PHYSIOLOGICAL REGULATION OF GENE ACTIVITY BY OXYGEN (O_2)

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Contents

Summary

All eukaryotic cells sense the decrease in oxygen level and respond to hypoxic-stress by regulating transcription of a wide variety of genes by the heterodimeric Hypoxia-Inducible Factor (HIF). Perhaps 1–4% of all human genes are expressed in response to hypoxia in a HIF-dependent manner. Both subunits of HIF, HIFα and HIFβ are constitutively expressed, but the HIFα is quickly degraded in normally oxygenated cells and the activation of HIF target genes is blocked. During hypoxia degradation attenuates, HIFα is stabilized and binds to DNA with HIFβ leading to an activation of array of genes involved in hypoxic adaptation. The Von Hippel-Lindau protein (pVHL) is required for the ubiquitination of HIFα once these proteins have physically interacted and this targets HIFα to proteasomal degradation. The mechanism of cellular oxygen sensing is bound to this interaction, since the recognition and binding of pVHL to HIFα requires oxygen-dependent hydroxylation of specific prolyl residues on HIFα. Three human HIF prolyl hydroxylases (PHD1 to 3) able to hydroxylate HIFα have been identified. The activity of HIF prolyl hydroxylases is directly regulated by oxygen availability indicating that these enzymes function as cellular oxygen sensors.

Aerobically growing cells are exposed to reactive oxygen species (ROS) which damage proteins, lipids and DNA (see *Metabolism of Oxygen*). Oxidative stress occurs when cellular defense mechanisms are unable to cope with existing ROS, and it has been associated with a number of pathologies including cancer and cardiovascular disease.

Since aerobic life is restricted within a very narrow range of oxygen concentrations, cells have been forced to develop highly sophisticated mechanisms to respond to fluctuations in oxygen tension. In this review, cellular mechanisms to cope with both hypoxic and hyperoxic stresses are discussed.

1. Introduction

For all aerobic life molecular oxygen (O_2) is essential for survival, since the energy production of aerobic organisms fundamentally depends on the oxygen bound cell respiration. In the process of oxidative phosphorylation, where the oxidoreductive energy of mitochondrial electron transport is converted into the chemical energy of ATP, oxygen functions as the ultimate electron acceptor. At the final step of the electron transport chain, cytochrome c reduces oxygen into water; this completes the process by which glucose is oxidized to carbon dioxide and water.

Decrease in oxygen availability directly impairs energy production and hence, cell viability. On the other hand excessive oxygen level is highly toxic through the generation of reactive oxygen species (ROS) which directly damage proteins and lipids in cell membranes as well as carbohydrates and genetic material. Thus aerobic life is restricted within a very narrow range of oxygen concentration. Because of its crucial role in sustaining aerobic life elaborated strategies have evolved to respond to decreased (hypoxia) or increased (hyperoxia) oxygen tensions. Cells have developed highly sophisticated systems to respond to variations in oxygen tension, leading to the activation of specific intracellular programs by induction of defined transcription factors and expression of appropriate target genes.

Fluctuations in oxygen levels in the blood stream are sensed by the carotic body (CB), the small organ located at the bifurcation of the common carotid artery close to the heart. Freshly oxygenated blood returns from the lung circulation pumped by the heart to the brain via the carotid artery. Even a small decrease in the blood oxygen level is sensed instantaneously, involving only rapid modifications of pre-existing proteins. Signals launched initiate systemic responses including respiratory and cardiovascular reflexes to ensure proper oxygenation in the vital organs. Prolonged decrease in oxygen availability activates chronic responses and initiates metabolic alterations to ensure cell and organ viability and functionality under hypoxic stress. These responses include stimulation of red blood cell production (erythropoiesis) as well as formation of new blood vessels (angiogenesis) into the hypoxic tissues, improving the efficiency of oxygen delivery.

The gene expression profile of hypoxic cells differs distinctively from normoxic cells. Cell specific sets of hypoxia-responsive genes are activated and production of proteins required to cope with lowered oxygen concentration is induced. Almost all genes controlled by low oxygen tension harbour the Hypoxia Response Element; (HRE) in their regulatory regions. In hypoxia the heterodimeric transcription factor, Hypoxia-Inducible Factor (HIF) binds to this conserved consensus sequence, leading to transcriptional activation of the particular gene.

Normally tissues and cells are not challenged by excess oxygen (hyperoxic stress). Administration of high concentration of pressurised oxygen (hyperbaric oxygen therapy,

HBO_2) is used as a supportive treatment for patients suffering from severe respiratory failure or chronic ischemic wounds. Despite the benefits of HBO_2 it does have its drawbacks, since prolonged HBO_2 treatment increases tissue damage because of high generation of ROS. Rapid reperfusion of ischemic tissue is extremely critical for restoring normal organ functions. However, a sudden burst of oxygenated blood may produce a progressive destruction of reversibly damaged cells leading to sustained tissue dysfunction. This reperfusion injury has an extremely important role in pathology of the brain during stroke and the heart during myocardial infarction.

2. HIF: transcriptional regulator of hypoxic responses

HIF is the most important and best-described factor controlling cellular responses to low oxygen. HIF was originally discovered as being responsible for the regulation of erythropoietin (EPO) expression in hypoxia. Since its discovery, over 100 genes have been identified, which are regulated by HIF and efficiently responding to hypoxia.

Many of the hypoxia-regulated genes play a central roles in acute or chronic adaptation to hypoxia. These genes are implicated in a vast variety of different cellular functions such as cell survival, apoptosis, cell motility, cytoskeletal structure, cell adhesion, angiogenesis, erythropoiesis, vasculature tone, transcriptional regulation, epithelial homeostasis, drug resistance, nucleotide-, amino acid-, iron-, glucose-, energy metabolism and pH regulation.

HIF is ubiquitously expressed and its activity is induced by hypoxia in almost all cell types. The expression of the majority of HIF target genes are induced tightly in a cell-type-specific manner. It is evident that HIF do not alone account for this cell type restricted gene expression. The functional interactions of HIF with other transcription factors determine the subgroup of HIF target genes which is activated in any particular cell type. HIF should be viewed as a messenger that is sent to the nucleus to activate transcriptional responses to hypoxia. The fine details of this response are determined by the developmental and physiological programming of each cell. As a result, the total number of HIF target genes cannot be ascertained by analysis of one or a few cell types. Perhaps 1–4% of all human genes are expressed in response to hypoxia in one or more cell types in a HIF-dependent manner.

HIF is transcriptionally active only as a heterodimeric protein complex composed of two subunits: HIFα and HIFβ (also known as ARNT which stands for Aryl hydrocarbon Receptor Nuclear Translocator). HIFα members exclusively dimerize with HIFβ family members, whereas the β subunits can dimerize with various non-HIF transcription factors. Both subunits contain a basic helix-loop-helix (bHLH) domain and two Per-ARNT-Sim (PAS) domains, designated PAS-A and PAS-B. The intact bHLH and PAS domains are required for protein dimerization and DNA binding. There are three HIFα family members HIF-1α, HIF-2α and HIF-3α and three β family members (HIF1β/ARNT1, HIF2β/ARNT2, HIF3β/ARNT3). HIF-1α and HIF-2α are expressed in a variety of human cell lines. There is concordance between the HIF-1α and HIF-2α responses as well as their regulation. HIF-1α is the predominant protein in epithelial cells, whereas HIF-2α is the main form in endothelial and fibroblast cells. Recently it has become evident that HIF-1α and HIF-2α have somewhat different and specialized

functions. HIF-3α has high similarity with HIF-1α and HIF-2α in the bHLH and PAS domains. One of the HIF-3α isoforms (HIF-3α2) referred as IPAS lacks structures required for transcriptional activation. Its expression is up-regulated in hypoxia, it dimerizes with HIFβ/ARNT and binds to the HRE core sequence, but it suppresses hypoxia-inducible HIF-mediated gene expression and appears to be the negative regulator of hypoxia-inducible gene expression. The overall importance of HIF-3α as a regulator of hypoxia-induced transcription remains to elucidated.

HIFα/HIFβ dimer binds to consensus DNA sequence (A/G)CGTG (HRE: Hypoxia Response Element) common for almost all genes induced by low oxygen tension. Transcriptional activation and interaction with co-activators such as p300 and CBP are mediated by two transactivation domains in the C-terminal half of HIFα, termed the N-terminal (N-TAD) and C-terminal (C-TAD) transactivation domains. HIFβ also contains a TAD but it is not required for transcriptional activity.

Both *hif-1α* and *hif-1β* genes are constitutively expressed but only HIF-1β protein is found in cells under all oxygen conditions, whereas HIF-1α is undetectable in normal oxygen conditions. Negative regulation of HIF-1α under normoxic conditions occurs via the oxygen-dependent degradation domain (ODDD). The ODDD domain contains prolyl residues that are hydroxylated in normoxia. This results in the binding of the Von Hippel–Lindau protein (pVHL), which subsequently targets the HIF-1α protein for rapid degradation by the ubiquitin-proteasome system. HIF-2α, as well as some of the splice variants of HIF-3α proteins contains the conserved VHL-binding domain and they are substrates for pVHL and proteasomal degradation (see Figure 1).

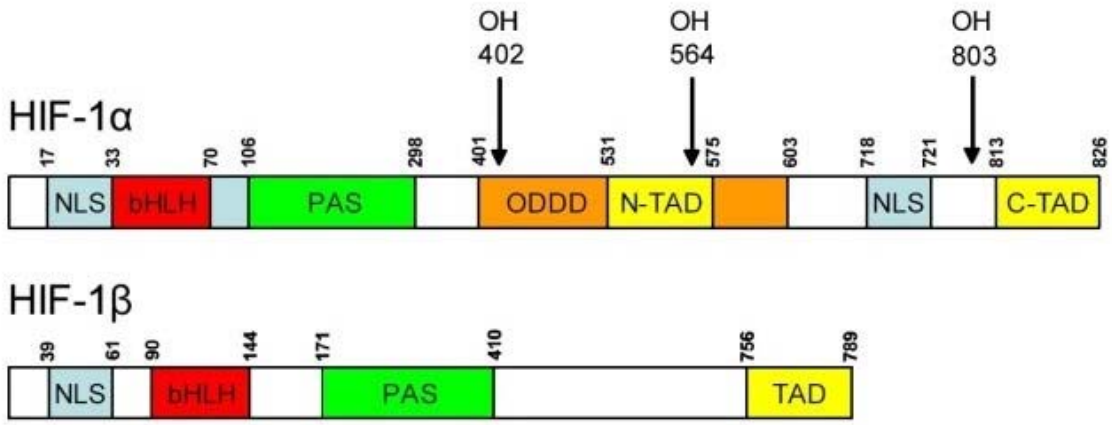

Figure 1. Protein domains of HIFα and HIFβ.
Functional domains are shown. Hydroxylation sites mentioned in the text are indicated. ODDD, oxygen-dependent degradation domain; N-TAD and C-TAD, N- and C-terminal transactivation domain; NLS, nuclear localization signal; PAS, Per-ARNT-Sim.

3. Oxygen-dependent regulation of HIF

Being the most crucial regulator of hypoxic responses, the activity of HIF is tightly controlled at multiple levels. The regulation is mostly targeted to the α subunits and the

main regulatory mechanisms involve oxygen-dependent protein stabilization and transcriptional activation. Moreover, activity is further fine-tuned via post-translational modifications, nuclear translocation, dimerization and interactions with other regulatory proteins. In normoxic conditions, when HIFα and HIFβ are constitutively expressed, the abrogation of HIF activity results mainly from constitutive HIFα degradation.

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Biographical Sketch

Juha-Pekka Pursiheimo, PhD, was born in 1970 in the town of Pori located in the Western part of Finland. He studied genetics and biochemistry in the University of Turku and received his Master of Science degree in 1995. In 1997 he joined in the Proteoglycan research team of Professor Markku Jalkanen located in the Turku Centre for Biotechnology (TCB) and in 2002 he finished his Doctoral thesis entitled "*Protein Kinase A: Regulator of Growth Factor-Induced Responses*". During the year 2002 he worked as a senior scientist in the drug development company Juvantia Pharma oy. He returned to the Academic research community in 2003 when he joined the Docent Panu Jaakkolas' research group (currently located in TCB) studying the cellular response mechanisms to hypoxia. In 2005 he received the postdoctoral fellowship of Academy of Finland and currently he is continuing the groundbreaking hypoxia research in the Turku Centre for Biotechnology. His special interests in research are the identification of a new and novel hydroxylation targets and the role of PHDs controlling the function of these putative target proteins and hence the vital cellular functions. He has contributed several papers in refereed journals and more are to come in the near future.