

Author's Accepted Manuscript

The regulation of transcriptional repression in hypoxia

Miguel A.S. Cavadas, Alex Cheong, Cormac T. Taylor

PII: S0014-4827(17)30076-9
DOI: <http://dx.doi.org/10.1016/j.yexcr.2017.02.024>
Reference: YEXCR10481

To appear in: *Experimental Cell Research*

Received date: 10 January 2017
Revised date: 14 February 2017
Accepted date: 15 February 2017

Cite this article as: Miguel A.S. Cavadas, Alex Cheong and Cormac T. Taylor, The regulation of transcriptional repression in hypoxia, *Experimental Cell Research*, <http://dx.doi.org/10.1016/j.yexcr.2017.02.024>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The regulation of transcriptional repression in hypoxiaMiguel A. S. Cavadas¹, Alex Cheong², Cormac T. Taylor^{3,4}¹ Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 2780-156 Oeiras, Portugal.² Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK.³ Systems Biology Ireland, University College Dublin, Dublin 4, Ireland.⁴ Conway Institute of Biomolecular and Biomedical Research, School of Medicine and Medical Sciences and Systems Biology Ireland, University College Dublin, Dublin 4, Ireland.**Abstract**

A sufficient supply molecular oxygen is essential for the maintenance of physiologic metabolism and bioenergetic homeostasis for most metazoans. For this reason, mechanisms have evolved for eukaryotic cells to adapt to conditions where oxygen demand exceeds supply (hypoxia). These mechanisms rely on the modification of pre-existing proteins, translational arrest and transcriptional changes. The hypoxia inducible factor (HIF; a master regulator of gene induction in response to hypoxia) is responsible for the majority of induced gene expression in hypoxia. However, much less is known about the mechanism(s) responsible for gene repression, an essential part of the adaptive transcriptional response. Hypoxia-induced gene repression leads to a reduction in energy demanding processes and the redirection of limited energetic resources to essential housekeeping functions. Recent developments have underscored the importance of transcriptional repressors in cellular adaptation to hypoxia. To date, at least ten distinct transcriptional repressors have been reported to demonstrate sensitivity to hypoxia. Central among these is the Repressor Element-1 Silencing Transcription factor (REST), which regulates over 200 genes. In this review, written to honor the memory and outstanding scientific legacy of Lorenz Poellinger, we provide an overview of our existing knowledge with respect to transcriptional repressors and their target genes in hypoxia.

Keywords: Hypoxia, Repressor, Transcription, REST

1- The activation and resolution of adaptive responses to hypoxia.

The evolution of mitochondrial respiration along with the associated increase in cellular metabolic capacity, allowed the development of multicellular organisms¹. Oxygen, the final electron acceptor in the oxidation of organic compounds, is thus essential to sustain essentially all complex life. Hypoxia is the condition which arises when metabolic oxygen demand exceeds its supply. Exposure to hypoxia can elicit adaptation, such as that observed during ascent to high altitude, where erythropoiesis is induced to counteract the reduced atmospheric oxygen supply².

Hypoxia is commonly associated with a range of pathophysiological states including ischemia, chronic inflammatory disease and cancer³. Taking inflammatory diseases as an example, upon tissue invasion by a pathogen, resident and recruited inflammatory cells mount

a protective response that requires the oxygen-demanding synthesis of inflammatory mediators and oxidative burst therefore creating a hypoxic environment⁴. When inflammation chronic, the associated fibrosis and thrombosis also results in diminished blood delivery, thus exacerbating the hypoxic state⁵. Physiological hypoxic microenvironments are also common and can occur in the developing embryo and the adult³. Hypoxic regions arise in a spatio-temporally controlled way in the developing embryo and function as signal to orchestrate embryonic development^{6,7}. Physiological hypoxia is also important in the adult where hematopoietic stem cells reside in the most hypoxic niches of the bone marrow^{8,9}. Therefore, hypoxia is a relatively commonly encountered threat to the maintenance of metabolic homeostasis in health and disease.

Adaptation to hypoxia involves 3 major responses (Figure 1): (1) increased glycolysis to cope with ATP depletion, (2) increased oxygen delivery to restore oxidative phosphorylation, and (3) inhibition of energy-demanding processes, to direct the scarce energetic resources to key house-keeping functions. This can be achieved by acute responses that rely on the modification of pre-existing proteins, translational inhibition and transcriptional changes (Figure 1) that will be discussed in detail in the next sections.

1.1-Modulation of the activity of pre-existing proteins

The strategies used by a cell to adapt to hypoxia are dependent upon the extent and duration of the challenge. Acute hypoxia can induce rapid and transient effects on the activity of pre-existing proteins¹⁰. This can be achieved through the regulation of post-translational modifications such as hydroxylation¹¹⁻¹³ and phosphorylation¹⁴, or through a direct effect on enzymes that requires oxygen such haem oxygenase-2 (HO-2). HO-2 activity is reduced in the carotid bodies in response to systemic hypoxia, leading to the modulation of ion channel activity and the subsequent generation of neuronal signals that promote increased depth and rate of breathing to restore systemic oxygen levels to normal¹⁵. Finally, hypoxia-induced changes in cellular metabolism can indirectly affect enzyme activity. For example, the AMP-activated protein kinase (AMPK), a sensor of AMP:ATP ratios, is activated when ATP levels drop in response to hypoxia, and promotes energetic homeostasis by activating catabolic process and inhibiting anabolic metabolism^{16,17}. While the acute responses to hypoxia are mostly mediated by changes in the activity of pre-existing proteins, long lasting adaptation to chronic hypoxia (e.g. high altitude) is achieved through delayed, yet sustained, changes in translation and transcription.

1.2-Inhibition of translation

Down-regulation of energy consuming processes under hypoxic conditions is thought to be part of an energetic adaptation strategy, aimed at redirecting the available energy supply

(ATP) to house-keeping functions, especially the maintenance of ionic and osmotic homeostasis in order to prevent membrane depolarization and subsequent cell swelling and necrosis¹⁸. Protein translation is one of the most energy consuming processes that occurs in cells. In rat cardiomyocytes it can take up to 27% of the total cellular energy capacity. RNA synthesis and Na⁺/K⁺ ATPase activity are the two other major energy sinks under normoxic conditions taking 20% and 22% of total energy capacity, respectively¹⁹. When energy capacity is reduced, translational rates are among the first cellular processes to be down-regulated.²⁰⁻²² Hypoxia induces translational arrest by activation of PERK and inhibition of mTOR, two major modulators of the translational machinery^{22,23}. Therefore, by reducing the rate of protein translation in hypoxia, energy is conserved and reserved for essential processes.

1.3-Transcriptional adaptation

1.3.1-Gene induction in hypoxia

The discovery of the hypoxia inducible factor (HIF)^{24,25} opened the way to the study of transcriptional regulation as a means for adaptation to hypoxia. HIF is responsible for adaptation to hypoxia through mechanisms including the induction of angiogenesis and erythropoiesis. HIF promotes such adaptive processes through the induction of VEGF and EPO respectively. HIF also promotes an increase in glycolysis to compensate for loss of ATP production during oxidative phosphorylation, through the induction of many of the enzymes in the glycolytic pathway (e.g. LDHA), and also through the induction of PDK1, which inhibits the conversion of pyruvate to acetyl Coenzyme A and entry into the tricarboxylic acid cycle²⁶.

The HIF pathway evolved as an adaptive mechanism, but it is maladaptive in cancer, where it plays an oncogenic role through the up-regulation of numerous genes that promote tumor growth. For example, increased glycolysis is thought to facilitate diversion of glycolytic intermediates into nucleoside and amino acid biosynthetic pathways facilitating cellular proliferation²⁷, and increased expression of the collagen remodeling LOX genes promotes metastasis²⁸. While HIF is the master regulator of gene induction in hypoxia (transcriptomic studies have revealed that HIF regulates 29% to 52% of induced genes²⁹⁻³¹), other transcription factors also contribute to the full-spectrum of transcriptional changes. Indeed, at least 20 other transcriptional activators are known to be regulated by hypoxia (Table S1)³².

A fundamental aspect of the HIF response is its resolution, which is necessary to prevent detrimental effects on cells and tissues, including tissue acidification, increased haematocrit³³⁻³⁵ and chronic HIF stabilization which has been associated with a number of cancers³³. An example of a non-resolving HIF response is causative in the von Hippel-Lindau (VHL) disease, a hereditary human cancer syndrome predisposing patients to highly angiogenic

tumours³⁶. In these patients loss of VHL causes HIF stabilization under normoxic conditions and expression of tumor promoting HIF target genes³⁶.

1.3.2-Gene repression in hypoxia

It is clear from transcriptomic studies that in response to hypoxia, concomitant with transcriptional up-regulation, there is transcriptional repression of comparable numbers of genes (Table S2). The mechanisms underpinning transcriptional repression is poorly understood relative to gene induction where HIF transcription factors are of central importance. Nonetheless transcriptional gene repression in hypoxia are a central part of the overall adaptive transcriptional response, as illustrated by the common repression between different transcriptional studies of several gene families involved in energy-demanding processes such as transcription, translation, mRNA processing and splicing, cell cycle and proliferation (Table S3). Hypoxia-repressed (but not hypoxia-induced) *in vitro* derived transcriptional signatures have prognostic value in breast cancer³⁷. This fact alone highlights the need for a better understanding of the mechanisms of gene repression in hypoxia. The clinical importance of hypoxia-repressed genes has not been as thoroughly investigated as the importance of induced genes. This historical bias is probably influenced by the large amount of attention focused on the HIF pathway which regulates the transcriptional induction of hundreds of target genes³⁷.

Transcriptional down-regulation of these energy-demanding processes under hypoxic conditions might be an essential part of a defense mechanism to direct the scarce energy available to essential house-keeping functions¹⁸. As described above, protein translation is regulated by hypoxia due to the action of the mTOR and PERK (Figure 1). However, long-term changes in translation are determined at the transcriptional level, likely so that the cells don't waste valuable resource in making new RNA that cannot be translated into protein due to the energy deficit. Consistent with this notion, genes related to translation are among the ones most commonly found to be repressed in transcriptomic studies (Table S3). For example, the translation initiation factor *EIF5A* is one of the five most down-regulated genes in hypoxia (>6 fold)³¹.

Most transcriptomic studies measure steady state mRNA level, but changes in mRNA stability influence the expression levels. Transcriptomic studies on the effect of hypoxia on *de novo* (nascent) mRNA pools revealed 196 differentially expressed genes, 43 of which were repressed in RAW264.7 macrophages³⁸. In another study performed in kidneys from ischemic mice, 1219 genes were differentially expressed (642 up- and 577 down-regulated), combinatorial analysis of steady state and nascent mRNA revealed that the majority of the genes (947) were *de facto* transcriptional changes rather than mRNA

stabilization/destabilization³⁹. Thus, gene repression in hypoxia is at least in part mediated by *de facto* transcriptional changes, resulting from the activation of transcriptional repressors.

A better understanding of the mechanism leading to repression of the genes involved in multiple energy-demanding processes (Table S3), would allow a better understanding of the adaptation to hypoxia, and potentially new avenues for therapeutic intervention. In this review we aim to provide an overview of the known existing mechanisms for transcriptional gene repression in hypoxia mediated by the activation of transcriptional repressors, an area that has been much less under the scrutiny of the scientific community, than gene induction in hypoxia and its master regulator HIF. Initially we will focus on REST, a transcriptional repressor that has been implicated by several studies to be a key transcription factor in hypoxia, and possibly a master regulator of gene repression⁴⁰.

2-Mechanisms of transcriptional repression in hypoxia

Transcriptional repressors are a large and diverse group of proteins⁴¹. They can repress transcription through one, or a combination of several mechanisms: inhibition of the basal transcriptional machinery, inhibition of transcriptional activators or remodeling of chromatin structure⁴¹. Repressors play essential roles in physiology, from embryonic development^{42,43} to adaptation to stress⁴⁴, and pathophysiology from neurological disorders⁴⁴ to cancer⁴⁵. Hypoxia is a major stress to the cells, that requires gene repression for adaptation. In the following sections we will summarize existing evidence for roles played by transcriptional repressors in hypoxia.

2.1-The Repressor Element 1-Silencing Transcription Factor (REST)

REST, also known as neuron restrictive silencer factor (NRSF), is a C2H2- or Krüppel-type zinc finger, one of the largest classes of transcription factors in humans⁴⁶. REST is recruited to target genes containing the 21 base pair Repressor Element 1 (RE1), and inhibits transcription by interaction with the basal transcriptional machinery or by regulation of chromatin structure⁴². REST was originally discovered as a master regulator of neuronal development^{47,48}, and is the first example of a transcriptional repressor that regulates a large repertoire of cell-type specific genes⁴⁹. During embryonic development, neuronal precursor cells gradually lose REST expression through proteasomal degradation, as a result, REST target genes are expressed in mature neurons, conferring them their unique phenotype, while repressing it everywhere else⁴².

Several lines of evidence indicate that the simple picture of REST being a neuronal switch, is far more complex, with REST being expressed in some neurons within the brain, where it serves a neuro-modulatory role, in response to stimuli including seizure^{50,51}, ischemia^{52,53}, oxidative stress and ageing⁴⁴. In addition, in non-neuronal cells REST is involved in

immunity⁵⁴, cardiac function⁵⁵ and cellular proliferation⁵⁶, being also heavily involved in cancer development⁴⁵.

Calderone and collaborators, first reported on the contribution of REST up-regulation (mRNA and nuclear protein) to ischemia-induced neuronal cell death⁵², the mechanisms involved repression of glutamate receptor subunit GluR2^{52,57}. Subsequent studies revealed that REST induction by ischemia, had more pronounced effects on gene expression, leading to the epigenetic remodeling and suppression of several neuronal genes, which ultimately lead to cell death⁵³. Studies using cultured neuroblastoma cell lines, subjected to an in vitro model of ischemia, oxygen and glucose deprivation, also exhibited increased REST mRNA and protein⁵⁸. These studies were the first to identify a potential link between low oxygen and REST regulation (Table 2). Recently it has been shown that REST is a hypoxia-responsive transcriptional repressor, acting as a master regulator of gene repression in hypoxia, regulating 20% of all hypoxia-repressed genes in HEK293 cells exposed to 1% oxygen⁴⁰. Upon exposure to hypoxia, REST accumulates in the nucleus (almost 4-fold increase over normoxia at 16 and 24 hours), and this can be rapidly reversed by re-oxygenation, REST protein stability was not affected by hypoxia, and REST mRNA was only modestly and transiently increased (at 2 hours of hypoxia), suggesting that the main mechanism for REST nuclear accumulation involves a nuclear translocation regulated by a post-translational regulation mechanism⁴⁰. A recent study by Rios and collaborators, has also reported REST accumulation in the nucleus of Marrow-isolated multilineage inducible (MIAMI) cells exposed to 3% oxygen for 7 days, with only a very modest <1.5-fold increase in REST mRNA.⁵⁹ While the mechanisms responsible for nuclear translocation of REST in hypoxia is currently unknown, REST has been shown to require several proteins such as dynactin p150(Glued), huntingtin, HAP1, and RILP/Prickle1 to form a complex involved in its the translocation into the nucleus⁶⁰. REST is post-transcriptionally regulated by phosphorylation^{61,62} (Casein-kinase II⁶³) and ubiquitylation (β -TRCP^{61,62}, HAUSP⁶⁴ and DYRK1A⁶⁵), a promising topic to study the regulation of REST in hypoxia, as these post-translational modifications are used by hypoxia to modulate the activity of several hypoxia responsive proteins (e.g. HIF and NF κ B pathways)⁶⁶⁻⁶⁸. Two studies reported a decrease in REST expression, in total cell lysates after 72 hours of 2% oxygen, in prostate cancer LNCaP cells⁶⁹, and 5% oxygen in prostate cancer PC-3 cells and neural crest cells⁷⁰. The mechanism for REST down-regulation involved hypoxia-induced miR-106b~25⁷⁰. This suggests that similarly to the HIF response which is resolved in prolonged hypoxia, to prevent over-activation of its target genes^{71,72}, the REST response to hypoxia also needs to be resolved. In summary, several lines of evidence suggest that REST is regulated by oxygen deprivation, in both hypoxic cell lines and ischemic rodents (Table 2).

2.2.1-Physiological relevance of REST regulation by hypoxia

Originally discovered as regulator of neuronal development, REST has since be found to be a very versatile and context specific transcription factor^{45,73}. For example, in non-neuronal cancers of the breast, lung and colon REST is a tumor suppressor^{45,73}, while in neuronal cancer REST is an oncogene⁷³⁻⁷⁵. In the adult brain REST can also play contradictory roles, being neurotoxic in ischemia^{52,53,57}, epilepsy^{50,51} and Huntington's disease,^{76,77} and neuroprotective in aging and Alzheimer's disease⁴⁴.

Regarding the role of REST in hypoxia and in oxygen-deprived conditions such as ischemia, the roles and genes regulated by REST also point to diverse and cell-type specific roles (Table 3). Global ischemia is able to induce REST protein and mRNA in hippocampal neurons, REST-epigenetic reprogramming and repression of neuronal genes (e.g. GluR2, GluA2) leading to cell death^{52,53,57}. In HEK293 cell we have showed it contributes to the resolution of the HIF-response, and regulates HIF-dependent metabolic adaptation to hypoxia (glycolytic genes)⁷². HIF-signaling is also negatively regulated by REST in prostate cancer cells⁶⁹. Thus regulation of HIF-signaling is a key feature of REST in hypoxia.

In a study by Lin and collaborators, REST downregulation after 3 days in hypoxia (2% oxygen) was required for the neuroendocrine phenotype of prostate cancer cells exposed to hypoxia, namely neuron-like extensions and AMPK activation (phospho-Thr172)⁶⁹. Suggesting that tumor hypoxia in solid prostate cancer down-regulates REST as a mechanism to induce the neuroendocrine phenotype, which is observed in the most advanced, treatment-refractory stages of this cancer⁶⁹. Notably, their comparison of the transcriptomic changes induced by REST RNAi in normoxic cells, and cells exposed for 3 days of hypoxia, revealed a 20% overlap in the induced genes, suggesting that REST down-regulation in prolonged/chronic hypoxia is a mechanism involved in late transcriptional up-regulation in hypoxia⁶⁹.

REST is overexpressed in neuronal cancers, and has oncogenic properties, it inhibits apoptosis and promotes tumorigenesis^{74,75}. Interestingly, hypoxic neuroblastoma tumors and cells exposed to hypoxia (1% oxygen) down-regulate neuronal markers^{78,79}, and REST protein is induced in oxygen/glucose deprived SK-N-SH neuroblastoma cells⁵⁸. Thus, it is plausible that REST induction by the hypoxic microenvironment known to be present in neuronal tumours⁸⁰, contributes to its oncogenic role. Interestingly our RNA-seq analysis revealed REST dependent repression of cell adhesion genes, which might be important for metastasis as hypoxic suppression of adhesion molecules allows cancer cells to escape their stressed environment^{81,82}. Several of the REST target genes we identified in our RNA-seq analysis where part of cancer pathways (RB1, MET, MYCBP, WNT5A, HDAC1, PRKCB, LAM4A,

LAMC1 and LAMB1). HIF itself is implicated in cancer, and has been shown to be repressed by REST in HEK293⁷² and prostate cancer cells⁶⁹. The physiological relevance of REST regulation of all these cancer-related genes in hypoxia, beyond the *in vitro* cell culture paradigm, will most certainly be context- and cell- type specific as REST is well known to play both tumor suppressor and oncogenic roles, a feature it shares with NOTCH signalling⁸³, E-cadherin⁸⁴ and MYC⁸⁵. Overall, existing evidence suggests that the regulation of REST protein expression levels by hypoxia in cancer cells, both its induction in neuronal cancer and its down-regulation in prostate cancer, plays a pathogenic role.

During neuronal development, neuronal stem cells require REST down-regulation for the expression of neuron-specific genes⁴². Hypoxia is well known to be a signal required for proper embryonic development³, and is reported to promote neuronal development through an unknown mechanism⁸⁶. Using neural crest cells as a model to study neuronal development, Liang and collaborators showed that hypoxia promotes REST down-regulation through up-regulation of miR-106b~25⁷⁰. Hypoxia and REST RNAi treated cells induced to a similar level the expression of several neuronal markers: the transcription factor ASH-1, the downstream protein paired-like homeobox 2a (Phox2a), and tyrosine hydroxylase (TH)⁷⁰. Overall, this study suggests that hypoxic down-regulation of REST in neuronal precursor cells, is an important mechanism for neuronal development.

We recently published a deep analysis of the effect of REST on hypoxia regulated gene repression, we analyzed the transcriptomic changes by RNAseq, and found that REST RNAi rescued the hypoxic repression of 201 genes, 20% of the total hypoxia repressed genes⁴⁰. Thus we showed for the first time that REST is a master regulator of gene repression in hypoxia. Gene ontology analysis of the REST-repressed genes in hypoxia suggests that REST plays an important role in the cellular adaptation to hypoxia by suppressing genes involved in proliferation, cell cycle progression and transcription⁴⁰. Our own ChIP experiments confirmed the hypoxic regulation of SYJN1 encoding synaptojanin 1, a protein involved in clathrin-mediated endocytosis and ATP-intensive process⁴⁰. Thus repressing energy demanding processes in hypoxia is a plausible function for REST.

As discussed above, REST regulates HIF and HIF-signalling^{59,72}, including HIF-target genes involved in glucose metabolism⁷², biosynthesis of lipids and nucleic acids, and protein catabolism⁴⁰. Thus, REST seems to be heavily involved in the regulation of the metabolism of hypoxic cells.

2.2.2-Mechanism of REST-mediated gene repression in hypoxia

In ischemic neurons, REST is recruited to the promoter of MOR-1⁵⁷ and GluA2⁵³, as assessed by Chromatin immunoprecipitation assays. Furthermore, GluA2 promoter exhibited recruitment of the REST co-repressor complexes CoREST and mSin3, and marks of

epigenetic silencing, that where dependent on REST⁵³. CoREST and mSin3 are multiprotein co-repressor complexes that contain both chromatin remodelers (BRG1, SWI/SNF) and histone modifiers (histone deacetylases 1 and 2, histone methyl-transferase G9a)⁴² This suggests that REST recruitment, with co-repressor complex assembly and epigenetic silencing is a mechanism for hypoxic gene repression.

In hypoxic HEK293 cells, we have observed REST recruitment to the HIF-1 α ⁷² and SYNJ1⁴⁰ promoters, with co-repressor complex components CoREST and mSin3 also being recruited to the HIF-1 α promoter⁷². Interestingly, the GANAB gene had REST bound to the promoter in hypoxia, but there was no increase of REST binding with hypoxia compared to control cells (21% oxygen)⁴⁰. Despite the absence of REST recruitment, GANAB was regulated by REST in hypoxia, as assessed by rescue of GANAB repression in hypoxia by REST RNAi⁴⁰. Thus, we propose that, while REST recruitment is required for transcriptional repression of some genes (e.g. SYNJ1, HIF-1 α), for other genes (e.g. GANAB) REST might already be bound to its target gene in normoxia in a low affinity state, “poised to act,” with its repressive activity triggered only in hypoxia, possibly by co-factor recruitment⁴⁰. This hypothesis finds support in studies by Otto and colleagues⁸⁷, where many of REST-occupied genes were still expressed while others were repressed, potentially due to their functional relevance to the cell.

Using publically available REST ChIP-seq datasets we found that 64% of our identified normoxic REST target genes and 18% of our hypoxic REST target genes were previously described in these datasets⁴⁰. This suggests that in hypoxia, REST regulates more genes indirectly than by direct binding. Consistent with indirect regulation we found that REST repressed the expression of transcription factors and transcriptional machinery in hypoxia⁴⁰.

In conclusion, 3 potential mechanisms account for REST mediated gene repression in hypoxia, direct REST recruitment to the gene promoter, activation of REST activity in genes where REST is “poised-to-act”, and indirect mechanisms.

3- Other hypoxia responsive transcriptional repressors

3.1 DEC1 and DEC2

DEC1 (Differentially expressed in chondrocytes protein 1) was found to be inducible by hypoxia in a screen for factors dependent on the VHL- HIF pathway, and its expression is dependent on HIF-1 α , as cells lacking functional HIF1 α do not up-regulate DEC1 in response to hypoxia⁸⁸. The role of DEC1 activation in hypoxia is best described in melanocytes, where HIF-1 α induced DEC1 is recruited to the M-MTIF promoter (melanocyte-specific isoform of

the microphthalmia-associated transcription factor) and represses its transcription, resulting in an inhibition of the oncogenic properties of M-MITF (the)⁸⁹.

Tumor hypoxia down regulates key genes of the DNA mismatch repair system, leading to genomic instability and tumor progression. A study in multiple cancer cell lines, has shown that hypoxic repression of the key mismatch repair gene (MLH1) is transcriptionally regulated by DEC1 and DEC2, which are induced by HIF-1 α in hypoxia⁸⁹⁻⁹¹.

3.2 Bach1

The transcription repressor Bach1 (BTB and CNC homology 1, basic leucine zipper transcription factor 1), is transcriptionally induced by hypoxia in several human cell lines, and to promotes transcriptional repression of HO-1 (heme oxygenase-1). The physiological relevance of this mechanism is not known. HO-1 is induced by and promotes degradation of heme, in a protective response against oxidative stresses¹⁰⁸.

3.3 ID1 and ID2

The protein inhibitor of DNA binding 2 (ID2), while not being a transcriptional repressor, is a negative regulator of the activity of bHLH transcription factors⁷⁸. ID2 is involved in normal neural crest development and inhibits pro-neuronal bHLH proteins. Hypoxia leads to a reduction of neuronal gene expression in neuroblastoma, and ID2 mRNA is quickly induced (3-fold after 2 hours) in SK-N-BE(2) neuroblastoma cell lines, and transcriptionally dependent on HIF-1 α ⁷⁸. Hypoxic induction of ID2 may contribute to the neuroblastoma de-differentiation into a neuronal crest-like phenotype (loss of neuronal markers) associated with increased tumor growth and aggressiveness⁷⁸. ID1 is also induced by hypoxia in multiple cell types, but with unreported function^{78,92}.

3.4 ZEB1/ZEB2

E-cadherin loss of function is a hallmark of metastatic cancer. Renal clear cell carcinoma (RCC) is characterized by loss of function of the von Hippel-Lindau tumor suppressor (VHL), which negatively regulates (HIF-1 α)⁹³. Loss of E-cadherin expression in VHL-null RCC4 cells can be corrected by rescue of VHL expression, or inhibition of HIF-1 α using a dominant negative mutant or RNAi⁹³. Because the mRNA expression of ZEB1 and ZEB2, well-described transcriptional repressors of E-cadherin, were reduced upon HIF inhibition, the authors suggest that loss of E-Cadherin in Renal clear cell carcinoma is due to HIF-dependent induction of ZEB1/ZEB2.⁹³

3.5 Snail

Snail, like ZEB1 and ZEB2, is a well-described transcriptional repressor of E-Cadherin. Studies in ovarian cancer cell lines have shown that Snail mRNA is induced by hypoxia, suggesting it might contribute to hypoxia induced epithelial mesenchymal transition and metastasis in ovarian cancer by repressing E-cadherin.⁹⁴

3.6 CtBP

The transcriptional corepressor C-terminal-binding protein (CtBP) is able to sense free nuclear NADH and transduce this into changes in gene expression⁴². In cancer cell lines, hypoxia increased free NADH levels, promoting CtBP recruitment to the E-cadherin promoter. In addition, hypoxic repression of E-cadherin and increased cell migration, were rescued with CtBP RNAi⁹⁵. Thus, hypoxic recruitment of CtBP to the E-Cadherin promoter is a mechanism for E-Cadherin repression and increased cancer cell migration in hypoxia.

3.7-HIF-dependent gene repression

Microarray data combined with siRNA against HIF-1/-2 indicate that there are HIF-dependent and HIF-independent hypoxia repressed genes³¹. For example, *EIF5A* is 6-fold repressed in hypoxia, but only 1.5-fold repressed in the presence of HIF-2 siRNA³¹. While there are some cases of hypoxic gene repression by HIF binding to gene promoters (e.g. CFTR⁹⁶, ADK51⁹⁷ and APC⁹⁸), these seem to be the exception rather than the rule, as studies combining genome wide ChIP-seq and microarray data indicate that while HIF is enriched in the promoters of genes induced in a HIF-dependent way, it is not enriched in the promoters of genes that are repressed in hypoxia, thus indicating the presence of HIF-independent and/or indirect HIF-dependent mechanisms governing gene repression in hypoxia⁹⁹. Supporting this notion, transcriptional regulation in hypoxia exhibits temporal waves of regulation, with a second late wave of transcriptional regulation by transcription factors that were transcriptionally induced by hypoxia in the first early wave of regulation⁶⁶.

3.8-Hydroxylase dependent gene repression

Some studies have revealed that hypoxia repressed genes can also be repressed by DMOG, a 2-oxoglutarate analogue, commonly used as a “hypoxia mimetic” drug that activates HIF through the inhibition of the Prolyl hydroxylase domain containing (PHD) enzymes. An example is the DNA repair gene *BRC A2*, in hypoxic breast cancer cells¹⁰⁰. Transcriptomic studies comparing the effect of hypoxia and DMOG, revealed a striking overlap, with 77% of the hypoxia induced genes also induced by DMOG³¹. This is consistent with the perceived central role of PHD/HIF in hypoxia induced gene expression²⁶. When the effects on hypoxia repressed genes were analyzed, the overlap was 36%³¹. This suggests that the PHD/HIF pathway does not play such a predominant role in gene repression as it does in gene

induction. In addition, PHD and other 2-oxoglutarate-dependent enzymes could contribute to gene repression in hypoxia, possibly through the hydroxylation of transcriptional repressors. This exciting hypothesis remains to be tested.

Another class of hydroxylases that can play a role in gene repression in hypoxia are the JmJc demethylases, reviewed in¹⁰¹. These hydroxylases require molecular oxygen to de-methylate histones, leading to epigenetic silencing. Interestingly, more than twenty JmJc demethylases have been shown to be induced by hypoxia / HIF, thus raising the possibility that they contribute to gene repression in hypoxia through epigenetic silencing mechanisms¹⁰¹.

Thus, while the underlying mechanisms are only recently being explored, hydroxylases might play a role in gene repression in hypoxia.

4-Cross-talk between REST and HIF: master regulators of gene repression in hypoxia

As mentioned above, REST negatively regulates HIF-expression and HIF-signaling in HEK293⁷² and MIAMI⁵⁹ cells. Other reports in the literature suggest that REST and HIF cross-talk to fine tune target gene expression. Namely, the NCX1 gene (Na⁺/Ca²⁺ exchanger 1), coding for a plasma membrane protein regulating intracellular calcium and sodium homeostasis in the brain, is epigenetically repressed in a rat model of brain ischemia by the REST/Sp3 complex and induced in brain ischemic preconditioning by the HIF-1/Sp1 complex¹⁰². Thus, in addition to regulating specific cohorts of hypoxia induced and hypoxia repressed genes, HIF and REST, share some transcriptional targets, working together to fine-tune the transcriptional response to hypoxic challenges.

5-Conclusions

Cellular adaptation to hypoxia involves multiple responses ranging from changes in the activity of pre-existing proteins, inhibition of protein synthesis, and also modulation of the transcriptional landscape (Figure 1). Our understanding of the mediators of transcriptional changes in hypoxia is rapidly expanding. While HIF has earned and deserves to retain its place as the master regulator of gene induction in hypoxia, evidence also exists for at least 20 other transcriptional activators in hypoxia (Table S1). Transcriptional repression in hypoxia, on the other hand, is less well understood. We now know that at least 10 transcriptional repressors are regulated by hypoxia (Table 1). Most of these factors have been identified to regulate cancer related genes M-MTIF, MLH1 and E-Cadherin, and to contribute to hypoxic modulation of cancer cell metastasis and DNA mismatch repair response (Figure 2). For most transcriptional repressors (DEC1, DEC2, ID1, ID2, ZEB1, ZEB2, Snail, Bach1, REST) hypoxia regulates their expression (protein or mRNA level) using HIF-dependent and

independent mechanisms (Figure 2). In the case of DEC1, CtBP and REST, hypoxia also seems to promote their recruitment to the promoter of its target genes (Figure 2). REST is undoubtedly the most studied and best understood transcriptional repressor in contexts of oxygen deprivation, including hypoxic exposure of cell lines *in vitro* and ischemia in rodents. REST induction by oxygen deprivation plays a pathogenic role in ischemic neurons and oxygen/glucose deprived neuroblastoma cells (Table 2), where REST-repression of neuronal genes contributes to cell death (CART, MOR-1, GluR2, GluA2). In addition to these genes, REST is recruited to the promoter of 12 other genes in ischemic neurons, the relevance of which is unknown (Table 3). In HEK293 cells and MIAMI cells, REST regulates HIF signaling, promoting resolution of the HIF-1 α response, which can be damaging when over-activated³³⁻³⁶. Another mechanisms for REST-mediated gene regulation in hypoxia, is repression of REST protein and subsequent de-repression (induction) of target genes. This mechanisms seems to play a role in the neuroendocrine and neuronal differentiation of prostate cancer cells and neuronal crest cells, respectively (Table 3). However, evidence is lacking for this mechanism, as no gene was yet identified as induced by hypoxia in a REST-dependent manner using genetic modulation of REST expression. The most common mechanism for REST regulation of gene expression in hypoxia, seems to rely on the induction of REST expression (mRNA and/or protein), nuclear accumulation and recruitment to the target gene (as accessed by Chromatin immunoprecipitation assays). Several genes have been identified in which REST inhibition (RNAi or dominant negative REST expression) rescues their repression in hypoxia (Table 3). These include HIF-1 α , with effects on glycolytic metabolism and the neuronal genes CART, MOR-1, GluA2, GluR2, with effects on neuronal cell death. Other genes validated to be REST-repressed in hypoxia include GANAB, RAB3C, and SYNJ1, the last one being involved in endocytic processes and energy demanding process, suggesting that REST might contribute to the transcriptional repression of energy-demanding processes in hypoxia. This is further substantiated by our recent transcriptomics (RNAseq) analysis, where we found that REST RNAi rescued the hypoxic repression of 201 genes, including genes involved in energy demanding processes such as transcription, metabolism, proliferation and cell cycle progression.

The hypoxia-repressed transcriptomic signature of cancer cells has prognostic value in cancer³⁷. Expanding our understanding of the mechanisms of gene repression in hypoxia will open new avenues for developing diagnostic, prognostic and potentially therapeutic approaches. REST inhibitors are already being developed for the treatment of nuerological disorders, where REST plays a pathogenic role¹⁰³. These are expected to be useful in situation where inhibiting REST activity in hypoxia might be beneficial, for example, REST

contributes to neuronal cell death in ischemic neurons, thus its inhibition is predicted to be beneficial in ischemic diseases (e.g. stroke).

We are only starting to unravel the physiological relevance and the mechanisms of gene repression in hypoxia. The transcriptional repressor REST, seems to play a prominent role in gene repression in hypoxia, it is the most studied and best described repressor, and can regulate up to 20 % of hypoxia repressed genes⁴⁰. Thus, REST is the first master regulator of gene repression in hypoxia.

Acknowledgements

Work carried out in the authors lab is funded by funding from Science Foundation Ireland (11/PI/1005) and the European Union (ERACoSysMed translational call).

References

1. Lane N, Martin W. The energetics of genome complexity. *Nature*. 2010;467(7318):929-934.
2. Prabhakar NR, Semenza GL. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. *Physiological Reviews*. 2012;92(3).
3. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. *Nature Reviews Molecular Cell Biology*. 2008;9(4):285-296.
4. Campbell EL, Bruyninckx WJ, Kelly CJ, et al. Transmigrating Neutrophils Shape the Mucosal Microenvironment through Localized Oxygen Depletion to Influence Resolution of Inflammation. *Immunity*. 2014;40(1):66-77.
5. Fluck K, Fandrey J. Oxygen sensing in intestinal mucosal inflammation. *Pflügers Archiv-European Journal of Physiology*. 2016;468(1):77-84.
6. Dunwoodie SL. The Role of Hypoxia in Development of the Mammalian Embryo. *Developmental Cell*. 2009;17(6):755-773.
7. Lord EM, Harwell L, Koch CJ. Detection of hypoxic cells by monoclonal antibody recognizing 2-nitroimidazole adducts. *Cancer Research*. 1993;53(23):5721-5726.
8. Cipolleschi MG, Dellosbarba P, Olivotto M. The role of hypoxia in the maintenance of hematopoietic stem cells. *Blood*. 1993;82(7):2031-2037.
9. Parmar K, Mauch P, Vergilio J-A, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104(13):5431-5436.
10. Kumar GK, Klein JB. Analysis of expression and posttranslational modification of proteins during hypoxia. *Journal of Applied Physiology*. 2004;96(3):1178-1186.

11. Guo J, Chakraborty AA, Liu P, et al. pVHL suppresses kinase activity of Akt in a proline-hydroxylation-dependent manner. *Science*. 2016;353(6302):929-932.
12. Scholz CC, Cavadas MAS, Tambuwala MM, et al. Regulation of IL-1 beta-induced NF-kappa B by hydroxylases links key hypoxic and inflammatory signaling pathways. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(46):18490-18495.
13. Scholz CC, Rodriguez J, Pickel C, et al. FIH Regulates Cellular Metabolism through Hydroxylation of the Deubiquitinase OTUB1. *Plos Biology*. 2016;14(1).
14. Kumar GK, Prabhakar NR. Post-translational modification of proteins during intermittent hypoxia. *Respiratory Physiology & Neurobiology*. 2008;164(1-2):272-276.
15. Prabhakar NR. Sensing hypoxia: physiology, genetics and epigenetics. *Journal of Physiology-London*. 2013;591(9):2245-2257.
16. Jung S-N, Yang WK, Kim J, et al. Reactive oxygen species stabilize hypoxia-inducible factor-1 alpha protein and stimulate transcriptional activity via AMP-activated protein kinase in DU145 human prostate cancer cells. *Carcinogenesis*. 2008;29(4):713-721.
17. Taylor CT, Pouyssegur J. Oxygen, hypoxia, and stress. *Stress Responses in Biology and Medicine: Stress of Life in Molecules, Cells, Organisms, and Psychosocial Communities*. 2007;1113:87-94.
18. Boutilier RG. Mechanisms of cell survival in hypoxia and hypothermia. *Journal of Experimental Biology*. 2001;204(18):3171-3181.
19. Casey TM, Pakay JL, Guppy M, Arthur PG. Hypoxia causes downregulation of protein and RNA synthesis in noncontracting mammalian cardiomyocytes. *Circulation Research*. 2002;90(7):777-783.
20. Kenneth NS, Rocha S. Regulation of gene expression by hypoxia. *Biochemical Journal*. 2008;414:19-29.
21. Liu LP, Cash TP, Jones RG, Keith B, Thompson CB, Simon MC. Hypoxia-induced energy stress regulates mRNA translation and cell growth. *Molecular Cell*. 2006;21(4):521-531.
22. Rocha S. Gene regulation under low oxygen: holding your breath for transcription. *Trends in Biochemical Sciences*. 2007;32(8):389-397.
23. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nature Reviews Cancer*. 2008;8(11):851-864.
24. Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proceedings of the National Academy of Sciences of the United States of America*. 1991;88(13):5680-5684.
25. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Molecular and Cellular Biology*. 1992;12(12):5447-5454.
26. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell*. 2012;148(3):399-408.
27. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell*. 2011;144(5):646-674.

28. Wong CC-L, Gilkes DM, Zhang H, et al. Hypoxia-inducible factor 1 is a master regulator of breast cancer metastatic niche formation. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(39):16369-16374.
29. Manalo DJ, Rowan A, Lavoie T, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood*. 2005;105(2):659-669.
30. Benita Y, Kikuchi H, Smith AD, Zhang MQ, Chung DC, Xavier RJ. An integrative genomics approach identifies Hypoxia Inducible Factor-1 (HIF-1)-target genes that form the core response to hypoxia. *Nucleic Acids Research*. 2009;37(14):4587-4602.
31. Elvidge GP, Glenny L, Appelhoff RJ, Ratcliffe PJ, Ragoussis J, Gleadle JM. Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition - The role of HIF-1 alpha, HIF-2 alpha, and other pathways. *Journal of Biological Chemistry*. 2006;281(22).
32. Cummins EP, Taylor CT. Hypoxia-responsive transcription factors. *Pflugers Archiv-European Journal of Physiology*. 2005;450(6).
33. Colgan SP, Taylor CT. Hypoxia: an alarm signal during intestinal inflammation. *Nature Reviews Gastroenterology & Hepatology*. 2010;7(5):281-287.
34. Howell K, Preston RJ, McLoughlin P. Chronic hypoxia causes angiogenesis in addition to remodelling in the adult rat pulmonary circulation. *Journal of Physiology-London*. 2003;547(1):133-145.
35. Kato Y, Ozawa S, Miyamoto C, et al. Acidic extracellular microenvironment and cancer. *Cancer Cell International*. 2013;13.
36. Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999;399(6733):271-275.
37. Starmans MHW, Chu KC, Haider S, et al. The prognostic value of temporal in vitro and in vivo derived hypoxia gene-expression signatures in breast cancer. *Radiotherapy and Oncology*. 2012;102(3).
38. Igwe EI, Essler S, Al-Furoukh N, Dehne N, Brune B. Hypoxic transcription gene profiles under the modulation of nitric oxide in nuclear run on-microarray and proteomics. *Bmc Genomics*. 2009;10.
39. Kenzelmann M, Maertens S, Hergenahn M, et al. Microarray analysis of newly synthesized RNA in cells and animals. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104(15):6164-6169.
40. Cavadas MAS, Mesnieres M, Crifo B, et al. REST is a hypoxia-responsive transcriptional repressor. *Scientific Reports*. 2016;6.
41. Gaston K, Jayaraman PS. Transcriptional repression in eukaryotes: repressors and repression mechanisms. *Cellular and Molecular Life Sciences*. 2003;60(4):721-741.
42. Ooi L, Wood IC. Chromatin crosstalk in development and disease: lessons from REST. *Nature Reviews Genetics*. 2007;8(7).
43. Reynolds N, O'Shaughnessy A, Hendrich B. Transcriptional repressors: multifaceted regulators of gene expression. *Development*. 2013;140(3):505-512.

44. Lu T, Aron L, Zullo J, et al. REST and stress resistance in ageing and Alzheimer's disease. *Nature*. 2014;507(7493):448-454.
45. Negrini S, Prada I, D'Alessandro R, Meldolesi J. REST: an oncogene or a tumor suppressor? *Trends in Cell Biology*. 2013;23(6):289-295.
46. Tadepally HD, Burger G, Aubry M. Evolution of C2H2-zinc finger genes and subfamilies in mammals: Species-specific duplication and loss of clusters, genes and effector domains. *Bmc Evolutionary Biology*. 2008;8.
47. Chong JHA, Tapiaramirez J, Kim S, et al. REST - A mammalian silencer protein that restricts sodium-channel gene-expression to neurons. *Cell*. 1995;80(6):949-957.
48. Schoenherr CJ, Anderson DJ. The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes. *Science*. 1995;267(5202):1360-1363.
49. Schoenherr CJ, Paquette AJ, Anderson DJ. Identification of potential target genes for the neuron-restrictive silencer factor. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;93(18):9881-9886.
50. Palm K, Belluardo N, Metsis M, Timmusk T. Neuronal expression of zinc finger transcription factor REST/NRSF/XRB gene. *Journal of Neuroscience*. 1998;18(4):1280-1296.
51. Spencer EM, Chandler KE, Haddley K, et al. Regulation and role of REST and REST4 variants in modulation of gene expression in in vivo and in vitro in epilepsy models. *Neurobiology of Disease*. 2006;24(1):41-52.
52. Calderone A, Jover T, Noh KM, et al. Ischemic insults derepress the gene silencer REST in neurons destined to die. *Journal of Neuroscience*. 2003;23(6).
53. Noh K-M, Hwang J-Y, Follenzi A, et al. Repressor element-1 silencing transcription factor (REST)-dependent epigenetic remodeling is critical to ischemia-induced neuronal death. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;109(16).
54. Scholl T, Stevens MB, Mahanta S, Strominger JL. A zinc finger protein that represses transcription of the human MHC class II gene, DPA(1,2). *Journal of Immunology*. 1996;156(4):1448-1457.
55. Kuwahara K, Saito Y, Ogawa E, et al. The neuron-restrictive silencer element-neuron-restrictive silencer factor system regulates basal and endothelin 1-inducible atrial natriuretic peptide gene expression in ventricular myocytes. *Molecular and Cellular Biology*. 2001;21(6):2085-2097.
56. Cheong A, Bingham AJ, Li J, et al. Downregulated REST transcription factor is a switch enabling critical potassium channel expression and cell proliferation. *Molecular Cell*. 2005;20(1):45-52.
57. Formisano L, Noh K-M, Miyawaki T, Mashiko T, Bennett MVL, Zukin RS. Ischemic insults promote epigenetic reprogramming of mu opioid receptor expression in hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104(10):4170-4175.
58. Zhang J, Wang S, Yuan L, et al. Neuron-restrictive Silencer Factor (NRSF) Represses Cocaine- and Amphetamine-regulated Transcript (CART) Transcription and Antagonizes cAMP-response Element-binding Protein

- Signaling through a Dual NRSE Mechanism. *Journal of Biological Chemistry*. 2012;287(51):42574-42587.
59. Rios C, D'Ippolito G, Curtis KM, et al. Low Oxygen Modulates Multiple Signaling Pathways, Increasing Self-Renewal, While Decreasing Differentiation, Senescence, and Apoptosis in Stromal MIAMI Cells. *Stem Cells and Development*. 2016;25(11):848-860.
60. Shimojo M. Huntingtin Regulates RE1-silencing Transcription Factor/Neuron-restrictive Silencer Factor (REST/NRSF) Nuclear Trafficking Indirectly through a Complex with REST/NRSF-interacting LIM Domain Protein (RILP) and Dynactin p150(Glued). *Journal of Biological Chemistry*. 2008;283(50):34880-34886.
61. Guardavaccaro D, Frescas D, Dorrello NV, et al. Control of chromosome stability by the beta-TrCP-REST-Mad2 axis. *Nature*. 2008;452(7185):365-U310.
62. Westbrook TF, Hu G, Ang XL, et al. SCF beta-TRCP controls oncogenic transformation and neural differentiation through REST degradation. *Nature*. 2008;452(7185):370-U311.
63. Kaneko N, Hwang J-Y, Gertner M, Pontarelli F, Zukin RS. Casein Kinase 1 Suppresses Activation of REST in Insulted Hippocampal Neurons and Halts Ischemia-Induced Neuronal Death. *Journal of Neuroscience*. 2014;34(17):6030-6039.
64. Huang Z, Wu Q, Guryanova OA, et al. Deubiquitylase HAUSP stabilizes REST and promotes maintenance of neural progenitor cells. *Nature Cell Biology*. 2011;13(2):142-U191.
65. Lu M, Zheng L, Han B, et al. REST Regulates DYRK1A Transcription in a Negative Feedback Loop. *Journal of Biological Chemistry*. 2011;286(12):10755-10763.
66. Lendahl U, Lee KL, Yang H, Poellinger L. Generating specificity and diversity in the transcriptional response to hypoxia. *Nature Reviews Genetics*. 2009;10(12):821-832.
67. Cummins EP, Berra E, Comerford KM, et al. Prolyl hydroxylase-1 negatively regulates I kappa B kinase-beta, giving insight into hypoxia-induced NF kappa B activity. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(48).
68. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-Inducible Factors and the Response to Hypoxic Stress. *Molecular Cell*. 2010;40(2):294-309.
69. Lin TP, Chang YT, Lee SY, et al. REST reduction is essential for hypoxia-induced neuroendocrine differentiation of prostate cancer cells by activating autophagy signaling. *Oncotarget*. 2016;7(18):26137-26151.
70. Liang HZ, Studach L, Hullinger RL, Xie J, Andrisani OM. Down-regulation of RE-1 silencing transcription factor (REST) in advanced prostate cancer by hypoxia-induced miR-106b similar to 25. *Experimental Cell Research*. 2014;320(2):188-199.
71. Cavadas MA, Nguyen LK, Cheong A. Hypoxia-inducible factor (HIF) network: insights from mathematical models. *Cell Commun Signal*. 2013;11(1):42.
72. Cavadas MA, Mesnieres M, Crifo B, et al. REST mediates resolution of HIF-dependent gene expression in prolonged hypoxia. *Sci Rep*. 2015;5:17851.

73. Majumder S. REST in good times and bad - Roles in tumor suppressor and oncogenic activities. *Cell Cycle*. 2006;5(17).
74. Conti L, Crisafulli L, Caldera V, et al. REST Controls Self-Renewal and Tumorigenic Competence of Human Glioblastoma Cells. *Plos One*. 2012;7(6).
75. Huang Z, Bao S. Ubiquitination and deubiquitination of REST and its roles in cancers. *Febs Letters*. 2012;586(11).
76. Buckley NJ, Johnson R, Zuccato C, Bithell A, Cattaneo E. The role of REST in transcriptional and epigenetic dysregulation in Huntington's disease. *Neurobiology of Disease*. 2010;39(1):28-39.
77. Zuccato C, Tartari M, Crotti A, et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nature Genetics*. 2003;35(1).
78. Lofstedt T, Jogi A, Sigvardsson M, et al. Induction of ID2 expression by hypoxia-inducible factor-1 - A role in dedifferentiation of hypoxic neuroblastoma cells. *Journal of Biological Chemistry*. 2004;279(38):39223-39231.
79. Jogi A, Ora I, Nilsson H, et al. Hypoxia alters gene expression in human neuroblastoma cells toward an immature and neural crest-like phenotype. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;99(10):7021-7026.
80. Kaur B, Khwaja FW, Severson EA, Matheny SL, Brat DJ, Van Meir EG. Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. *Neuro-Oncology*. 2005;7(2):134-153.
81. Hasan NM, Adams GE, Joiner MC, Marshall JF, Hart IR. Hypoxia facilitates tumour cell detachment by reducing expression of surface adhesion molecules and adhesion to extracellular matrices without loss of cell viability. *British Journal of Cancer*. 1998;77(11):1799-1805.
82. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature*. 2006;441(7092):437-443.
83. Lobry C, Oh P, Aifantis I. Oncogenic and tumor suppressor functions of Notch in cancer: it's NOTCH what you think. *Journal of Experimental Medicine*. 2011;208(10):1931-1935.
84. Rodriguez FJ, Lewis-Tuffin LJ, Anastasiadis PZ. E-cadherin's dark side: Possible role in tumor progression. *Biochimica Et Biophysica Acta-Reviews on Cancer*. 2012;1826(1):23-31.
85. Uribealago I, Aznar Benitah S, Di Croce L. From oncogene to tumor suppressor The dual role of Myc in leukemia. *Cell Cycle*. 2012;11(9):1757-1764.
86. Morrison SJ, Csete M, Groves AK, Melega W, Wold B, Anderson DJ. Culture in reduced levels of oxygen promotes clonogenic sympathoadrenal differentiation by isolated neural crest stem cells. *Journal of Neuroscience*. 2000;20(19):7370-7376.
87. Otto SJ, McCorkle SR, Hover J, et al. A new binding motif for the transcriptional repressor REST uncovers large gene networks devoted to neuronal functions. *Journal of Neuroscience*. 2007;27(25):6729-6739.
88. Wykoff CC, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ. Identification of novel hypoxia dependent and independent target genes of the von Hippel-

- Lindau (VHL) tumour suppressor by mRNA differential expression profiling. *Oncogene*. 2000;19(54):6297-6305.
89. Feige E, Yokoyama S, Levy C, et al. Hypoxia-induced transcriptional repression of the melanoma-associated oncogene MITF. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(43):E924-E933.
 90. Miyazaki K, Kawamoto T, Tanimoto K, Nishiyama M, Honda H, Kato Y. Identification of functional hypoxia response elements in the promoter region of the DEC1 and DEC2 genes. *Journal of Biological Chemistry*. 2002;277(49):47014-47021.
 91. Nakamura H, Tanimoto K, Hiyama K, et al. Human mismatch repair gene, MLH1, is transcriptionally repressed by the hypoxia-inducible transcription factors, DEC1 and DEC2. *Oncogene*. 2008;27(30):4200-4209.
 92. Jiang B, Kamat A, Mendelson CR. Hypoxia prevents induction of aromatase expression in human trophoblast cells in culture: Potential inhibitory role of the hypoxia-inducible transcription factor Mash-2 (mammalian achaete-scute homologous protein-2). *Molecular Endocrinology*. 2000;14(10):1661-1673.
 93. Krishnamachary B, Zagzag D, Nagasawa H, et al. Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFH1A, and ZFH1B. *Cancer Research*. 2006;66(5):2725-2731.
 94. Imai T, Horiuchi A, Wang CJ, et al. Hypoxia attenuates the expression of E-cadherin via up-regulation of SNAIL in ovarian carcinoma cells. *American Journal of Pathology*. 2003;163(4):1437-1447.
 95. Zhang QH, Wang SY, Nottke AC, Rocheleau JV, Piston DW, Goodman RH. Redox sensor CtBP mediates hypoxia-induced tumor cell migration. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(24):9029-9033.
 96. Zheng W, Kuhlicke J, Jaeckel K, et al. Hypoxia inducible factor-1 (HIF-1)-mediated repression of cystic fibrosis transmembrane conductance regulator (CFTR) in the intestinal epithelium. *Faseb Journal*. 2009;23(1):204-213.
 97. Morote-Garcia JC, Rosenberger P, Kuhlicke J, Eltzhig HK. HIF-1-dependent repression of adenosine kinase attenuates hypoxia-induced vascular leak. *Blood*. 2008;111(12):5571-5580.
 98. Newton IP, Kenneth NS, Appleton PL, Naethke I, Rocha S. Adenomatous Polyposis Coli and Hypoxia-inducible Factor-1 alpha Have an Antagonistic Connection. *Molecular Biology of the Cell*. 2010;21(21):3630-3638.
 99. Schoedel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood*. 2011;117(23):E207-E217.
 100. Fanale D, Bazan V, Caruso S, et al. Hypoxia and Human Genome Stability: Downregulation of BRCA2 Expression in Breast Cancer Cell Lines. *Biomed Research International*. 2013.
 101. Melvin A, Rocha S. Chromatin as an oxygen sensor and active player in the hypoxia response. *Cellular Signalling*. 2012;24(1):35-43.
 102. Formisano L, Guida N, Valsecchi V, et al. Sp3/REST/HDAC1/HDAC2 Complex Represses and Sp1/HIF-1/p300 Complex Activates ncx1 Gene

- Transcription, in Brain Ischemia and in Ischemic Brain Preconditioning, by Epigenetic Mechanism. *Journal of Neuroscience*. 2015;35(19):7332-7348.
103. Charbord J, Poydenot P, Bonnefond C, et al. High Throughput Screening for Inhibitors of REST in Neural Derivatives of Human Embryonic Stem Cells Reveals a Chemical Compound that Promotes Expression of Neuronal Genes. *Stem Cells*. 2013;31(9):1816-1828.
 104. Frederich M, O'Rourke MR, Furey NB, Jost JA. AMP-activated protein kinase (AMPK) in the rock crab, *Cancer irroratus*: an early indicator of temperature stress. *Journal of Experimental Biology*. 2009;212(5):722-730.
 105. Taylor CT. Mitochondria and cellular oxygen sensing in the HIF pathway. *Biochemical Journal*. 2008;409:19-26.
 106. Bertout JA, Patel SA, Simon MC. The impact of O₂ availability on human cancer. *Nature Reviews Cancer*. 2008;8(12):967-975.
 107. Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR Complex 1 Pathway by Nutrients, Growth Factors, and Stress. *Molecular Cell*. 2010;40(2):310-322.
 108. Kitamuro T, Takahashi K, Ogawa K, et al. Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. *Journal of Biological Chemistry*. 2003;278(11):9125-9133.
 109. Leonard MO, Howell K, Madden SF, et al. Hypoxia Selectively Activates the CREB Family of Transcription Factors in the In Vivo Lung. *American Journal of Respiratory and Critical Care Medicine*. 2008;178(9):977-983.
 110. Taylor CT, Furuta GT, Synnestvedt K, Colgan SP. Phosphorylation-dependent targeting of cAMP response element binding protein to the ubiquitin/proteasome pathway in hypoxia. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97(22):12091-12096.
 111. Salnikow K, Kluz T, Costa M, et al. The regulation of hypoxic genes by calcium involves c-Jun/AP-1, which cooperates with hypoxia-inducible factor 1 in response to hypoxia. *Molecular and Cellular Biology*. 2002;22(6):1734-1741.
 112. Laderoute KR. The interaction between HIF-1 and AP-1 transcription factors in response to low oxygen. *Seminars in Cell & Developmental Biology*. 2005;16(4-5):502-513.
 113. Koumenis C, Alarcon R, Hammond E, et al. Regulation of p53 by hypoxia: Dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. *Molecular and Cellular Biology*. 2001;21(4):1297-1310.
 114. Fei PW, Wang WG, Kim SH, et al. Bnip3L is induced by p53 under hypoxia, and its knockdown promotes tumor growth. *Cancer Cell*. 2004;6(6):597-609.
 115. Sermeus A, Michiels C. Reciprocal influence of the p53 and the hypoxic pathways. *Cell Death & Disease*. 2011;2.
 116. Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. HIF-1 alpha induces cell cycle arrest by functionally counteracting Myc. *Embo Journal*. 2004;23(9):1949-1956.

117. Corn PG, Ricci MS, Scata KA, et al. Mxi1 is induced by hypoxia in a HIF-1-dependent manner and protects cells from c-Myc-induced apoptosis. *Cancer Biology & Therapy*. 2005;4(11):1285-1294.
118. Kaluz S, Kaluzova M, Stanbridge EJ. Expression of the hypoxia marker carbonic anhydrase IX is critically dependent on SP1 activity. Identification of a novel type of hypoxia-responsive enhancer. *Cancer Research*. 2003;63(5):917-922.
119. Discher DJ, Bishopric NH, Wu XS, Peterson CA, Webster KA. Hypoxia regulates beta-enolase and pyruvate kinase-M promoters by modulating Sp1/Sp3 binding to a conserved GC element. *Journal of Biological Chemistry*. 1998;273(40):26087-26093.
120. Sun L, Li H, Chen J, et al. PIASy mediates hypoxia-induced SIRT1 transcriptional repression and epithelial-to-mesenchymal transition in ovarian cancer cells. *Journal of Cell Science*. 2013;126(17):3939-3947.
121. Yan SF, Mackman N, Kiesel W, Stern DM, Pinsky DJ. Hypoxia/hypoxemia-induced activation of the procoagulant pathways and the pathogenesis of ischemia-associated thrombosis. *Arteriosclerosis Thrombosis and Vascular Biology*. 1999;19(9):2029-2035.
122. Yan SF, Lu JS, Zou YS, et al. Protein kinase C-beta and oxygen deprivation - A novel Egr-1-dependent pathway for fibrin deposition in hypoxemic vasculature. *Journal of Biological Chemistry*. 2000;275(16):11921-11928.
123. Yan SF, Tritto I, Pinsky D, et al. Induction of interleukin 6 (IL-6) by hypoxia in vascular cells. Central role of the binding site for nuclear factor-IL-6. *Journal of Biological Chemistry*. 1995;270(19):11463-11471.
124. Shie JL, Wu GF, Wu JP, et al. RTEF-1, a novel transcriptional stimulator of vascular endothelial growth factor in hypoxic endothelial cells. *Journal of Biological Chemistry*. 2004;279(24):25010-25016.
125. Yamashita K, Discher DJ, Hu J, Bishopric NH, Webster KA. Molecular regulation of the endothelin-1 gene by hypoxia - Contributions of hypoxia-inducible factor-1, activator protein-1, GATA-2, and p300/CBP. *Journal of Biological Chemistry*. 2001;276(16):12645-12653.
126. Joung YH, Park JH, Park T, et al. Hypoxia activates signal transducers and activators of transcription 5 (STAT5) and increases its binding activity to the GAS element in mammary epithelial cells. *Experimental and Molecular Medicine*. 2003;35(5):350-357.
127. Jiang B, Mendelson CR. USF1 and USF2 mediate inhibition of human trophoblast differentiation and CYP19 gene expression by Mash-2 and hypoxia. *Molecular and Cellular Biology*. 2003;23(17):6117-6128.
128. Carriere A, Carmona MC, Fernandez Y, et al. Mitochondrial reactive oxygen species control the transcription factor CHOP-10/GADD153 and adipocyte differentiation - A mechanism for hypoxia-dependent effect. *Journal of Biological Chemistry*. 2004;279(39):40462-40469.
129. Zhang H, Akman HO, Smith ELP, et al. Cellular response to hypoxia involves signaling via Smad proteins. *Blood*. 2003;101(6):2253-2260.
130. Sanchez-Elsner T, Botella LM, Velasco B, Corbi A, Attisano L, Bernabeu C. Synergistic cooperation between hypoxia and transforming growth factor-beta pathways on human vascular endothelial growth factor gene expression. *Journal of Biological Chemistry*. 2001;276(42):38527-38535.

131. Peters CL, Morris CJ, Mapp PI, Blake DR, Lewis CE, Winrow VR. The transcription factors hypoxia-inducible factor 1 alpha and Ets-1 colocalize in the hypoxic synovium of inflamed joints in adjuvant-induced arthritis. *Arthritis and Rheumatism*. 2004;50(1):291-296.
132. Elvert G, Kappel A, Heidenreich R, et al. Cooperative interaction of hypoxia-inducible factor-2 alpha (HIF-2 alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). *Journal of Biological Chemistry*. 2003;278(9):7520-7530.
133. Yang M-H, Wu K-J. TWIST activation by hypoxia inducible factor-1 (HIF-1). *Cell Cycle*. 2008;7(14):2090-2096.
134. Liu L, Zhu X-D, Wang W-Q, et al. Activation of beta-Catenin by Hypoxia in Hepatocellular Carcinoma Contributes to Enhanced Metastatic Potential and Poor Prognosis. *Clinical Cancer Research*. 2010;16(10):2740-2750.
135. Mense SM, Sengupta A, Zhou M, et al. Gene expression profiling reveals the profound upregulation of hypoxia-responsive genes in primary human astrocytes. *Physiological Genomics*. 2006;25(3):435-449.
136. Costello CM, Howell K, Cahill E, et al. Lung-selective gene responses to alveolar hypoxia: potential role for the bone morphogenetic antagonist gremlin in pulmonary hypertension. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2008;295(2):L272-L284.
137. Bosco MC, Puppo M, Santangelo C, et al. Hypoxia modifies the transcriptome of primary human monocytes: Modulation of novel immune-related genes and identification of CC-chemokine ligand 20 as a new hypoxia-inducible gene. *Journal of Immunology*. 2006;177(3):1941-1955.
138. Pajukanta P, Lilja HE, Sinsheimer JS, et al. Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). *Nature Genetics*. 2004;36(4):371-376.
139. Aprelikova O, Wood M, Tackett S, Chandramouli GVR, Barrett JC. Role of ETS transcription factors in the hypoxia-inducible factor-2 target gene selection. *Cancer Research*. 2006;66(11):5641-5647.
140. Weigand JE, Boeckel J-N, Gellert P, Dimmeler S. Hypoxia-Induced Alternative Splicing in Endothelial Cells. *Plos One*. 2012;7(8).
141. Staib F, Robles AI, Varticovski L, et al. The p53 tumor suppressor network is a key responder to microenvironmental components of chronic inflammatory stress. *Cancer Research*. 2005;65(22):10255-10264.
142. Baze MM, Schlauch K, Hayes JP. Gene expression of the liver in response to chronic hypoxia. *Physiological Genomics*. 2010;41(3):275-288.
143. Chi JT, Wang Z, Nuyten DSA, et al. Gene expression programs in response to hypoxia: Cell type specificity and prognostic significance in human cancers. *Plos Medicine*. 2006;3(3):395-409.

Figure 1. Molecular mechanisms of adaptation to hypoxia. Adaptation to hypoxia involves 3 major responses: increased glycolysis to cope with ATP depletion, increased oxygen

delivery to restore oxidative phosphorylation, and inhibition of energy-demanding processes, to direct the scarce energetic resources to key house-keeping functions. This can be achieved by acute responses that rely on the modification of pre-existing proteins (e.g. AMPK and HO-2), translational inhibition (mediated by mTOR and PERK) and transcriptional changes (mediated by HIF and largely unknown mechanisms for transcriptional repression). Depicted are examples of these mechanisms (from left to right): **(1)** Hypoxia decreases ATP production (energetic stress), this activates AMPK that restores energetic homeostasis by inducing catabolic pathways and inhibiting anabolic pathways. Adapted from^{104,105}. **(2)** Haem oxygenase-2 (HO-2)-generated carbon monoxide (CO) is reduced under hypoxia, CO inhibits cystathionine- γ -lyase (CSE)-regulated hydrogen sulfide generation, thus in hypoxia more H₂S is generated, inhibiting potassium channels (K⁺ channel), this leads to increased Ca²⁺ influx through Ca²⁺ channels, which initiates neuronal signaling to the central nervous system, ultimately increasing the rate and depth of breathing. Abbreviations: NT, neurotransmitter(s); and NT-R, neurotransmitter receptor. Adapted from¹⁵. **(3)** Hypoxia activates TSC1/2 via AMPK or HIF-1 α /REDD1, TSC1/2 inhibits mTOR leading to an inhibition of 4EBP1, a negative regulator of the eIF4E, thus suppressing protein translation. mTOR also activates P70S6K, which activates ribosomal protein S6. Adapted from^{23,106,107}. **(4)** Alternatively hypoxia can activate the effector of the UPR, the kinase PERK, which phosphorylates and inactivates eIF-2 α , thus leading to suppression of protein translation. Adapted from^{23,106,107}. **(5)** HIF is the master regulator of gene induction in hypoxia. HIF is stabilized by inhibition of the oxygen-dependent PHD enzymes. **(6)** Transcriptional repression is an essential component of adaptation to hypoxia through the inhibition of energy demanding process. However, the mechanisms for transcriptional gene repression in hypoxia are poorly understood.

Figure 2. The landscape of transcriptional repressors in hypoxia. Depicted are the known hypoxia-regulated transcriptional repressors, their target genes and the biological processes they regulate in hypoxia (see text for details). For most transcriptional repressors (DEC1, DEC2, ID1, ID2, ZEB1, ZEB2, Snail, Bach1, REST) hypoxia regulates their expression (protein or mRNA level) using HIF-dependent and independent mechanisms. In the case of DEC1, CtBP and REST, hypoxia also seems to promote their recruitment to the promoter of its target genes. REST is the best-described transcriptional repressor in hypoxia. The most common mechanism for REST regulation of gene expression in hypoxia, seems to rely on the induction of REST expression (mRNA and/or protein), nuclear accumulation and recruitment to the target gene. At least 9 genes are regulated by REST in hypoxia, these were validated using chromatin immunoprecipitation assays to show REST recruitment to their promoters, and REST RNAi to rescue their hypoxic repression.

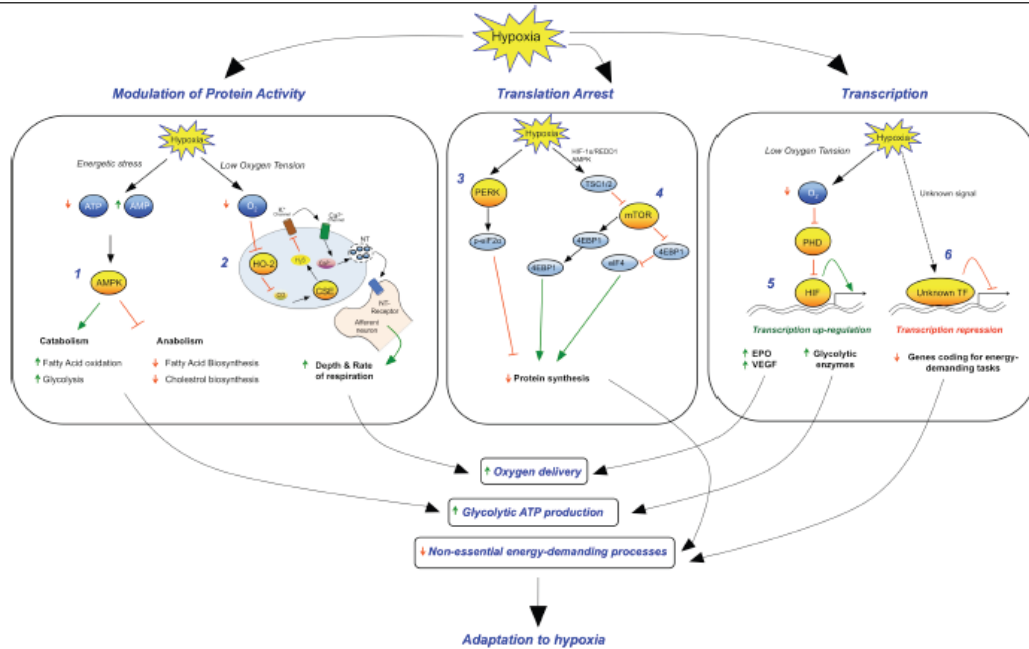
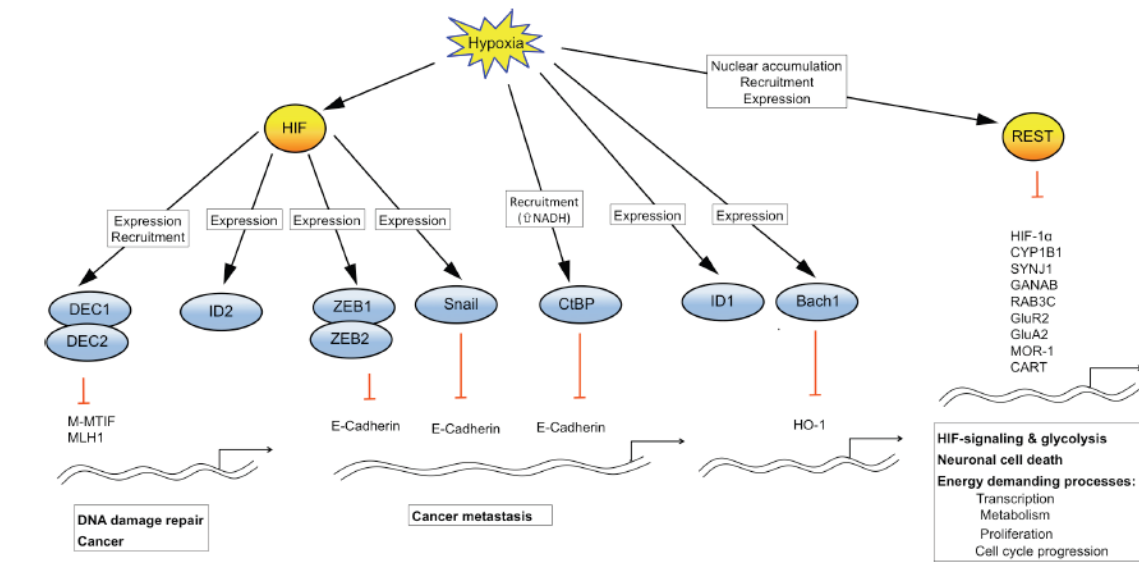


Table 1. Hypoxia responsive transcription repressors

Name	Description
DEC1/2	Induced by HIF-1 in hypoxia, repress MITF and MLH1 ⁸⁹⁻⁹¹
BACH1	Induced by hypoxia, represses HO-1 (haem oxygenase-1) ¹⁰⁸
ID2	Induced by hypoxia, HIF-1 dependent, may contribute to hypoxic neuroblastoma de-differentiation into a neuronal crest-like phenotype. ⁷⁸
ID1	Induced by hypoxia in multiple cell types. ^{78,92}
ZEB1 / ZEB2	Repressed in RCC4 cells in response to dn-HIF-1 α or VHL overexpression, but may be repressed by hypoxia. Known repressors of E-cadherin. ⁹³
SNAIL	Induced by hypoxia in ovarian cancer, may contribute to hypoxia induced EMT/metastasis by repressing E-cadherin. ⁹⁴
REST	Induced by hypoxia and ischaemia, it represses multiple genes in hypoxia, including HIF-1 α . See Table 2 and 3 for details.
CtBP	This co-repressor is recruited to the E-cadherin promoter, in response to increased NADH levels in hypoxia. ⁹⁵

Table 2. REST regulation in oxygen-deprived conditions

Experimental condition	Effect on REST	Function	Ref
Ischemic neurons ^(a)	↑ mRNA ↑ nuclear protein	Represses gluR2 leading to neuron death	52,57
Ischemic neurons ^(a)	↑ mRNA ↑ nuclear protein	Epigenetic remodelling and repression of neuronal genes	53
Oxygen/glucose deprived SK-N-SH cells ^(b)	↑ mRNA ↑ protein	Represses the neuropeptide CART leading to cell death	58
1% O ₂ (24 hrs), HEK293 cells	↑ nuclear protein	Represses >200 genes	40
3% O ₂ (7 days), MIAMI cells	↑ mRNA ↑ nuclear protein	Hypoxia-induced stem cell self-renewal	59
2% O ₂ (3 days), LNCaP cells ^(c)	↓ total protein mRNA not affected	Neuroendocrine phenotype of prostate cancer cells (AMPK activation, neurite growth)	69
5% O ₂ (3 days), LNCaP, PC-3 cells ^(c)	↓ total protein ↓ tmRNA	Neuroendocrine phenotype of prostate cancer cells	70
5% O ₂ (3 days), primary neural crest cells	↓ total protein ↓ tmRNA	Neuronal differentiation of neural crest cells	70

Notes and abbreviations: ^(a) Studies performed on hippocampal CA1 neurons from mouse model of ischaemia; ^(b) SK-N-SH cells are a neuroblastoma-derived cell line; ^(c) LNCaP and PC-3 are prostate cancer derived cell lines.

Table 3. REST target genes in oxygen-deprived conditions

Experiment	Target	Evidence	Ref
Ischemic neurons ^(a)	GluR2	REST RNAi rescues ischemic repression <i>Western Blott and Immunofluorescence</i>	52
Ischemic neurons ^(a)	MOR-1	Ischaemia promotes REST recruitment to the promoter ^(b) <i>Chromatin-Immunoprecipitation</i>	57
Ischemic neurons ^(a)	GluA2	Ischaemia promotes REST recruitment to the promoter and epigenetic silencing <i>Chromatin-Immunoprecipitation</i>	53
Ischemic neurons ^(a)	Peg12 Slc22a12 Nfkb2 GluA2 Chrb2 Grin1 Csrnp3 Nppa Slc22a13S cg2 Fdxr Nefh Syp	REST RNAi or dnREST ^(c) rescues ischemic repression <i>Western Blott and qRT-PCR</i>	53
OGD, SK-N-SH ^(e)	CART	REST RNAi rescues hypoxic repression <i>qRT-PCR</i>	58
1% O ₂ , HEK293	Hif-1 α	REST RNAi increases expression and activity in hypoxia <i>Western Blott and qRT-PCR</i>	72
		Hypoxia promotes REST recruitment to the promoter <i>Chromatin-Immunoprecipitation</i>	

1% O ₂ , HEK293	201 genes	REST RNAi rescues hypoxic repression	⁴⁰
			<i>RNA-seq</i>
	GANAB SYNJ1	REST is enriched in the promoter Hypoxia promotes REST recruitment to the promoter	
			<i>Chromatin-Immunoprecipitation</i>
	GANAB CYP1B1 RAB3C	REST RNAi rescues hypoxic repression	
			<i>qRT-PCR</i>
2% O ₂ , LNCaP	AMPK	REST RNAi increases AMPK activation	⁶⁹
			<i>WesternBlott</i>

Notes and abbreviations: ^(a) Studies performed on hippocampal CA1 neurons from mouse model of ischaemia; ^(b) MOR-1 is a well known REST target gene, that is repressed in ischaemia⁵⁷; ^(c) dominant negative REST; ^(d) 58 targets at 24 hrs, 50 targets at 48 hrs; ^(e) OGD, oxygen/glucose deprivation.

Highlights:

- Gene repression accounts for approximately 50% of the transcriptional response to hypoxia.
- A number of transcriptional repressors have been identified as hypoxia-sensitive.
- REST is a key transcriptional repressor in hypoxia