoietin.

Angiogenic growth factors

Higher organisms such as mammals have developed a complex vasculature to supply oxygen and other nutrients to their cell. The development of the vascular system appears to be under the control of many important influences one of which is likely to be hypoxia. Angiogenesis, the formation of new blood vessels from existing vasculature, appears to play a role in physiological processes such as placentation, the development of the corpus luteum and in wound healing. Excessive angiogenesis is associated with pathological states including proliferative retinopathies, atherosclerotic plaques, tumour growth and metastasis.

There are several observations that suggest that oxygen provision is an important determinant of vascular supply and of angiogenesis. The limited stores of oxygen compared with other metabolic substrates makes oxygen an attractive candidate for the signalling of vascular insufficiency. The metabolic requirements of a tissue are closely linked to its vascular supply. The capillary length density, a measure of vascular supply, of a wide range of muscles from different species and sites shows a close linear relationship with mitochondrial volume density, a marker of oxidative capacity (Adair et al., 1990). Further evidence for an important role of oxygen availability in determining vascular growth is also seen in pathological conditions. In premature infants exposed to high concentrations of inspired oxygen the retinal vasculature is attenuated. Upon return to normal oxygen, there is a profuse growth of disorganised, leaky vasculature with the subsequent development of retrolental fibroplasia.

Vascular Endothelial Growth Factor (VEGF) appears to be a particularly selective and potent angiogenic factor. Recent work has provided evidence suggesting an important role for VEGF in

physiological and pathological angiogenesis and that hypoxia is an important stimulus for expression of this gene (Shweiki et al., 1992). We have shown that hypoxic regulation of this and other angiogenic growth factors shares important similarities with erythropoietin regulation (Gleadle et al., 1995a), in particular stimulation by cobalt and iron chelation. Indeed, more recently, others have demonstrated the existence of cis-acting sequences in the VEGF gene which can confer hypoxic regulation. Levy and colleagues demonstrated a 12-fold increase in VEGF mRNA in hypoxically-stimulated PC12 cells (Levy et al., 1995). They went on to identify a 28-base pair element in the 5' promoter with sequence and binding similarities to the HIF-1 binding site within the erythropoietin 3'-enhancer (Levy et al., 1995).

This region is highly conserved in the human gene and was similarly identified by Liu and colleagues as an elementthat was necessary and sufficient to increase transcription in response to hypoxia (Liu et al., 1995). At odds with these results is another study which has defined hypoxia responsive elements at other sites in the 5' and 3' untranslated region of the VEGF gene which do not share homology with a HIF- 1 site (Minchenko et al., 1994).

Tyrosine hydroxylase

Tyrosine hydroxylase is the rate limiting step in the synthesis of catecholamines. Exposure of rats to 10% oxygen resulted in a significant increase in tyrosine hydroxylase mRNA in the carotid body Type I cells (Czyzyk-Krzeska et al., 1992). This increase appears to be mediated in part by an increase in gene transcription. The deletional analysis of the tyrosine hydroxylase promoter has demonstrated a sequence 2W150 from the start site which can confer hypoxic regulation to reporter genes. This region contains HIF-1 like and AP-1 recognition sequences, both of which show hypoxically regulated protein binding (Norris and Millhorn, 1995).

Nitric oxide synthase

The availability of oxygen and other nutrients to tissues is dictated both by the anatomy of the vascular bed and by vascular tone. One of the most important regulators of blood flow appears to be endothelially derived nitric oxide (NO). Some evidence has been provided for modulation of NO synthases by oxygen. Hypoxic exposure produced a fall in endothelial NO synthase mRNA levels in vascular endothelial cells that appeared to be mediated through a reduction in gene transcription and in mRNA stability (McQuillan et al., 1994). Melilio and colleagues examined the induction of ioducible NO synthase by the L-tryptophan metabolite Picolinic acid in interferon treated murine macrophages (Melilloet al., 1995). The cis-acting element responsible for this induction was analysed in transient transfection assays and contained a region with substantial homology to the hypoxia responsive enhancer from the erythropoietin gene, including an exact 13 bp match. Electrophoretic mobility shift assays demonstrated inducible binding to this sequence by both picolinic acid and by hypoxia. Hypoxia was then showed to induce inducible NO synthase mRNA. These observations are of interest not just because of the likelihood of HIF-1 dependent hypoxic regulation of inducible NO Synthase but also because picolinic acid can function as an iron chelator and this might underly its mechanism of action.

Endothelins

The endothelins are a family of widely expressed peptides which are among the most potent endogenous vasoconstrictors. Hypoxia is a potent inducer of endothelin-1 mRNA in human umbilical vein endothelial cells (HUVEC) (Kourembanas et al., 1991). This regulation also shares important similarities with the hypoxic regulation of erythropoietin mRNA. The hypoxic increase can be abrogated by carbon monoxide (Kourembanas et al., 1993) and can be mimicked by the transition metals nickel and manganese and to a less erextent cobalt (Bodi et al., 1995).

The regulation of genes by hypoxia in other organisms

In lower organisms, including yeast and bacteria, complex mechanisms exist for the sensing of oxygen and the modulation of gene expression. Direct homologies with the system involving the transcription factor HIF- 1 have not been demonstrated yet. In an attempt to demonstrate the existence of the mechanism of oxygen sensing which controls erythropoietin in lower organisms, Nagao et al examined for the presence of hypoxically induced DNA binding in embryonic drosophila cells (Nagao et al., 1996). They were able to demonstrate induction of binding to HIF- 1 recognition sequences by acomplex with similarities to mammatian HIF-1. Furthermore, they showed hypoxic regulation of the PGK gene. These observations support the idea that this

mechanism of oxygen sensing may be conserved in other organisms.

Two main lines of evidence have thus contributed to the idea that the system controlling erythropoietin in response to hypoxia also controls other genes. The demonstration of functionally active cis-acting sequences able to regulate the transcription of many genes in response to hypoxia together with responsiveness of these genes to stimulation with cobaltous ions and iron chelators. More recently a furtherline of evidence has been provided (Wood et al., 1996). Cells exist which are deficient for one component of HIF-1. They are unable to form a functional HIF-1 complex in response to hypoxia. In contrast to the wild type cells from which they were derived, they show marked impairment in the hypoxic induction of glycolytic enzymes such as Phosphoglycerate kinase, glucose transporters such as Glut-1 and angiogenic factors such as VEGF(Wood et al., 1996). These results provide further strong support for the existence of a widespread mechanism of oxygen sensing which acts via the transcription factor HIF-1 and regulates the expression of many genes in response to hypoxia.

Outstanding questions

The system which regulates erythropoietin and these other genes has largely been defined in cell culture. It remains to be determined whether these in vitro observations have relevance in vivo. One approach enabling a study of the effects of HIF-1 deficiency in vivo is by the xenotransplantation of the cells lacking ARNT introduced subcutaneously into nude mice. Preliminary experiments suggest significant differences in the growth characteristics, vascularity and VEGF expression of tumours formed from the c4, ARNT deficient, cells when compared with wild type.

The molecular basis of oxygen sensing still requires definition. As alredy discussed a haem based sensor accommadates many of the observations underlying erythropoietin production. A precedent for a haemoprotein oxygen sensor is provided by the Nif protein in Rhizobium meliloti. However, even if a haemoprotein does play a critical role in oxygen sensing it may not be via the liganding of oxygen and undergoing a conformational change. Several lines of evidence are difficult to accommodate in this simple model of a liganding haemoprotein sensor. The effect of iron chelators in inducing erythropoietin, HIF-1 and other hypoxically regulated genes suggests that chelatable iron is closely involved with the mechanism of oxygen sensing. Haem iron is not chelatable. In some bacteria the FNR protein appears to function as an oxygen sensor. It contains an iron-sulphur cluster and iron chelation produces an aerobic pattern of gene expression. This effect illustrates one way in which iron chelation could interact with an oxygen sensing molecule. Altemative models of oxygen sensing rely upon the redox properties of oxygen. It is envisaged that oxygen is rate limiting in the production of reduced oxygen species such as superoxide and hydrogen peroxide. Observations of the effects of catalase and hydrogen peroxide on erythropoietin expression by Fandrey and colleagues provide some support for such a mechanism (Fandrey et al., 1994) as does the inhibition of hypoxic induction of erythropoietin and other genes by iodonium compounds (Gleadle et al., 1995b). The in vitro redox sensitivity of HIF-1 has been demonstrated suggesting a mechanism by which this might occur (Wang et al., 1995).

The mode of regulation of the transcriptional complex HIF-1 is not clear. Potential mechanisms of regulation include protein phosphorylation, redox alterations and direct ligand binding. Using chimeric fusion genes containing HIF-lα, we have recently been able to confer oxygen regulated activity to heterologous transcription factors. This strategy should provide information about the sequences of HIF-lα which are capable of conferring regulated transactivation permitting insights into the likely mechanism of activation.

The system regulating erythropoietin appears to play an important role in the regulation of genes involved in oxygen supply and consumption. The regulation of other genes involved in other processes such as development and with protective functions seems highly plausible. The increasing realisation of the widespread nature of this system and similar systems in more primitive organisms, emphasises the interest in defining such genes and in the molecular mechanisms underlying oxygen sensing.

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