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Gene regulation under low oxygen: holding your breath for transcription

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Oxygen is both an environmental and developmental signal that governs important cellular pathways. Therefore, hypoxia (or low oxygen tensions) is part of both physiological and pathological processes. To deal with hypoxic conditions, cells and organisms have evolved exquisite mechanisms for adaptation and survival. The cellular responses are reliant on controlled transcriptional and post-transcriptional events, where certain genes are positively regulated and others either remain inactive or are actively repressed. It has been known for some time that, during hypoxia, transcription is mainly regulated by the hypoxia inducible factor (HIF). However, recently it has been demonstrated that additional transcription factors are also activated and that non-HIF-dependent processes are involved in the hypoxic stress response. Therefore, gene expression following hypoxia is the result of combined effects on transcription, translation and adjustment mechanisms such as the induction of microRNAs and changes in chromatin.

Dealing with low oxygen

When faced with low oxygen supply, the cell orchestrates a coordinated response with the intent of restoring oxygen homeostasis. Alteration of gene expression is one of the most effective and fundamental methods by which a cell can respond to extracellular signals. Gene transcription is a complex cellular process that usually results from the interaction between transcription factors and their target consensus sequences within DNA. For the majority of extracellular stimuli, the signal must be transduced from outside the cell to the nucleus, resulting in the activation of specific transcription factors and their target genes. Relative to other stimuli, oxygen presents itself in a unique manner because it can diffuse easily into the cell and initiate cellular responses. Over the past decade, the identification of the putative oxygen sensors of the cell and the transcription factors responsible for the cellular response to hypoxia has been the subject of intense research. In addition, the identification of hypoxia as a component of many human diseases has made the field important in therapeutic terms [1]. In light of recent results showing that changes in translation effect replication and induction of microRNAs, I briefly review the hypoxia inducible factor (HIF)-dependent regulation of gene expression but focus primarily on the current understanding of how gene expression is regulated and achieved under low oxygen tensions by non-HIF-dependent processes.

The HIF transcription factor family

More than a decade ago, the Semenza group identified HIF as the key mediator of erythropoietin (EPO) expression following hypoxia [2]. HIF is a heterodimeric complex consisting of an α and a β subunit, which belong to a family characterized by the presence of basic helix–loop–helix Per/Arnt/Sim domains (bHLH-PAS) [3]. There are at least three identified α subunits (1, 2 and 3α) (Figure 1) and a β subunit (with several splice variants [4]).

Of the three HIF-α-subunits, HIF-1α is the best studied and characterized to date. Nevertheless, the understanding of HIF-2α (also known as endothelial PAS domain protein 1, EPAS-1) function has increased dramatically, whereas the most recently identified and consequently less-well studied subunit is HIF-3α [5,6]. The past five years has helped established that HIF-1α and HIF-2α subunits possess non-redundant functions. Mouse knockout studies have shown the vital importance of HIF-1α for development and survival, and that HIF-2α$^{-/-}$ mice have different phenotypes depending on their genetic background, thus illustrating the importance of HIF-2α and the context-dependent nature of its activity [7–9]. In addition, a recent study has demonstrated that HIF-2α cannot functionally substitute for HIF-1α in embryonic stem cells [10].

HIF-β (also known as aryl hydrocarbon receptor nuclear translocator, ARNT) is not controlled by oxygen levels and is found constitutively expressed in all cell types. By contrast, α-subunit levels are under tight control. In response to changing oxygen levels, the control of HIF-α subunit expression is achieved by regulating protein level, although other stimuli such as oncogene activation and cytokines can induce both transcription and protein synthesis increases of the α subunits [11].

The mechanism of HIF regulation in hypoxia

The mechanism behind oxygen-dependent regulation of HIF-α was revealed with the identification of a novel class of prolyl hydroxylases (PHDs), of which four isoforms (PHD1, PHD2, PHD3 and PHD4) have been identified at this time [12,13]. In the presence of oxygen, PHDs catalyse hydroxylation of HIF-α. The hydroxylated HIF-α subunits are then polyubiquitylated and targeted for proteasome-mediated degradation (Figure 2), thus lowering the

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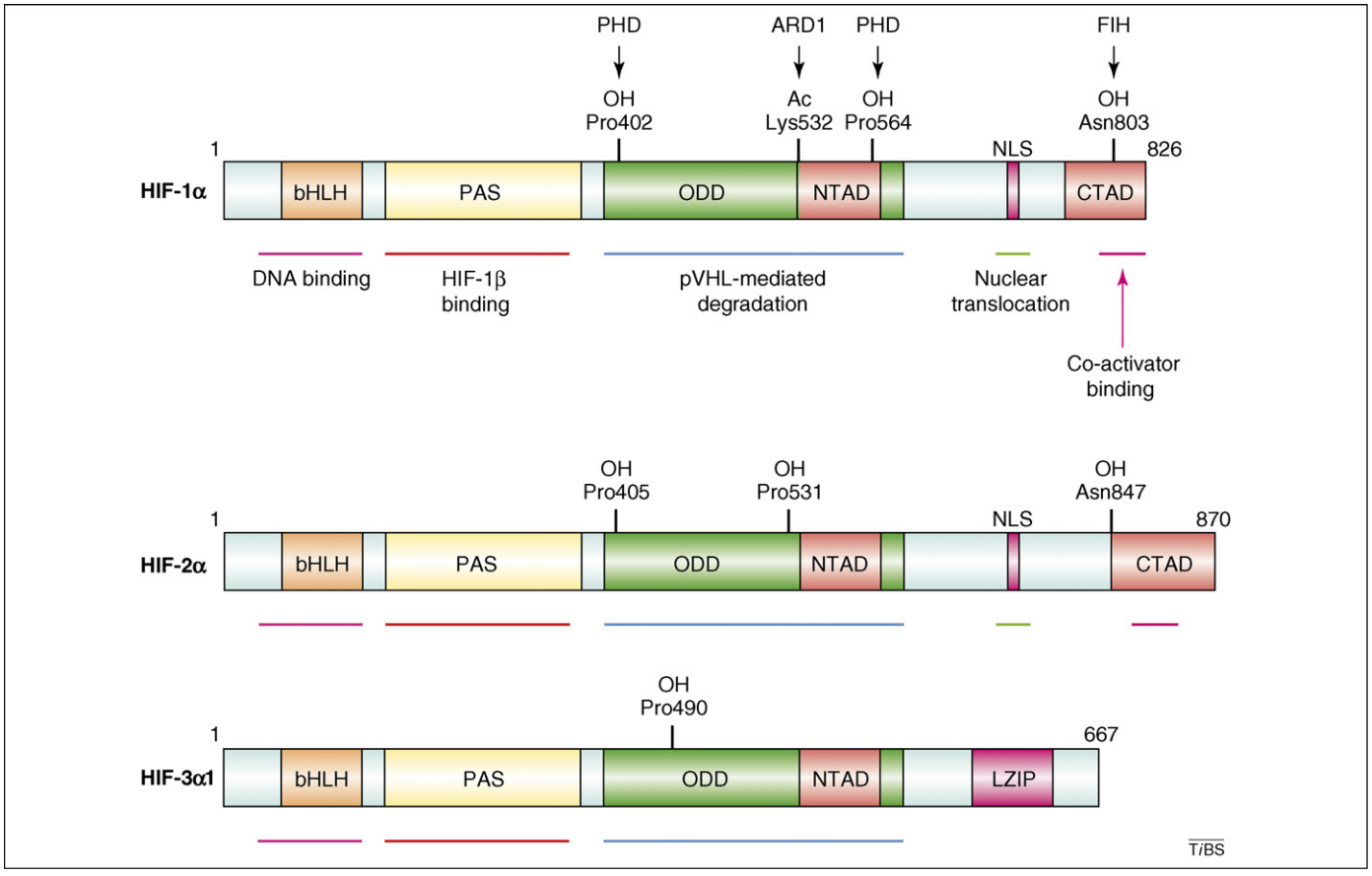

Figure 1. The HIF-α proteins: a schematic diagram showing the similarities and differences between HIF-α family members HIF-1α, HIF-2α and HIF-3α. The coloured boxes represent the different functional domains identified in these proteins. The modifications of specific residues are noted above each, and the proteins that perform those modifications are indicated. Coloured bars below each protein delineate particular interaction regions within HIF proteins that are important for their function. Abbreviations: bHLH, basic helix–loop–helix; CTAD, C-terminal transactivation domain; LZIP, leucine zipper; NLS, nuclear localization signal; NTAD, N-terminal transactivation domain; ODD, oxygen-dependent-degradation domain; PAS, Per/ARNT/Sim domain.

cellular levels of the protein. An extra oxygen-dependent hydroxylation event mediated by factor inhibiting HIF-1 (FIH) takes place at the C-terminal transactivation domain of HIF-α. This modification prevents the association between HIF-α and p300/CBP (CREB-binding protein) [14–17] (Figure 2). The identification of HIF hydroxylation as a control mechanism has led to the search for novel substrates of the PHDs and FIH. Several different studies have identified novel and putative substrates for these enzymes, including several ankyrin-repeat-containing proteins such as nuclear factor-κB (NF-κB) and one of its inhibitory proteins IκBα [18]. In addition, a recent study implicated PHD1 in the activation of inhibitor of κB kinase β (IKKβ, the NF-κB upstream and activating kinase) following hypoxia [19]. The next few years should produce interesting new examples of how hydroxylation modulates additional cellular functions.

Hypoxia-induced gene transactivation

To activate the majority of its target genes, HIF must associate with the transcriptional coactivator proteins p300 or CBP [16,17] (Figure 2). However, recent evidence has shown that this is not required for all HIF genes, indicating that additional activating mechanisms or binding partners must also exist [20,21]. To date, >70 HIF target genes have been identified and validated in humans [22]. However, this number is probably under-estimated and there are likely to be hundreds of both primary and secondary (i.e. indirect) HIF target genes. The direct target genes validated so far can be grouped within several categories, such as cellular metabolism, cell growth and apoptosis, and restoration of the oxygen supply (Table 1). Importantly, all of these target genes ensure that either the cell restores oxygen homeostasis (during which cells survive with minimal energy production obtained from glycolysis) or that cells die due to persistent lack of energy. The introduction of siRNA technology has helped elucidate the contribution of HIF-1 and HIF-2 in the cellular response to hypoxia. Furthermore, it has also led to the identification of novel and HIF-independent hypoxia-inducible genes [23–31] (Table 1). More extensive analyses, in different cellular backgrounds (for example normal versus transformed cells), will further characterize the gene-expression profile induced by hypoxic stress.

HIF-independent responses

When the cell is exposed to hypoxia, a coordinated response is mounted, which relies heavily on gene expression (Figures 3,4). Gene expression is not only achieved by transcriptional activation but it is also achieved by modulation of the translational process. Translation is one of the most energy-consuming processes in the cell and in hypoxia; when energy production is reduced, translational rate is one of the first aspects of cellular biology to be

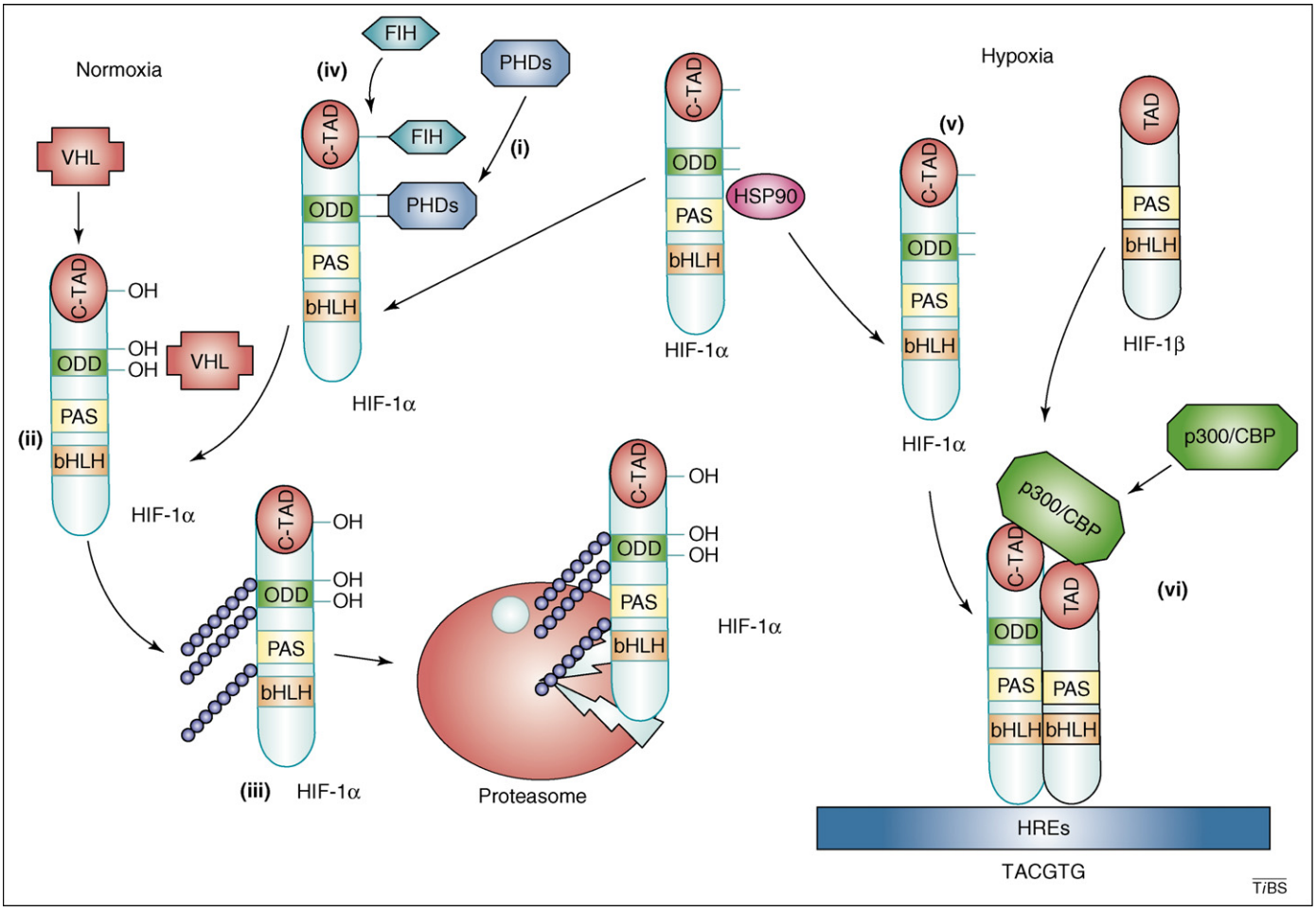

Figure 2. The HIF system and its regulation: a schematic diagram of the HIF-α-degradation and -stabilization mechanism following oxygen changes. **(i)** In the presence of oxygen (e.g. during normoxia), PHDs (dark blue) bind to HIF-α and catalyse the Fe(II)-dependent hydroxylation of specific proline residues within the ODD domain (Pro402 and Pro564 in HIF-1α; Pro405 and Pro531 in HIF-2α). **(ii)** Once hydroxylated, HIF-α binds rapidly to the VHL tumour-suppressor protein (an E3 Ligase), which results in its polyubiquitylation (dark blue spheres). **(iii)** This targets HIF-α for proteasome-mediated degradation. An extra oxygen-dependent hydroxylation event takes place on HIF-α, which concerns a single asparagine residue within the C-terminal transactivation domain (Asn803 in HIF-1α and Asn847 in HIF-2α). **(iv)** Asparagine hydroxylation is mediated by FIH and this modification prevents the association between HIF-α and p300/CBP [14–17]. **(v)** In the presence of low oxygen, HIF-α is stabilized and can translocate to the nucleus. **(vi)** HIF-α dimerizes with its partner HIF-1β and associates with co-activator proteins p300/CBP to transactivate target genes containing hypoxia-responsive elements. Note, molecules are not drawn to scale. Abbreviations: FIH, factor inhibiting HIF; HREs, hypoxia-responsive element; p300/CBP, CREB-binding protein; PHD, prolyl hydroxylases; VHL, von Hippel–Lindau tumour-suppressor protein.

affected. Moreover, DNA replication – another energy-demanding process – is also modulated by low oxygen levels. These effects are grossly independent of the HIF system, even though HIF can contribute to the final cellular response.

Translational control

Effects on translation and overall protein synthesis have been mostly observed following severe lack of oxygen, ranging from 0.5% to 0.0% O_2 [32–34]. However, a recent study has demonstrated that moderate hypoxia (1–5% O_2) can produce the same effects but with delayed kinetics [35]. Translation is a complex process with multiple levels of regulation [36]. It is also sensitive to several cellular stresses [36]. Mechanistically, the process of mRNA translation can be divided into three steps: initiation, elongation and termination. In eukaryotes, initiation is the most complex step and is tightly controlled by the eukaryotic initiation factors (eIFs). Most translation in eukaryotes is termed 'cap-dependent', whereby the assembly of an active eIF-4F complex at the ^{m7}GpppN cap structure located at the 5′-end of the mRNA is required. The eIF-4F complex consists of the cap-binding protein, eIF-4E, a scaffold protein, eIF-4G and an ATP-dependent helicase eIF-4A. Formation of this complex facilitates the recruitment of the 43S pre-initiation complex, which, in addition to a few other initiation factors, includes the small 40S ribosomal subunit and the ternary complex (eIF-2–GTP–Met-tRNA$_i$) [36]. Once assembled, this initiation complex scans through the 5′-untranslated region (UTR) of the mRNA until it encounters the AUG start codon, where the hydrolysis of the eIF-2-bound GTP into GDP occurs, resulting in the delivery of the Met-tRNAi to the start site of translation. The initiation factors are released and recruitment of the large 60S ribosomal subunit leads to start of the elongation phase of translation.

The eIF-4F and ternary complexes constitute the two major control points in eukaryotic initiation of translation. The protein levels of eIF-4E are rate limiting and the formation of the eIF-4F complex can be prevented by a family of eIF-4E-binding proteins (4E-BPs), which compete with eIF-4G. The association of the 4E-BPs with eIF-4E is

Table 1. HIF-dependent and independent target genes involved in the response to hypoxia

Cellular process	HIF-dependent genes[a]	HIF-independent genes[a]
Oxygen supply	*EPO* (erythropoietin)	None known
	FECH (ferrochelatase)	
	ABCG2 (breast cancer resistance protein)	
	TF (transferring)	
	CP (ceruloplasmin)	
	VEGFA [vascular endothelial growth factor (VEGF)]	
	FLT1 (VEGF receptor/fms-related tyrosine kinase 1)	
	LEP (Lep-Leptin)	
	NOS2A (inducible nitric oxide synthase)	
	NOS3 (endothelial nitric oxide synthase)	
	HO1 (haem oxygenase 1)	
	END1 (endothelin 1)	
HIF control	*EGLN1* (prolyl hydroxylase domain-containing protein 2)	None known
	EGLN3 (prolyl hydroxylase domain-containing protein 3)	
	CITED2 (CBP/p300-interacting transactivator with Glu/Asp rich C-terninal domain 2)	
	HIF3A (inhibitory PAS domain protein)	
Transcription	*CITED2*	*ATF3* (activating transcription factor 3)
	ID2 (inhibitor of differentiation 2)	*EGR1* (early growth response 1)
	ETS1 (erythroblastosis virus E26 oncogene homolog 1)	*ATF4* [activating transcription factor 4 (tax-responsive enhancer element B67)]
	BHLHB2 (differentially expressed in chondrocytes 1)	
	BHLHB3 (differentially expressed in chondrocytes 2)	
Cellular metabolism	*PFK* (phosphofructokinase)	*ASPH* (aspartate β-hydroxylase)
	ALDOA (aldolase A)	
	GAPDH (glyceraldehyde-3-phosphate dehydrogenase)	
	PGK1 (Phosphoglycerate kinase 1)	
	ENO1 (enolase 1)	
	PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3)	
	PFKFB4 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4)	
	GLUT1 (glucose transporter 1)	
	GLUT3 (glucose transporter 3)	
	CAIX (carbonic anhydrase 9)	
	ABCB1 (multidrug resistance gene 1)	
Cell growth	*IGFBP1* (insulin-like growth factor-binding protein 1)	*RBM3* (RNA-binding motif (RNP1, RRM) protein 3)
	TGFB3 (transforming growth factor β3)	*CIRP* (cold-inducible RNA-binding protein)
	ENG (endoglin)	*PIM1* (pim-1 oncogene)
	CTGF (connective tissue growth factor)	*MET* [met proto-oncogene (hepatocyte growth factor receptor)]
	TFF3 (intestinal trefoil factor)	*GDF15* (growth differentiation factor 15)
	NT5E (5′-nucleotidase, ecto CD73)	*CADM1* (immunoglobulin superfamily member 4)
	CXCL12 (stromal cell-derived factor 1)	*MMP* (matrix metallopeptidase)
	CXCR4 (chemokine (C-X-C motif) receptor 4)	
	MET [met proto-oncogene (hepatocyte growth factor receptor)]	
	TERT (telomerase reverse transcriptase)	
Cell death	*BNIP3* (BCL2/adenovirus E1B 19kDa interacting protein 3)	*PHLDA1* (pleckstrin homology-like domain, family A, member 1)
	BNIP3L (BCL2/adenovirus E1B 19kDa interacting protein 3-like)	*BNIP3L* (BCL2/adenovirus E1B 19kDa interacting protein 3-like)
	PPP5C (protein phosphatase 5, catalytic subunit)	*CADM1* (immunoglobulin superfamily member 4)
	MCL1 [myeloid cell leukemia sequence 1 (BCL2 related)]	*DDIT3* (C/EBP homologous protein)
	NPM1 (nucleophosmin)	
	PPP1R10 (phosphatase 1 nuclear targeting subunit)	
	PMAIP1 (phorbol-12-myristate-13-acetate-induced protein 1; also known as Noxa)	

[a]The protein encoded by the gene is given in brackets.

regulated by phosphorylation, mediated mainly by the mammalian target of rapamycin (mTOR), a master integrator of several signalling pathways [37]. mTOR is a Ser/Thr kinase that responds to changes in energy status, nutrient availability, insulin and growth factors, and its activity is associated with cell-cycle progression and cell growth. mTOR-mediated phosphorylation of 4E-BPs reduces its affinity for eIF-4E, therefore, enabling eIF-4E to preferentially bind eIF-4G, thus enabling initiation of translation to proceed. In addition, mTOR also phosphorylates S6-kinase (S6K), which also stimulates translation via the ribosomal protein S6 and the ribosomal-recruitment protein eIF-4B.

In hypoxia, mTOR activity is inhibited (Figure 5). This can be seen by the hypophosphorylation of S6K, S6 ribosomal protein and 4E-BP1 [35]. The mechanism of mTOR inhibition seems to be rather complicated (Figure 5). However, the data published so far indicate that hypoxia can induce a decrease in cellular energy levels, leading to activation of the energy-responsive 5′ AMP-activated

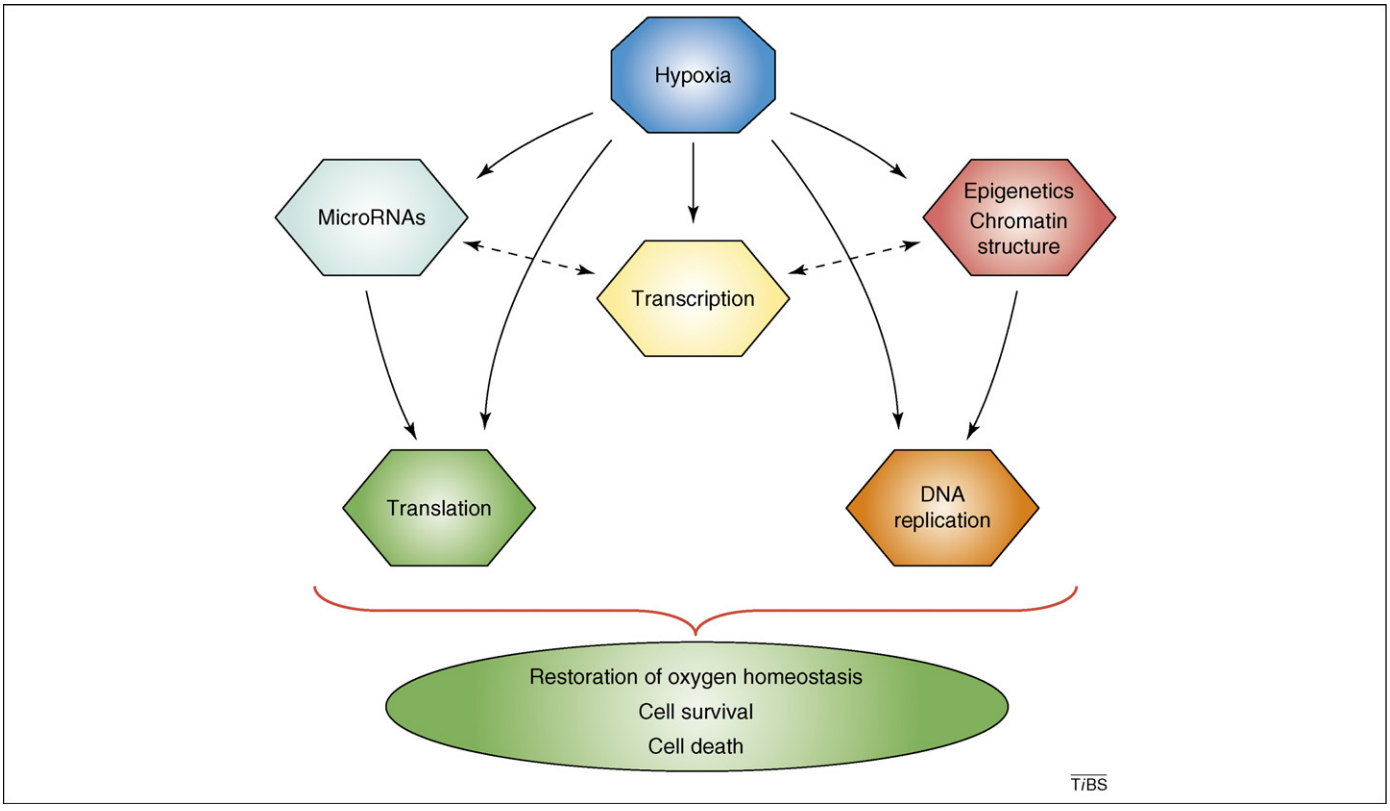

Figure 3. Hypoxia induces a global change in gene expression. Numerous cellular processes (e.g. transcription, translation and DNA replication) are affected by low oxygen, giving rise to a coordinated response to hypoxia. This response results in either the restoration of oxygen homeostasis and cell survival or apoptosis and cell death.

protein kinase (AMPK) [35]. AMPK phosphorylates the tuberous sclerosis complex (TSC) 1/2, which downregulates mTOR activity. AMPK activation is mainly regulated by the tumour-suppressor protein kinase LKB1 [38]. However, in moderate hypoxia, LKB1 alone cannot explain AMPK activation, which indicates that additional kinases are involved [35]. An alternative or parallel level of mTOR regulation comes from the protein REDD1 (also called RTP8001). REDD1 is induced following hypoxia in a HIF-dependent manner [39] (Figure 5). REDD1 activates the TSC1/2 complex, which then inhibits mTOR. Therefore, hypoxia can regulate mTOR by two independent pathways.

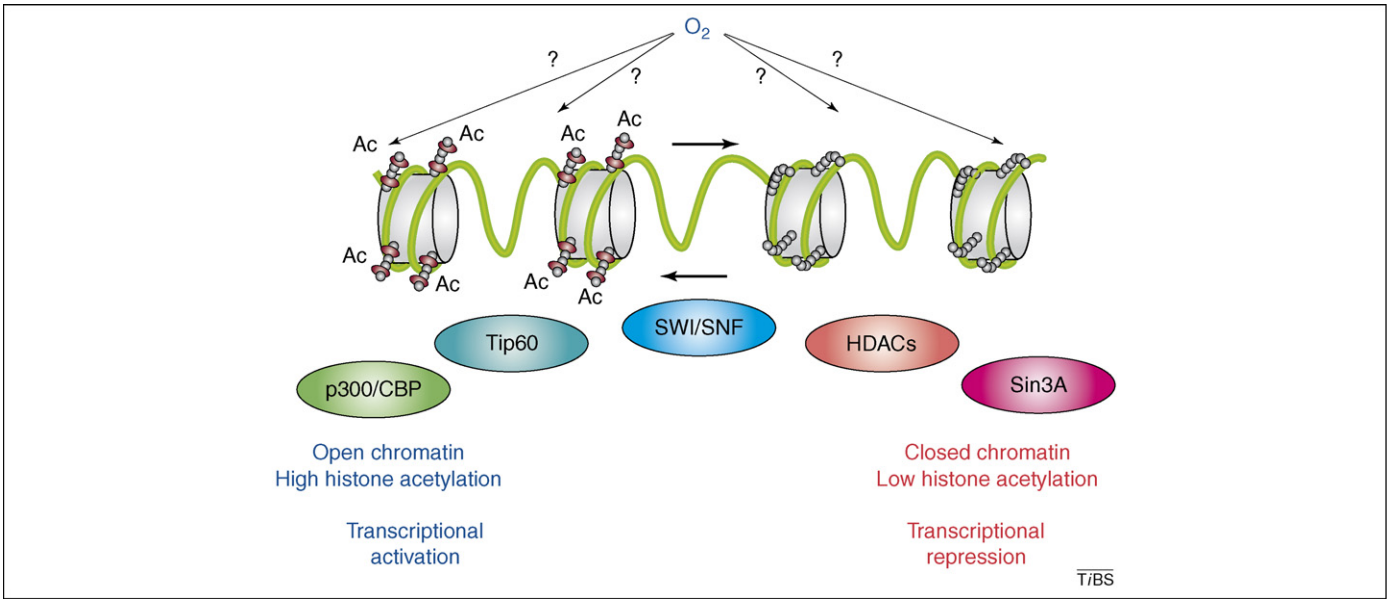

Figure 4. How does oxygen influence chromatin dynamics and structure? The figure shows chromatin-changing molecules that facilitate transcription and how oxygen and specific factors involved in the hypoxia response can influence transcriptional responses. Co-activators and the co-repressor complex have important roles in controlling transcription. Chromatin remodellers and histone modifications (Ac, histone tail acetylation) assure that chromatin is accessible for transcription factor and polymerase binding.

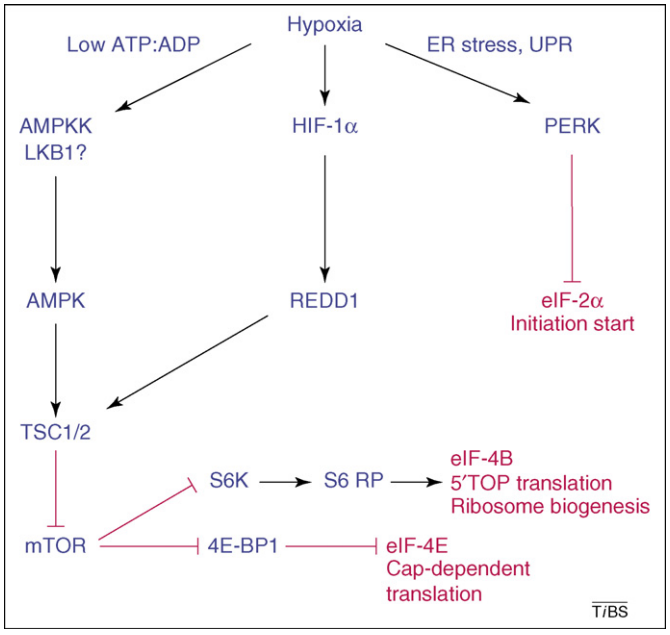

Figure 5. How does oxygen influence eukaryotic translation? Hypoxia activates several different pathways that result in the inhibition of the initiation of translation. (i) Hypoxia induces a reduction in the ATP:ADP ratio within the cell, resulting in the activation of 5′ AMP-activated protein kinase (AMPK). AMPK inhibits mTOR, which regulates the activity of two important initiation factors, eIF-4E and eIF-4B, which are involved in the mRNA cap-binding process and the scanning mechanism of the ribosome, respectively. (ii) When induced by hypoxia, HIF-1 activates REDD1, which also inhibits mTOR and, therefore, also blocks translation in the same manner. (iii) Finally, when induced by hypoxia, endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) result in the activation of the double-stranded RNA-activated protein kinase-like ER kinase (PERK), which directly phosphorylates eIF-2α. This ultimately blocks translation by preventing the delivery of Met-tRNA$_i$ to the pre-initiation complex.

In addition to the effects on eIF-4F and 4E-BPs, hypoxia also induces hyperphosphorylation of Ser51 of the α subunit of eIF-2 [33,35]. This also results in translation inhibition because phosphorylation of eIF-2α at this site prevents the exchange of GDP for GTP. Several kinases have mediate this phosphorylation event: PKR (interferon inducible, double-stranded RNA-activated kinase), HRI (heam-regulated inhibitor of translation), GCN2 (kinase activated by nutrient starvation) and PERK (double-stranded RNA-activated protein kinase-like ER kinase), all of which are stress-responsive [28]. Seminal work by the groups of Koumenis and Wouters (for example, Refs [28,40]) led to the identification of PERK as the kinase responsible for eIF-2α phosphorylation following hypoxia (Figure 5). These studies resulted in the identification of the unfolded protein response (UPR) as a component of the cellular response to hypoxia, leading to more mechanistic insights as to how cells survive such harsh conditions. Mouse knockout studies for PERK and ATF4 (a transcription factor that is upregulated following hypoxia in a HIF-independent manner and is also involved in the UPR) demonstrated that these proteins contribute to overall survival. Cells deficient for these factors are more sensitive to hypoxia-induced cell death. Furthermore, tumour xenograft models demonstrated that PERK contributes to tumour growth *in vivo* [33,40]. Given these results, targeting the UPR component of the hypoxia response could provide a new avenue for therapy both as sole and combined treatment regimens in solid tumours.

MicroRNAs

One of the paradoxes of hypoxia-induced protein-synthesis inhibition is that this inhibition is selective. In other words, the translation block is not general and several proteins are highly expressed and preferentially translated [34]. One possible explanation is the differential dependency or affinity of certain transcripts for the translational machinery [41]. Another possibility is the hypoxic induction of microRNAs (miRNAs). miRNAs are a class of 22-nucleotide non-coding small RNAs that influence mRNA stability and translation [42]. Recent studies have demonstrated that solid tumours have specific miRNA signatures and it is suggested that these miRNAs contribute to the phenotype, grade and overall response of these tumours [43,44]. Recently, three independent studies have shown that hypoxia does indeed induce miRNAs [45–47]. One study focused on the identified target of the miRNAs, vascular endothelial growth factor (VEGF), and determined the possible combinations of miRNAs that could modulate VEGF expression [45]. The other studies identified a set of miRNAs induced by hypoxia but did not identify their targets [46,47]. However, these latter studies did provide several important insights. First, it was demonstrated that a subset of hypoxia-induced miRNAs provides a survival advantage for the cell; second HIF-1 was implicated in the regulation of some of the miRNAs induced. In addition, several hypoxia-responsive transcription factors have been shown to induce miRNAs, such as NF-κB and p53 [42]. Because these studies are very recent, and the area of miRNA biology is still being developed, much is still unknown about hypoxia-induced miRNAs. The next few years of research will most certainly provide new clues about how gene expression is controlled by miRNAs, and the nature of their targets and affects.

Replication stress

Another energy-demanding aspect of cell biology is DNA replication. This is the process by which genetic material is replicated once, and once only, during the cell cycle. Initiation of DNA replication starts when the origin recognition complex (ORC) binds to DNA, recruiting helicase complexes (Mcm2–7), polymerases and additional cofactors [48]. It is vital to the cell that this is tightly controlled and regulated. Errors in the process of DNA replication can prove catastrophic for the viability of the cell and can give rise to malignant mutations. Cells possess cellular checkpoints during the S-phase (during which DNA is synthesized), which provide mechanisms to stall replication forks and correct any errors in the DNA. There are several functional levels of response for the S-phase checkpoint. Upon recognition of a problem, DNA-checkpoint kinases are activated, namely ATM (ataxia telangiectasia mutated) and ATR (ATM- and Rad3-related) [49], resulting in a cascade of events leading to cell-cycle arrest, DNA repair and/or apoptosis [49].

It is of note that the majority of studies performed to investigate hypoxia-induced replication stress have used severe hypoxic to anoxic conditions (see, for example, Refs [50,51]). However, other ranges of oxygen also induce cell-cycle arrest at the G1/S-phase boundary. The effects of hypoxia on the replication-arrest-induced checkpoint

started with seminal work from the Giaccia group. In several studies, Giaccia and colleagues identified several similarities and differences between hypoxia-induced replication stress and other stresses such as aphidicolin and hydroxyurea [50,52]. All of these stresses induce activation of ATR and its downstream target checkpoint kinase 1 (Chk1), although the exact mechanism of ATR activation is currently unknown. Hypoxia also induces phosphorylation of several other ATR targets such as the histone 2A variant, H2A.X, Rad17 and the tumour-suppressor protein p53 [50,52]. However, hypoxia-induced replication stress is different from other stresses because it does not induce DNA damage *per se*. In addition, the exact mechanism of hypoxia-induced replication arrest is currently not well defined. Several possible explanations exist such as shortage of deoxynucleotide precursors due to inhibition of dihydroorotate dehydrogenase and of ribonucleotide reductase (see Ref. [53] and references therein). Replication arrest caused by hypoxia has been described to be caused by an inhibition of replication initiation [53] because it does not seem to inhibit active replication clusters [53]. To further corroborate this concept, an additional study of chromatin-bound replication proteins indicated that the classical pre-replication complex was formed in hypoxia but was not processive [54].

More recently, ATM and Chk2 (which are involved in a branch of the DNA-damage checkpoint that had been previously shown to respond to re-oxygenation) have also been implicated in the hypoxia response [51]. This was discovered because much longer time courses than typical (between 48–96 h following hypoxia exposure) were used. Recent studies in the field of DNA-damage response have revealed that ATR and ATM activate each other following a stimulus that previously had been shown to activate only one of the mentioned kinases [55]. Furthermore, ATR has also been shown to activate Chk2 in response to certain stimuli such as ionizing radiation and UV light [56]. Late induction of ATM and Chk2 could, therefore, be a consequence of early ATR activation; however, additional studies are required to determine if this is the case.

Physiologically, activation of these checkpoints enables cells to survive through the stress of low oxygen conditions. As such, targeting these pathways could prove to be a good strategy for therapy. In fact, inhibition of ATR and/or Chk1 has been shown to sensitize cells to hypoxia–re-oxygenation-induced cell death [57]. A more-detailed and comprehensive analysis of the mechanisms of hypoxia-induced replication arrest would be a useful step towards the generation of novel and targeted therapies.

Epigenetics and chromatin changes in the hypoxia response

Although the cellular response to hypoxia is largely determined by transcription, and several DNA-binding transcription factors have been identified, much is still unknown about the mechanisms involved.

For transcription to occur, transcription factors must bind to specific sequences within the promoters and enhancers of their target genes. However, these can be inaccessible if they are contained within chromatin structures. Chromatin is composed of several levels of organization and compaction [58]. The nucleosome is the basic unit of chromatin, which consists of around 147 base pairs of DNA wrapped around a histone octamer comprising two H2A–H2B heterodimers and an H3–H4 tetramer. Given the number of DNA–histone contacts, the nucleosome, once assembled, is a very stable complex [58]. Nucleosome arrays are then coiled into a 30-nm fibre that is stabilized by the linker histone H1. The most compact forms of chromatin do not enable the activation of genes and so changes must occur to permit not only transcription factor binding but also recruitment of the polymerase and elongation of transcribed strands, with parallel restructuring of the already transcribed areas.

Three possible ways of changing chromatin structure are known: nucleosome remodelling [59], covalent modifications of histone tails within the nucleosome [60] and replacement of one or more of the core histones by histone variants [61]. Nucleosome remodelling is mediated by a family of ATP-dependent enzymes, for example, the SWI/SNF complex (SWI refers to yeast mating type switching, and SNF is an abbreviation for sucrose non-fermenting). The action of complexes such as SWI/SNF can result in the movement of the nucleosome away from the DNA sequences required for transcription-factor binding [59]. Covalent modifications of histone tails, including methylation, phosphorylation, acetylation, sumoylation and ubiquitylation, are carried out by a diverse array of enzymes. These modifications can change chromatin structure and facilitate the recruitment of other chromatin remodellers. In most cases, chromatin close to transcriptionally active sites is enriched for acetylation. Furthermore, the identification of histone variants that become incorporated in the nucleosome has added an additional level of epigenetic control of gene expression. These histone variants can, for example, signal stalled replication forks or areas of DNA damage. Over the past five years, the histone code (specific signature histone-tail modifications) has helped explain how higher-order chromatin structure can be selectively disassembled or reassembled to enable the activation or repression of specific genes [62,63].

Therefore, it is surprising to find that almost nothing is known about chromatin changes occurring following hypoxic stress or low oxygen tensions. However, there is compelling evidence that indicates that chromatin structure and chromatin changes are of great importance to the regulation of gene expression under low oxygen tensions. For example, is it well established that HIF-1 associates with p300 (which has histone acetyl transferase activity) to fully activate its target genes [16,17]. Several studies have also investigated the effects of histone deacetylase (HDAC) inhibitors in HIF-1 function [64,65]. One of these studies identified HDAC7 as a HIF-1α interacting partner that is important for HIF-1α activation [64].

The only study performed to date regarding the involvement of chromatin-remodelling enzymes in hypoxia has shown that the SWI/SNF complex is required for hypoxic-mediated activation of the gene encoding EPO [66]. This study also demonstrated that histones at the EPO gene were acetylated upon hypoxic stress [66]. The finding that an ATP-dependent chromatin-remodelling complex is required to achieve EPO transcription might seem to be

a paradox because, under low oxygen, ATP production is severely impaired. However, it points to the importance of the activation of certain genes above others, and how the cell has evolved decision-making programs to set priorities for gene expression. Despite the exciting finding of SWI/SNF requirement for hypoxia-mediated EPO expression, little has been done to truly address the question of how chromatin structure is modulated to enable transcription to occur during low oxygen tensions. Furthermore, epigenetic studies might also answer the question of how the pro-apoptotic function of HIF-1 is often repressed in cancer cells. For example, a recent study demonstrated that one of the pro-death target genes of HIF, BNIP3 (Bcl-2/adenovirus E1B 19-kDa-interacting protein 3), is selectively silenced by both de-acetylation and methylation in colorectal cancer [67]. Interestingly, hypoxic stress induces a global increase in dimethylated histone H3 Lys9 (a marker of transcriptional repression), which is accompanied with a reduction in acetylation at this site [68]. A novel class of demethylases has recently been identified, JHDM2A (Jumonji C domain-containing histone demethylase 2A), that requires iron and 2-oxoglutarate, much like the PHDs and FIH enzymes. Because oxygen is required for the activity of these demethylases, under hypoxia, JHDM2A would be inhibited [68]. Additional studies would be extremely useful for the understanding of gene regulation under low oxygen tensions and how specific gene expression is achieved under such harsh conditions.

Concluding remarks

Low oxygen tensions activate a specific gene-expression program within the cell to enable adaptation, reinstatement of oxygen homeostasis or cell death. Research during the past decade has led to the identification of the key transcription factors and mechanisms controlling their function. However, as more is known about the players involved, the next step should lead to the identification of the mechanisms of gene expression and how specific genes are activated and repressed in the context of chromatin structure (Figures 3,4). Epigenetic studies will answer many of the questions regarding cell and tissue specificity that cannot be simply explained by transcription-factor availability. With such information, decisions on therapeutic approaches to be undertaken can be made simpler and more adapted to the individual. The modulation of other cellular processes such as translational blocks and DNA-replication arrest (Figure 3) indicates that combinational therapies targeting these pathways can be, under certain circumstances, synergistic.

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