RNA-binding proteins implicated in the hypoxic response

Kiyoshi Masuda, Kotb Abdelmohsen, Myriam Gorospe *

Laboratory of Cellular and Molecular Biology, National Institute on Aging-Intramural Research Program, National Institutes of Health, Baltimore, MD, USA

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Abstract

In cells responding to low oxygen levels, gene expression patterns are strongly influenced by post-transcriptional processes. RNA-binding proteins (RBPs) are pivotal regulators of gene expression in response to numerous stresses, including hypoxia. Here, we review the RBPs that modulate mRNA turnover and translation in response to hypoxic challenge. The RBPs HuR (human antigen R) and PTB (polypyrimidine tract-binding protein) associate with mRNAs encoding hypoxia-response proteins such as HIF-1α/H9251 and VEGF mRNAs, enhance their expression after hypoxia and play a major role in establishing hypoxic gene expression patterns. Additional RBPs such as iron-response element-binding proteins (IRPs), cytoplasmic polyadenylation-element-binding proteins (CPEBs) and several heterogeneous nuclear ribonucleoproteins (hnRNPs) also bind to hypoxia-regulated transcripts and modulate the levels of the encoded proteins. We discuss the efficient regulation of hypoxic gene expression by RBPs and the mounting interest in targeting hypoxia-regulatory RBPs in diseases with aberrant hypoxic responses.

Keywords: stress • oxygen tension • post-transcriptional gene regulation • RNA-binding proteins • mRNA turnover • translational control • ribonucleoprotein complex • untranslated regions

Introduction

Mammalian cells confronted with low oxygen levels can efficiently alter the patterns of expressed proteins. These changes allow the cell to respond to the hypoxic challenge by triggering adaptive processes, such as halting cell division, undergoing necrotic or apoptotic death, increasing proliferation, mounting a survival response, or inducing cell motility, differentiation or senescence.

Many hypoxia-triggered changes in gene expression occur via transcriptional control. The hypoxia-inducible factor (HIF) plays a central role in the transcriptional activation of gene expression during adaptation to hypoxia [1]. A heterodimer transcription factor, HIF is composed of a constitutively expressed β-subunit and a hypoxia-inducible α-subunit, each consisting of three isoforms [2, 3]. Among the HIF α-isofoms, HIF-1α and HIF-2α regulate a similar set of target genes but only HIF-1α is expressed constitutively [4, 5]. In response to low oxygen levels (typically <2% O2), HIF-1α transcriptionally activates dozens of genes

*Correspondence to: Myriam GOROSPE, RNA Regulation Section, LCMB, NIA-IRP, NIH, 251 Bayview Blvd, Baltimore, MD 21224, USA.
Tel.: 410-558-8443
Fax: 410-558-8386
E-mail: myriam-gorospe@nih.gov

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encoding proteins that are implicated in angiogenesis, cell proliferation, cell survival and glucose metabolism; many of these genes have hypoxia-response elements (HREs) in their regulatory regions [6]. Additional HIF-1-activated gene transcription occurs independently of HREs, triggered instead by the interaction of HIF-1 with histone acetyltransferases p300, CBP and SRC-1 [7]. Other transcription factors proposed to participate in the transcriptional response to hypoxia include the activator protein-1 (AP-1) complex, the nuclear factor (NF)-\( \kappa B \) complex and the activating transcription factor ATF-4 (reviewed in [8, 9]).

Although hypoxia was proposed to increase pockets of gene transcription through histone modification, by acetylation (H3K9, H3K14, H4) and methylation (H4R3, H3K4, H3K79), total RNA levels and mRNA transcription were found to decrease markedly in response to low oxygen levels [10, 11]. This repression was also accompanied by changes in histone acetylation (methylation at H3K9, H3K27, H3K4, H3K9), suggesting that chromatin modifications help to suppress gene transcription globally in response to hypoxia. In addition, hypoxia triggers the ubiquitination and other post-translational modifications of RNA polymerase II, which alter its activity [12].

Given that transcription is globally reduced with hypoxia, as it occurs with many stress agents, the post-transcriptional regulation of pre-existing mRNAs is particularly important. The main post-transcriptional mechanisms affecting the levels of expressed proteins during hypoxia are mRNA turnover and translational control. These processes are modulated efficiently by both RNA-binding proteins (RBPs) and an emerging group of non-coding RNAs (ncRNAs). In this review, we will focus on the regulation of hypoxic gene expression by RBPs (Table 1).

### Table 1 RBPs that modulate gene expression during hypoxia

| RBP   | Target mRNA-binding site | Conditions for association | Influence on mRNA | References |
|-------|--------------------------|----------------------------|-------------------|------------|
| HuR   | HIF-1α (5’UTR)           | CoCl2                      | Translation ↑     | [54]       |
|       | HIF-1α (3’UTR)           | Hypoxia                    | Stability ↑       | [59]       |
| PTB   | VEGF (3’UTR)             | Hypoxia                    | Stability ↑       | [70]       |
|       | HIF-1α (3’UTR)           | CoCl2                      | Translation ↑     | [106]      |
|       | VEGF (3’UTR)             | Hypoxia                    | Stability ↑       | [107]      |
|       | Insulin (3’UTR)          | Hypoxia                    | Stability ↑       | [113]      |
| IRPs  | HIF-2α (5’UTR)           | Normoxia                   | Translation ↓     | [120, 121] |
| CPEBs | HIF-1α (3’UTR)           | Hypoxia + insulin          | Translation ↑     | [44]       |
|       | Thioredoxin (3’UTR)      | Hypoxia                    | Translation ↑     | [45]       |
|       | GLUT1 (3’UTR)            | Normoxia                   | Translation ↓?    | [41]       |
|       | GLUT1 (3’UTR)            | Normoxia                   | Stability ↓?      | [41]       |
|       | SG-associated mRNAs      | Hypoxia                    | Translation ↓?    | [123]      |
|       | Erythropoietin (3’UTR)    | Hypoxia                    | Stability ↑       | [31, 32]   |

RBPs whose function has been implicated in the cellular response to hypoxia are listed. For each RBP, the reported examples of hypoxia-regulated target mRNAs and the regions of association are indicated; the conditions that enhance binding of the RBP to the mRNA are specified. The influence of the RBP upon the target mRNA is described. ‘?’ denotes instances in which information is incomplete.

### Post-transcriptional gene regulation by hypoxia

#### Control of mRNA turnover

The half-life of an mRNA can vary widely in response to different stimuli. It can decrease or increase rapidly, thus altering dramatically the levels of mRNA present in the cell, often in the absence of transcriptional changes [13, 14]. In response to hypoxia, several RBPs have been shown to mediate changes in mRNA turnover, as reviewed in detail later.

#### mRNA decay

By this process, the relative half-life of an mRNA is reduced in response to a given stimulus. Although the mechanisms of mRNA
degradation are not fully understood, they involve enzymatic structures such as the *exosome* and processing (P)-bodies, which degrade mRNAs containing instability sequences. The *exosome* is a large multi-protein complex that performs 3′→5′ degradation of labile mRNAs following the recruitment by decay-promoting RBPs such as AUF1 (also known as hnRNP [heterogeneous nuclear ribonucleoprotein] D), TTP (tristetraprolin), BRF1 (butyrate response factor-1) and KSRP (KH domain-containing RBP) [15–18]. *P*-bodies are cytoplasmic structures that contain proteins involved in mRNA decapping and 5′→3′ degradation [19–23]; mRNAs appear to be recruited to PBs by RBPs, but also by microRNAs, a class of ~22-nt long ncRNAs, which associate with cellular mRNAs and repress gene expression by reducing their half-life and/or inhibiting their translation [23]. However, it remains to be studied if PBs and the exosome are involved in the hypoxia-triggered changes in mRNA turnover. It also remains to be shown if decay-promoting RBPs are directly implicated in promoting mRNA degradation after hypoxia, although some RBPs such as TTP help to maintain low constitutive levels of mRNA subsets that are inducible by hypoxia [24].

### mRNA stabilization

This process results in the accumulation of an mRNA through a reduction in its rate of decay. Stabilizing RBPs typically associate with an mRNA and enhance its half-life by competing with, and hence preventing the binding of, degradation-promoting RBPs and possibly also degradation-promoting miRNAs (e.g. [25], reviewed by [26]). RBPs that promote mRNA stabilization include Hu proteins [the ubiquitous HuR (HuA) and the primarily neuronal HuB (Hel-N1), HuC and HuD], nuclear factor (NF)90, the α-complex protein 1 (αCP1), nucleolin, heterogeneous ribonucleoprotein (hnRNP) C1/C2, PTB, CUG-binding protein 2 (CUG-BP2), the poly(A)-binding protein-interacting protein 2 (PAIP2) and erythropoietin mRNA-binding protein (ERBP) [26–33]. The hypoxia-induced binding of RBPs human antigen R (HuR), polyymidine tract-binding protein (PTB), and ERBP to target mRNAs, and the hypoxia-triggered dissociation of hnRNP A2 and hnRBP L from target mRNAs – all have been implicated in mRNA stabilization, as discussed below.

### Control of translation

The relative utilization of a given mRNA for translation can also be regulated by increasing or decreasing the loading of ribosomes onto the mRNA (the translation *initiation* step) or the rates of polypeptide synthesis and dissociation from the ribosome (the *elongation* and *termination* steps, respectively). For a comprehensive review of the hypoxia-regulated pathways that alter global translation, see Wouters et al. [34].

### Translational inhibition

RBPs that lower the translation of specific target mRNAs include the T-cell intracellular antigen-1 (TIA-1), the TIA-1-related protein (TIAR), NF90, HuR, CUG-BP1, hnRNP A2, hnRNP L and the iron-response proteins (IRP1, IRP2) [26, 35–41]. Most mammalian microRNAs studied to date also repress translation, whether or not they also affect mRNA stability. RBPs and microRNAs can block translation by mobilizing mRNAs to subcellular sites where translation is inhibited, such as stress granules (SGs, cytoplasmic foci that form transiently in response to cell damage and harbour mRNAs that are typically stable and not translated), PBs and neuronal granules. In general, these RNA granules contain RBPs and microRNAs that can direct specific mRNAs between the translation and decay machineries [42, 43]. In response to hypoxia, translation is repressed globally by shutting off the general translational machinery [34]. However, hypoxia can also relieve the action of some inhibitory RBPs, such as IRPs, hnRNP A2 and hnRNP L, through a process termed post-transcriptional derepression. The action of hypoxia upon these RBPs is described later in this review.

### Translational activation

RBPs that promote translation include HuR, PTB, the cytoplasmic polyadenylation-element-binding protein (CPEB) and hnRNP A18 [26, 33, 44, 45]. These RBPs are believed to promote translation by competing with (or otherwise preventing the action of) RBPs or possibly microRNAs that repress translation. Hypoxic conditions activate numerous RBPs (e.g. HuR, PTB, hnRNP A18 and CPEB), which in turn ensures the selective up-regulation of proteins necessary for the hypoxic response, at a time when global translation is potently repressed.

### HuR

Also known as ELAVL1 (embryonic lethal, abnormal vision, Drosophila-like 1), HuR is a 36-kDa RB with three RNA recognition motifs (RRMs), through which it binds to numerous mRNAs bearing U- or AU-rich sequences, typically present in their 3′-untranslated regions (UTRs) [26, 27, 46]. HuR was shown to enhance the stability of many transcripts, such as those encoding p21, sirtuin 1, cyclin A2, cyclin B1, vascular endothelial growth factor (VEGF), eotaxin, MKP-1 (MAP kinase phosphatase-1) and heme oxygenase (HO)-1 [26, 27]. HuR also influences the translation of several target mRNAs; it enhances the translation of many target transcripts (e.g. those encoding p53, prothymosin-α, HIF-1α, cytochrome c, MKP-1 and HO-1), but it represses the translation of other target mRNAs (e.g. those encoding p27, Wnt5a and IGF-IR). Although HuR is predominantly nuclear, its influence on the stability and translation of target mRNAs is tightly linked to its translational process to the cytoplasm, a process that is modulated by numerous transport factors reviewed in 26, 27]. By altering the patterns of expressed proteins, HuR can affect major cellular processes such as proliferation, differentiation, carcinogenesis, senescence, apoptosis and the response to immune and environmental stresses [26, 27, 47–53].

In response to hypoxia, HuR potently elevates the expression of two major hypoxia-inducible proteins, VEGF and HIF-1α, as
described below. In addition, HuR likely regulates the levels and/or translation of other target mRNAs that encode hypoxia-inducible proteins (e.g. GLUT1, TGF-β, c-myc and p53). While exposure to a variety of stress agents increases the cytoplasmic levels of HuR [47], treatment with the hypoxia mimetic CoCl2 unexpectedly does not [54]. Instead, HuR actions on hypoxia-regulated targets might be influenced by other post-translational modifications of HuR, including phosphorylation by kinases Chk2 and PKC, which modulate HuR’s ability to bind target mRNAs [50, 55, 56]. Hypoxia activates Chk2 and PKC [57, 58], but it remains to be studied if these kinases modulate HuR activity during hypoxia.

**HIF-1α mRNA**

HuR associates with the 5’UTR of the HIF-1α mRNA and promotes HIF-1α translation in human cervical carcinoma cells [54]. In unstressed cells, HuR overexpression increases the relative abundance of HIF-1α mRNA in actively translating polysomes and increases the de novo HIF-1α translation. In cells treated with CoCl2, HuR binding to the HIF-1α 5’UTR increases dramatically and HuR is necessary for the induction of HIF-1α by CoCl2 treatment. The HIF-1α 5’UTR has an internal ribosome entry site (IRES) that contributes to enhancing the constitutive translation of HIF-1α; however, this IRES does not appear to enhance HIF-1α translation after hypoxia [54], nor does it seem to be affected by the presence of HuR or PTB (below). Even though HuR does not affect HIF-1α mRNA stability, HuR binding to HIF-1α 3’UTR appears to contribute to elevating HIF-1α mRNA levels in response to androgen treatment [59].

Although HIF-1α levels are strongly induced by translational up-regulation, the stabilization of HIF-1α protein is the best-understood mechanism to increase HIF-1α levels. In normoxia, the HIF-1α protein is extremely short-lived (with a half-life of < 10 min); it is rapidly degraded through the hydroxylation of conserved prolyl and asparagyl residues that are targeted for degradation by the von Hippel–Lindau protein (pVHL), the recognition factor of an E3 ubiquitin ligase complex [5, 60]. During hypoxia, these hydroxylases are quickly inhibited, resulting in the rapid and robust stabilization of HIF-1α [61]. Interestingly, pVHL levels are also regulated by HuR, which binds to the VHL mRNA, stabilizes it, and increases pVHL protein levels [51, 62, 63]. As pVHL is up-regulated by hypoxia [64], it is plausible that HuR contributes to elevating pVHL under such conditions (Fig. 1).

**VEGF mRNA**

VEGF plays a central role in neoangiogenesis (the formation of new blood vessels), acts on vascular endothelial cells to induce proliferation and promote cell migration, and is pivotal for enhancing tumour growth [65–67]. VEGF is extensively regulated by mRNA stabilization; the VEGF mRNA is highly unstable in normoxia, but its half-life increases rapidly in response to cytokines and stress conditions such as hypoxia [68–71]. Transcriptionally up-regulated by HIF-1 and HIF-1-related factors [72, 73], the VEGF mRNA was among the first HuR target transcripts ever reported [70]. HuR interacts with the VEGF 3’UTR and stabilizes the VEGF mRNA after hypoxia, as lowering HuR levels by antisense HuR transfection reduces VEGF mRNA stability and steady-state abundance [70].

**GLUT1, p53, TGF-β and c-myc mRNAs**

Numerous other hypoxia-inducible proteins are also encoded by HuR target mRNAs, although the role of HuR in their hypoxia-triggered increase has not been reported. The expression of GLUT1 (glucose transporter 1), which has a key function in the uptake of glucose, increases by hypoglycaemia and hypoxia, thereby ensuring the availability of intracellular glucose at times of low energy levels [74–76]. HIF-1 increases the expression of GLUT1 transcriptionally [77]; in addition, HuR increases GLUT1 levels by binding to the GLUT1 mRNA and enhancing its translation [78], although it was not reported if HuR promotes GLUT1 expression during hypoxia. Similarly, the levels of the tumour suppressor p53 increase with hypoxia [79, 80]. HuR binds the p53 mRNA and enhances its stability and translation in response to nutritional and genotoxic stresses [81, 82], but it is not known if the hypoxia-triggered increase in p53 requires HuR function. The levels of tissue growth factor (TG)β also increase after hypoxia [83, 84]; HuR associates with the TGF-β mRNA [85], but HuR’s role in enhancing TGβ levels after hypoxia has not been investigated. Another HuR target, the c-Myc mRNA [86], is also induced by hypoxia [87, 88]. Although HIF-1 appears to inhibit c-Myc [89], HIF-2α was shown to stimulate c-Myc activity [90]; whether HuR participates in the hypoxia-triggered increase in c-Myc levels is unknown. Collectively, these proteins influence processes such as cell survival, cell proliferation, carcinogenesis and differentiation.

Together with the aforementioned influence on pVHL expression, HuR could be proposed to control hypoxic gene expression patterns in two stages. Immediately after hypoxia, HuR helps to elevate HIF-1α expression, which in turn increases the transcription of hypoxia-inducible gene products (e.g. VEGF, TGF-β, p53, c-myc, GLUT1). During this time, HuR may further assist HIF-1α in the up-regulation of these target genes, as HuR binds to the encoded mRNAs and enhances their stability and/or translation rates. At later times, however, HuR likely helps to ‘shut off’ hypoxic gene expression as it also enhances the levels of pVHL, which targets HIF-1α for degradation, thereby helping to restore the levels of hypoxia-inducible proteins to basal conditions (Fig. 1).

**PTB**

Alternatively known as hnRNP I, PTB is a 57-kDa protein that contains four RRM s, all of which bind pyrimidine-rich sequences, preferentially those contained within longer pyrimidine tracts with cytosines [91, 92]. RRMM and RRMM interact extensively, forming
a compact globular structure, whereas the overall protein has an elongated configuration [93–95]. By binding to different sites on the same RNA molecule, including regions that may be distant in the primary sequence, PTB can lead to substantial restructuring of the RNA substrate and to the introduction of RNA loops [95, 96]. Such conformational changes are believed to be critical for PTB to recruit ribosomes onto IRESs to initiate translation, and for modulating the interaction of factors required for splicing [95, 96].

The three main isoforms of PTB are expressed at varying levels in different cell types and have different roles in splicing and IRES activity [97–100]. Through its RNA-binding properties, PTB has been shown to function in numerous cellular processes, including splicing, polyadenylation, mRNA stability and translation initiation [101–105]. During the hypoxic response, PTB promotes HIF-1α mRNA translation and stabilizes the VEGF and insulin mRNAs, as described below.

**HIF-1α mRNA**

Schipens and coworkers reported that the polypurimidine tract in the 5’UTR of the HIF-1α mRNA promoted its translation in human embryonic kidney cells exposed to hypoxia [106]. Using RNA affinity chromatography and UV-cross-linking experiments, the authors identified PTB as a protein that interacted with this region and proposed that PTB functioned as an IRES-transacting factor (ITAF), possibly promoting the formation of an IRES that facilitated HIF-1α translation during hypoxia [106]; however, subsequent studies
showed that the HIF-1α IRES did not appear to mediate the hypoxia-triggered increase in HIF-1α translation. In human cervical carcinoma cells, PTB also associates with the endogenous HIF-1α mRNA, primarily at the HIF-1α 3′UTR, and this interaction leads to a strong increase in HIF-1α translation after hypoxia or CoCl2 treatment [54]. Although the PTB-triggered increase in HIF-1α translation is independent of the HIF-1α IRES, it is strongly dependent on the presence of HuR, as HuR silencing severely reduces both the binding of PTB to HIF-1α mRNA after CoCl2 treatment and the PTB-stimulated translation of HIF-1α. Conversely, after lowering PTB levels, both HuR binding to the HIF-1α 3′UTR and HIF-1α translation are dramatically lower. This collective evidence supports a model whereby PTB and HuR, likely binding on multiple sites of the HIF-1α mRNA, together promote HIF-1α translation [54, 59, 106].

**VEGF mRNA**

The stability of VEGF mRNA was linked to its association with PTB, which formed a complex with the cold shock domain (CSD) protein [107]. The PTB/CSD complex promotes the stability of VEGF mRNA in both normoxic and hypoxic conditions, and similarly stabilizes other target mRNAs. Because CSD also regulates VEGF transcription, Coles and colleagues postulated that the PTB/CSD complex could regulate in tandem the transcription and stabilization of target mRNAs [107]. Moreover, the long and highly structured VEGF 5′UTR contains two IRESs (IRES A and IRES B), which contribute to enhancing VEGF translation after hypoxia [108]. PTB associates with IRES B, but this interaction does not appear to impact upon VEGF translation [108], even though PTB is a well-known regulator of some viral IRESs [109].

**Insulin mRNA**

The insulin mRNA is highly abundant in pancreatic β-cells, where it constitutes approximately 30% of the pancreatic β-cell mRNA [110]. The insulin mRNA is extremely stable, with a half-life of more than 24 hrs in resting conditions and an even longer half-life in the presence of high glucose levels [110, 111]. Studies to identify the specific mechanisms of insulin mRNA stabilization revealed that the 3′UTR was critical for the β-cell-specific glucose-mediated control of rat insulin II expression [112]. As shown by Tillmar and coworkers, PTB mRNA levels increase markedly in pancreatic β-cells cultured in high glucose concentrations; the authors propose that PTB is important for increasing insulin mRNA levels, as binding of PTB to the polypyrimidine-rich sequence in the rat insulin 3′UTR (ins-PRS) increases insulin mRNA stability [110, 113]. The discovery that binding was inhibited by rapamycin suggested that signalling through mTOR (mammalian target of rapamycin) controlled this process [114].

Hypoxia also increases insulin mRNA levels (33% by 6 hrs and 100% by 16 hrs) in a rapamycin-independent fashion, but accompanied by increased binding of PTB to the ins-rat PRS [113]. Interventions to disrupt the interaction of PTB with the ins-PRS (e.g. by mutation of the ins-PRS) were found to decrease the expression of insulin mRNA in both normoxia and hypoxia and significantly reduced the half-life of the insulin mRNA [110, 113, 115]. The mechanisms by which PTB protects insulin mRNA from degradation under hypoxic conditions are not clear, but it has been suggested that binding of PTB could mask destabilizing elements in the insulin 3′UTR in β-cells, leading to the stabilization of the insulin mRNA [113–115]. PTB is also proposed to stabilize other mRNAs that encode secretory granule components required for insulin storage [116–119]. Other effects of PTB, such as its ability to promote polyadenylation [33], may also contribute to the increased stability of the insulin mRNA.

**Other RBPs implicated in the response to hypoxia**

**IRPs**

Iron-responsive elements (IREs) are specialized stem-loop structures present on mRNAs that are subject to post-transcriptional gene regulation in response to changes in iron concentration [35]. IREs present in the 3′UTR have been shown to alter mRNA stability, whereas 5′UTR IREs block translation in a cap-independent fashion [35]. IREs were recently identified in the 5′-untranslated region (UTR) of HIF-2α and HIF-1α [120, 121]. The interaction of iron-regulatory proteins (IRP1, IRP2) with the HIF-2α IRE represses HIF-2α translation [120, 121]. Although exposure to hypoxia increases HIF-2α expression and function, in conditions of iron deficiency the interaction of IRP2 with the HIF-2α IRE can lead to a strong repression of HIF-2α translation [120]. Zimmer and colleagues showed that hypoxia increased HIF-2α expression by a process of translational derepression, elicited by relieving the interaction of IRP1 with IRE. The authors further identified several compounds that solidified IRP–IRE interactions, thereby blocking the hypoxia-triggered increase in HIF-2α translation and HIF-2α levels [121]. Whether IRP–IRE interactions through the 5′UTR of HIF-1α [35, 121] are also implicated in the regulation of HIF-1α expression by hypoxia awaits further study.

**CPEBs**

Hägele and coworkers recently demonstrated that CPEB1 and CPEB2 associate with the HIF-1α 3′UTR [44]. CPEB1 and CPEB2 repressed HIF-1α translation in untreated neuroblastoma cells; however, in response to insulin treatment, both CPEBs enhanced the hypoxia-triggered increase in HIF-1α levels. This effect was attributed to the ability of the CPEBs to promote translation of HIF-1α, as they induced the synthesis of a heterologous reporter bearing the HIF-1α 3′UTR. In keeping with the function of CPEBs, the elevation in HIF-1α translation by insulin was accompanied by an elongation of the HIF-1α mRNA poly(A) tail [44]. Further work is...
needed to determine if CPEBs affect HIF-1α translation irrespective of insulin levels.

**hnRNPs**

Several hnRNPs have been implicated in post-transcriptional gene regulation following hypoxia. As shown by Yang and colleagues, hnRNPA18 associated with the 3′ UTR of the thioredoxin mRNA and enhanced its translation [45]. The study further revealed that phosphorylation of hnRNPA18 by the hypoxia-activated kinase GSK3β (glycogen synthase kinase 3β) increased the binding of hnRNPA18 to thioredoxin mRNA. The authors propose that hnRNPA18 enhances translation by interacting with eIF4G, a component of the basic translational machinery that associates with the 5′ end of the mRNA, helping to form a loop between the 3′ and 5′ ends of the mRNA and leading to more efficient translation [45]. Further studies are warranted to elucidate if other targets of hnRNPA18, many of which encode stress-response proteins [122], are also translationally induced by hnRNPA18 in response to hypoxia.

Both hnRNPA2 and hnRNPL associated with the AU-rich elements in the GLUT1 3′ UTR in numerous cell types [41]. Hypoxia and other damaging stimuli, which enhance GLUT1 expression, selectively decreased the interaction of GLUT1 mRNA with hnRNPA2 and hnRNPL [41]. Hamilton and colleagues proposed that hnRNPA2 and hnRNPL lowered GLUT1 mRNA stability and translation; hypoxia triggered the dissociation from GLUT1 mRNA, thereby relieving the repressive influence of these hnRNPs [41].

**TIA-1, TIAR and RBPs present in SGs**

In a model of rat cerebral ischaemia (deprivation of oxygen, glucose and serum), TIA-1 was potently up-regulated [123]; conversely, TIA-1 levels were reduced by anti-apoptotic drugs in a model of retinal ischaemia [124]. Although these studies did not examine TIA-1 target mRNAs, they link TIA-1 to the cell death caused by hypoxia together with nutrient deprivation. TIA-1 forms RNP foci in oocytes under various damaging conditions, including anoxia [125], but the specific mRNA targets present in the RNPs were not identified. As hypoxia triggers the formation of SGs [9], other RBPs implicated in mRNA turnover and translation linked to SG metabolism (e.g. G3BP, HuR, CPEB, FMRP, FXR1, PMR1, TTP, BRF1, YB-1 and ZBP, reviewed by Anderson and Kedersha [126]) could also be involved in controlling gene expression in response to hypoxia.

**ERBP**

Identified as an RBP that binds the 3′ UTR of erythropoietin (EPO) mRNA, ERBP levels increased in response to hypoxia and its binding activity was modulated by sulphydryl groups [31]. Additional studies have shown that ERBP stabilized the EPO mRNA in normoxic cells, but more potently in response to hypoxia [32].

**Perspective**

Hypoxia triggers rapid and robust changes in gene expression patterns. The discovery that hypoxia reduces global gene transcription has fuelled efforts to identify post-transcriptional factors that interact with pre-existing pools of mRNAs in order to modulate the subsets of proteins expressed in the hypoxic cell. A host of such mRNA-interacting post-transcriptional regulatory factors have started to emerge. Many of them are non-coding RNAs (e.g. antisense RNAs and microRNAs [127]), but the best known group of interacting factors are RBPs. Collectively, the hypoxia-regulated RBPs described in this review (e.g. HuR, PTB, IRPs, CPEBs, hnRNPs) bind to specific subsets of mRNAs and ensure that they are selectively stabilized and/or translated under low oxygen conditions.

The studies to uncover the roles of RBPs during hypoxia are also prompting many questions for immediate consideration. For example, what signalling pathways control the expression and function of RBPs in response to hypoxia? Do hypoxia-triggered signalling cascades jointly control multiple RBPs? Can we identify systematically the subsets of mRNAs controlled by a given RBP in response to hypoxia? How do SGs affect the hypoxic response? Do microRNAs and other ncRNAs alter RBP function during hypoxia?

As we begin to answer these questions, the usefulness of RBPs and ncRNAs as therapeutic targets in hypoxic situations is also coming into focus [127–129]. Intervention to inhibit the hypoxic response is deemed to be beneficial in situations such as cancer. It is well recognized that tumours must induce angiogenesis in order to develop past microscopic dimensions, and that tumours have adapted to conditions of reduced oxygen by activating specific metabolic pathways [3]. Accordingly, therapies aimed at reducing the levels of factors like HIF-1α are being actively considered as therapeutic targets to impair tumour growth, as detailed in many excellent reviews [1, 3, 4, 67, 130–133]. On the other hand, interventions to favour angiogenesis are anticipated to be beneficial in situations such as myocardial infarction, cerebral ischaemia and wound healing [134–136]. In these conditions, the promotion of hypoxic factors in specific locations and for limited times could be advantageous, as it would prevent further hypoxic damage to the tissues. With increasing recognition of the central role of RBPs in hypoxic gene expression, interest is mounting to understand more thoroughly the processes that control their expression and function. Over the coming years, this knowledge will allow us to harness the potential of RBPs as therapeutic targets to intervene in diseases with hypoxic aetiology.

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