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

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 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-isoforms, 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 $<2\%$ O_2), HIF-1 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

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)	CoCl$_2$	Translation (↑)	[54]
	HIF-1α (3′UTR)	Hypoxia	Stability (↑)	[59]
	VEGF (3′UTR)	Hypoxia	Stability (↑)	[70]
PTB	HIF-α (5′UTR)	Hypoxia	Translation (↑)	[106]
	HIF-1α (3′UTR)	CoCl$_2$	Translation (↑)	[54]
	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]
hnRNP A18	Thioredoxin (3′UTR)	Hypoxia	Translation (↑)	[45]
hnRNP A2	GLUT1 (3′UTR)	Normoxia	Translation (↓)?	[41]
			Stability (↓)?	[41]
hnRNP L	GLUT1 (3′UTR)	Normoxia	Translation (↓)?	[41]
			Stability (↓)?	[41]
TIAR, TIA-1	SG-associated mRNAs	Hypoxia	Translation (↓)?	[123]
ERBP	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.

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)-κB 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).

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 $3' \rightarrow 5'$ 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* (PBs) are cytoplasmic structures that contain proteins involved in mRNA decapping and $5' \rightarrow 3'$ 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, heterogenous 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), polypyrimidine 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 s*tress 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 RBP 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 translocation 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 $CoCl_2$ 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 $CoCl_2$, HuR binding to the HIF-1α 5′UTR increases dramatically and HuR is necessary for the induction of HIF-1α by $CoCl_2$ 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 (TGF)-β also increase after hypoxia [83, 84]; HuR associates with the TGF-β mRNA [85], but HuR's role in enhancing TGF-β 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 RRMs, all of which bind pyrimidine-rich sequences, preferentially those contained within longer pyrimidine tracts with cytosines [91, 92]. RRM3 and RRM4 interact extensively, forming
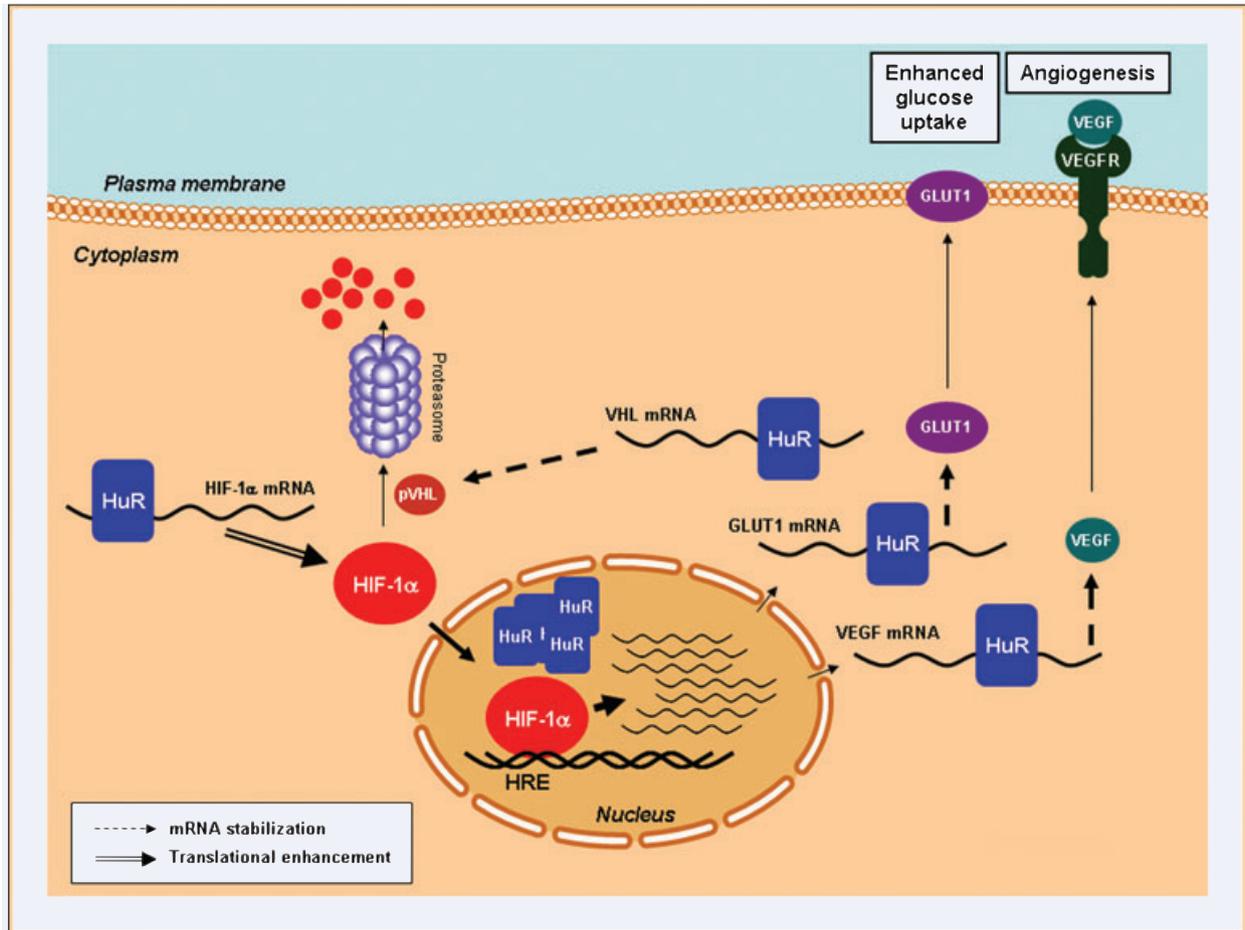
Fig. 1 HuR helps to implement hypoxic gene expression patterns and subsequently to shut it off. HuR interacts with the HIF-1α mRNA and promotes its translation; in turn, the HIF-1 complex transcriptionally elevates the levels of hypoxia-inducible mRNAs, including VEGF and GLUT1 mRNAs. Interestingly, HuR also interacts with these mRNAs, stabilizes them and contributes to increasing their abundance, which leads to an enhancement in angiogenesis and glucose uptake. HuR also interacts with the VHL mRNA, stabilizes it and promotes the expression of pVHL. As a component of the E3 ubiquitin ligase, pVHL in turn promotes the ubiquitin/proteasome-mediated degradation of HIF-1α, which helps to return gene expression patterns to those present during normoxia (see text for details).

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

Schepens and coworkers reported that the polypyrimidine 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 CoCl$_2$ 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 CoCl$_2$ 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, hnRNP A18 associated with the 3′UTR of the thioredoxin mRNA and enhanced its translation [45]. The study further revealed that phosphorylation of hnRNP A18 by the hypoxia-activated kinase GSK3β (glycogen synthase kinase 3β) increased the binding of hnRNP A18 to thioredoxin mRNA. The authors propose that hnRNP A18 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 hnRNP A18, many of which encode stress-response proteins [122], are also translationally induced by hnRNP A18 in response to hypoxia.

Both hnRNP A2 and hnRNP L 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 hnRNP A2 and hnRNP L [41]. Hamilton and colleagues proposed that hnRNP A2 and hnRNP L 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), TIAR was potently up-regulated [123]; conversely, TIAR levels were reduced by anti-apoptotic drugs in a model of retinal ischaemia [124]. Although these studies did not examine TIAR target mRNAs, they link TIAR 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 sulfhydryl 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.

Acknowledgements

References

1. **Rankin EB, Giaccia AJ.** The role of hypoxia-inducible factors in tumorigenesis. *Cell Death Differ.* 2008; 15: 678–85.

2. **Bracken CP, Whitelaw ML, Peet DJ.** The hypoxia-inducible factors: key transcriptional regulators of hypoxic responses. *Cell Mol Life Sci.* 2003; 60: 1376–93.

3. **Semenza GL.** Hypoxia-inducible factor 1 and cancer pathogenesis. *IUBMB Life.* 2008; 60: 591–7.

4. **Semenza GL.** Targeting HIF-1 for cancer therapy. *Nat Rev Cancer.* 2003; 3: 721–32.

5. **Schofield CJ, Ratcliffe PJ.** Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol.* 2004; 5: 343–54.

6. **Wenger RH, Stiehl DP, Camenisch G.** Integration of oxygen signaling at the consensus HRE. *Sci STKE.* 2005; 306: re12.

7. **Carrero P, Okamoto K, Coumailleau P, et al.** Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha. *Mol Cell Biol.* 2000; 20: 402–15.

8. **Shih SC, Claffey KP.** Hypoxia-mediated regulation of gene expression in mammalian cells. *Int J Exp Pathol.* 1998; 79: 347–57.

9. **Gardner LB, Corn PG.** Hypoxic regulation of mRNA expression. *Cell Cycle.* 2008; 7: 1916–24.

10. **Johnson AB, Barton MC.** Hypoxia-induced and stress-specific changes in chromatin structure and function. *Mutat Res.* 2007; 618: 149–62.

11. **Johnson AB, Denko N, Barton MC.** Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. *Mutat Res.* 2008; 640: 174–9.

12. **Ignacak ML, Harbaugh SV, Dayyat E, et al.** Intermittent hypoxia regulates RNA polymerase II in hippocampus and prefrontal cortex. *Neuroscience.* 2009; 158: 1436–45.

13. **Mitchell P, Tollervey D.** mRNA turnover. *Curr Opin Cell Biol.* 2001; 13: 320–5.

14. **Wilusz CJ, Wormington M, Peltz SW.** The cap-to-tail guide to mRNA turnover. *Nat Rev Mol Cell Biol.* 2001; 2: 237–46.

15. **Chen CY, Gherzi R, Ong SE, et al.** AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* 2001; 107: 451–64.

16. **Butler JS.** The yin and yang of the exosome. *Trends Cell Biol.* 2002; 12: 90–6.

17. **Mukherjee D, Gao M, O'Connor JP, et al.** The mammalian exosome mediates the efficient degradation of mRNAs that contain AU-rich elements. *EMBO J.* 2002; 21: 165–74.

18. **Gherzi R, Lee KY, Briata P, et al.** A KH-domain RNA-binding protein, KSRP, promotes ARE-directed mRNA turnover by recruiting the degradation machinery. *Mol Cell.* 2004; 14: 571–83.

19. **Ingelfinger D, Arndt-Jovin DJ, Luhrmann R, et al.** The human LSm1–7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrnl in distinct cytoplasmic foci. *RNA* 2002; 8: 1489–501.

20. **Eystathioy T, Jakymiw A, Chan EK, et al.** The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. *RNA.* 2003; 9: 1171–3.

21. **Cougot N, Babajko S, Seraphin B.** Cytoplasmic foci are sites of mRNA decay in human cells. *J. Cell Biol.* 2004; 165: 31–40.

22. **Kedersha N, Stoecklin G, Ayodele M, et al.** Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. *J Cell Biol.* 2005; 169: 871–84.

23. **Parker R, Sheth U.** P bodies and the control of mRNA translation and degradation. *Mol Cell.* 2007; 25: 635–46.

24. **Ciais D, Cherradi N, Bailly S, et al.** Destabilization of vascular endothelial growth factor mRNA by the zinc-finger protein TIS11b. *Oncogene.* 2004; 23: 8673–80.

25. **Zhao Z, Chang FC, Furneaux HM.** The identification of an endonuclease that cleaves within an HuR binding site in mRNA. *Nucleic Acids Res.* 2000; 28: 2695–701.

26. **Abdelmohsen K, Kuwano Y, Kim HH, et al.** Posttranscriptional gene regulation by RNA-binding proteins during oxidative stress: implications for cellular senescence. *Biol Chem.* 2008; 389: 243–55.

27. **Hinman MN, Lou H.** Diverse molecular functions of Hu proteins. *Cell Mol Life Sci.* 2008; 65: 3168–81.

28. **Shi L, Godfrey WR, Lin J, et al.** NF90 regulates inducible IL-2 gene expression in T cells. *J Exp Med.* 2007; 204: 971–7.

29. **Kiledjian M, Wang X, Liebhaber SA.** Identification of two KH domain proteins in the alpha-globin mRNP stability complex. *EMBO J.* 1995; 14: 4357–64.

30. **Onesto C, Berra E, Grépin R, et al.** Poly(A)-binding protein-interacting protein 2, a strong regulator of vascular endothelial growth factor mRNA. *J Biol Chem.* 2004; 279: 34217–26.

31. **Rondon IJ, Scandurro AB, Wilson RB, et al.** Changes in redox affect the activity of erythropoietin RNA binding protein. *FEBS Lett.* 1995; 359: 267–70.

32. **McGary EC, Rondon IJ, Beckman BS.** Post-transcriptional regulation of erythropoietin mRNA stability by erythropoietin mRNA-binding protein. *J Biol Chem.* 1997; 272: 8628–34.

33. **Sawicka K, Bushell M, Spriggs KA, et al.** Polypyrimidine-tract-binding protein: a multifunctional RNA-binding protein. *Biochem Soc Trans.* 2008; 36: 641–7.

34. **Wouters BG, van den Beucken T, Magagnin MG, et al.** Control of the hypoxic response through regulation of mRNA translation. *Semin Cell Dev Biol.* 2005; 16: 487–501.

35. **Leipuviene R, Theil EC.** The family of iron responsive RNA structures regulated by changes in cellular iron and oxygen. *Cell Mol Life Sci.* 2007; 64: 2945–55.

36. **Piecyk M, Wax S, Beck AR, et al.** TIA-1 is a translational silencer that selectively regulates the expression of TNF-α. *EMBO J.* 2000; 19: 4154–63.

37. **López de Silanes I, Galban S, Martindale JL, et al.** Identification and functional outcome of mRNAs associated with RNA-binding protein TIA-1. *Mol Cell Biol.* 2005; 25: 9520–31.

38. **Kim HS, Kuwano Y, Zhan M, et al.** Elucidation of a C-rich signature motif in target mRNAs of RNA-binding protein TIAR. *Mol Cell Biol.* 2007; 27: 6806–17.

39. **Xu YH, Busald C, Grabowski GA.** Reconstitution of TCP80/NF90 translation inhibition activity in insect cells. *Mol Genet Metab.* 2000; 70: 106–15.

40. **Paillard L, Legagneux V, Maniey D, et al.** c-Jun ARE targets mRNA deadenylation by an EDEN-BP (embryo deadenylation element-binding protein)-dependent pathway. *J Biol Chem.* 2002; 277: 3232–5.

41. **Hamilton BJ, Nichols RC, Tsukamoto H, et al.** hnRNP A2 and hnRNP L bind the 3′UTR of glucose transporter 1 mRNA and exist as a complex in vivo. *Biochem Biophys Res Commun.* 1999; 261: 646–51.

42. **Kedersha N, Anderson P.** Mammalian stress granules and processing bodies. *Methods Enzymol.* 2007; 431: 61–81.

43. **Anderson P, Kedersha N.** RNA granules. *J Cell Biol.* 2006; 172: 803–8.

44. **Hägele S, Kühn U, Böning M, *et al.*** Cytoplasmic polyadenylation-element-binding protein (CPEB)1 and 2 bind to the HIF-1alpha mRNA 3′-UTR and modulate HIF-1alpha protein expression. *Biochem J*. 2009; 417: 235–46.

45. **Yang R, Weber DJ, Carrier F.** Post-transcriptional regulation of thioredoxin by the stress inducible heterogenous ribonucleoprotein A18. *Nucleic Acids Res*. 2006; 34: 1224–36.

46. **López de Silanes I, Zhan M, Lal A, *et al.*** Identification of a target RNA motif for RNA-binding protein HuR. *Proc Natl Acad Sci USA*. 2004; 101: 2987–92.

47. **Wang W, Furneaux H, Cheng H, *et al.*** HuR regulates p21 mRNA stabilization by UV light. *Mol Cell Biol*. 2000; 20: 760–69.

48. **Lal A, Kawai T, Yang X, *et al.*** Anti-apoptotic function of RNA-binding HuR effected through prothymosin-alpha. *EMBO J*. 2005; 24: 1852–62.

49. **Abdelmohsen K, Lal A, Kim HH, *et al.*** Posttranscriptional orchestration of an anti-apoptotic program by HuR. *Cell Cycle*. 2007; 6: 1288–92.

50. **Abdelmohsen K, Pullmann R Jr, Lal A, *et al.*** Phosphorylation of HuR by Chk2 regulates SIRT1 expression. *Mol Cell*. 2007; 25: 543–57.

51. **Abdelmohsen K, Srikantan S, Kuwano Y, *et al.*** miR-519 reduces cell proliferation by lowering RNA-binding protein HuR levels. *Proc Natl Acad Sci USA*. 2008; 105: 20297–302.

52. **Gorospe M.** HuR in the mammalian genotoxic response: post-transcriptional multitasking. *Cell Cycle*. 2003; 2: 412–4.

53. **López de Silanes I, Fan J, Yang X, *et al.*** Role of the RNA-binding protein HuR in colon carcinogenesis. *Oncogene*. 2003; 22: 7146–54.

54. **Galbán S, Kuwano Y, Pullmann R Jr, *et al.*** RNA-binding proteins HuR and PTB promote the translation of hypoxia-inducible factor 1α. *Mol Cell Biol*. 2008; 28: 93–107.

55. **Doller A, Huwiler A, Müller R, *et al.*** Protein kinase C alpha-dependent phosphorylation of the mRNA-stabilizing factor HuR: implications for posttranscriptional regulation of cyclooxygenase-2. *Mol Biol Cell*. 2007; 18: 2137–48.

56. **Doller A, Akool el-S, Huwiler A, *et al.*** Posttranslational modification of the AU-rich element binding protein HuR by protein kinase Cdelta elicits angiotensin II-induced stabilization and nuclear export of cyclooxygenase 2 mRNA. *Mol Cell Biol*. 2008; 28: 2608–25.

57. **Gibson SL, Bindra RS, Glazer PM.** Hypoxia-induced phosphorylation of Chk2 in an ataxia telangiectasia mutated-dependent manner. *Cancer Res*. 2005; 65: 10734–41.

58. **Sahai A, Mei C, Pattison TA, *et al.*** Chronic hypoxia induces proliferation of cultured mesangial cells: role of calcium and protein kinase C. *Am J Physiol Renal Physiol*. 1997; 273: 954–60.

59. **Sheflin LG, Zou AP, Spaulding SW.** Androgens regulate the binding of endogenous HuR to the AU-rich 3′UTRs of HIF-1alpha and EGF mRNA. *Biochem Biophys Res Commun*. 2004; 322: 644–51.

60. **Kim WY, Kaelin WG.** Role of VHL gene mutation in human cancer. *J Clin Oncol*. 2004; 22: 4991–5004.

61. **Schofield CJ, Ratcliffe PJ.** Signalling hypoxia by HIF hydroxylases. *Biochem Biophys Res Commun*. 2005; 338: 617–26.

62. **Abdelmohsen K, Srikantan S, Yang X, *et al.*** Ubiquitin-mediated proteolysis of HuR by heat shock. *EMBO J*. 2009; 28: 1271–82.

63. **Danilin S, Sourbier C, Thomas L, *et al.*** von Hippel-Lindau tumor suppressor gene-dependent mRNA stabilization of the survival factor parathyroid hormone-related protein in human renal cell carcinoma by the RNA-binding protein HuR. *Carcinogenesis*. 2009; 30: 387–96.

64. **Turcotte S, Desrosiers RR, Béliveau R.** Hypoxia upregulates von Hippel-Lindau tumor-suppressor protein through RhoA-dependent activity in renal cell carcinoma. *Am J Physiol Renal Physiol*. 2004; 286: F338–48.

65. **Yancopoulos GD, Davis S, Gale NW, *et al.*** Vascular-specific growth factors and blood vessel formation. *Nature*. 2000; 407: 242–8.

66. **Neufeld G, Cohen T, Gengrinovitch S, *et al.*** Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J*. 1999; 13: 9–22.

67. **Veikkola T, Alitalo K.** VEGFs, receptors and angiogenesis. *Semin Cancer Biol*. 1999; 9: 211–20.

68. **Goodall GJ, Coles LS, Bartley MA, *et al.*** Post-transcriptional regulation of VEGF. In: Hoying JB, editors. Genetics of angiogenesis. BIOS Scientific Publishers, 2002; pp. 69–83.

69. **Pages G, Berra E, Milanini J, *et al.*** Stress-activated protein kinases (JNK and p38/HOG) are essential for vascular endothelial growth factor mRNA stability. *J Biol Chem*. 2000; 275: 26484–91.

70. **Levy NS, Chung S, Furneaux H, *et al.*** Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. *J Biol Chem*. 1998; 273: 6417–23.

71. **Shih SC, Mullen A, Abrams K, *et al.*** Role of protein kinase C isoforms in phorbol ester-induced vascular endothelial growth factor expression in human glioblastoma cells. *J Biol Chem*. 1999; 274: 15407–14.

72. **Ema M, Taya S, Yokotani N, *et al.*** A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1 regulates VEGF expression and is potentially involved in lung and vascular development. *Proc Natl Acad Sci USA*. 1997; 94: 4273–8.

73. **Flamme I, Frohlich T, von Reutern M, *et al.*** HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 alpha and developmentally expressed in blood vessels. *Mech Dev*. 1997; 63: 51–60.

74. **Ouiddir A, Planès C, Fernandes I, *et al.*** Hypoxia upregulates activity and expression of the glucose transporter GLUT1 in alveolar epithelial cells. *Am J Respir Cell Mol Biol*. 1999; 21: 710–8.

75. **Simpson IA, Appel NM, Hokari M, *et al.*** Blood-brain barrier glucose transporter: effects of hypo- and hyperglycemia revisited. *J Neurochem*. 1999; 72: 238–47.

76. **Qi C, Pekala PH.** The influence of mRNA stability on glucose transporter (GLUT1) gene expression. *Biochem Biophys Res Commun*. 1999; 263: 265–9.

77. **Chen C, Pore N, Behrooz A, *et al.*** Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. *J Biol Chem*. 2001; 276: 9519–25.

78. **Gantt KR, Cherry J, Richardson M, *et al.*** The regulation of glucose transporter (GLUT1) expression by the RNA binding protein HuR. *J Cell Biochem*. 2006; 99: 565–74.

79. **Graeber TG, Peterson JF, Tsai M, *et al.*** Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol Cell Biol*. 1994; 14: 6264–77.

80. **Alarcón R, Koumenis C, Geyer RK, *et al.*** Hypoxia induces p53 accumulation through MDM2 down-regulation and inhibition of E6-mediated degradation. *Cancer Res*. 1999; 59: 6046–51.

81. **Mazan-Mamczarz K, Galbán S, López de Silanes I, *et al.*** RNA-binding protein HuR enhances p53 translation in response to

ultraviolet light irradiation. *Proc Natl Acad Sci USA*. 2003; 100: 8354–9.

82. **Zou T, Mazan-Mamczarz K, Rao JN, *et al.*** Polyamine depletion increases cytoplasmic levels of RNA-binding protein HuR leading to stabilization of nucleophosmin and p53 mRNAs. *J Biol Chem*. 2006; 281: 19387–94.

83. **Nunoyama H, Kusachi S, Ninomiya Y, *et al.*** Hypoxia increases transforming growth factor-β1 concomitantly with types I and III collagen without further enhancement by reoxygenation in cultured rat cardiac fibroblasts. *Connective tissue*. 1998; 30: 207–11.

84. **Yu AL, Fuchshofer R, Birke M, *et al.*** Hypoxia/reoxygenation and TGF-β increase αB-crystallin expression in human optic nerve head astrocytes. *Exp Eye Res*. 2007; 84: 694–706.

85. **Nabors LB, Gillespie GY, Harkins L, *et al.*** HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3′ untranslated regions of cytokine and angiogenic factor mRNAs. *Cancer Res*. 2001; 61: 2154–61.

86. **Lafon I, Carballès F, Brewer G, *et al.*** Developmental expression of AUF1 and HuR, two c-myc mRNA binding proteins. *Oncogene*. 1998; 16: 3413–21.

87. **Knies-Bamforth UE, Fox SB, Poulsom R, *et al.*** c-Myc interacts with hypoxia to induce angiogenesis in vivo by a vascular endothelial growth factor-dependent mechanism. *Cancer Res.* 2004; 64: 6563–70.

88. **Mizukami Y, Fujiki K, Duerr EM, *et al.*** Hypoxic regulation of vascular endothelial growth factor through the induction of phosphatidylinositol 3-kinase/Rho/ROCK and c-Myc. *J Biol Chem*. 2006; 281: 13957–63.

89. **Dang CV, Kim, JW, Gao P, *et al.*** The interplay between MYC and HIF in cancer. *Nat Rev Cancer*. 2008; 8: 51–6.

90. **Patel SA, Simon MC.** Biology of hypoxia-inducible factor 2a in development and disease. *Cell Death Differ*. 2008; 15: 628–34.

91. **Auweter SD, Oberstrass FC, Allain FHT.** Solving the structure of PTB in complex with pyrimidine tracts: an NMR study of protein-RNA complexes of weak affinities. *J Mol Biol*. 2007; 367: 174–86.

92. **Oh YL, Hahm B, Kim YK, *et al.*** Determination of functional domains in polypyrimidine-tract-binding protein. *Biochem J*. 1998; 331: 169–75.

93. **Oberstrass FC, Auweter SD, Erat M, *et al.*** Structure of PTB bound to RNA: specific

94. **Simpson PJ, Monie TP, Szendroi A, *et al.*** Structute and RNA interactions of the N-terminal RRM domains of PTB. *Structure*. 2004; 12: 1631–43.

95. **Petoukhov MV, Monie TP, Allain FHT, *et al.*** Conformation of polypyrimidine tract binding protein in solution. *Structure*. 2006; 14: 1021–7.

96. **Mitchell SA, Spriggs KA, Coldwell MJ, *et al.*** The Apaf-1 internal ribosome entry segment attains the correct structural conformation for function via interactions with PTB and unr. *Mol Cell*. 2003; 11: 757–71.

97. **Ghetti A, Pinolroma S, Michael WM, *et al.*** hnRNP-1, the polypyrimidine tract-binding protein: distinct nuclear localization and association with hnRNAs. *Nucleic Acids Res*. 1992; 20: 3671–8.

98. **Gil A, Sharp PA, Jamison SF, *et al.*** Characterization of cDNAs encoding the polypyrimidine tract-binding protein. *Genes Dev*. 1991; 5: 1224–36.

99. **Wollerton MC, Gooding C, Robinson F, *et al.*** Differential alternative splicing activity of isoforms of polypyrimidine tract binding protein (PTB). *RNA*. 2001; 7: 819–32.

100. **Wagner EJ, Carstens RP, Garcia-Blanco MA.** A novel isoform ratio swich of the polypyrimidine tract binding protein. *Electrophoresis*. 1999; 20: 1082–6.

101. **Garcia-Blanco MA, Jamison SF, Sharp PA.** Identification and purification of a 62,000-dalton protein that binds specifically to the polypyrimidine tract of introns. *Genes Dev*. 1989; 3: 1874–86.

102. **Lou H, Helfman DM, Gagel RF, *et al.*** Polypyrimidine tract-binding protein positively regulates inclusion of an alternative 3′-terminal exon. *Mol Cell Biol*. 1999; 19: 78–85.

103. **Castelo-Branco P, Fugrer A, Wollerton MC, *et al.*** Polypyrimidine tract binding protein modulates efficiency of polyadenylation. *Mol Cell Biol*. 2004; 24: 4174–83.

104. **Wollerton MC, Gooding C, Wagner EJ, *et al.*** Autoregulation of polypyrimidine tract binding protein by alternative splicing leading to nonsense-mediated decay. *Mol Cell*. 2004; 13: 91–100.

105. **Jang SK, Wilmmer E.** Cap-independent translation of encephalomyocarditis virus RNA: structural elements of the internal ribosome entry site and involvement of a cellular 57-kDa RNA-binding protein. *Genes Dev*. 1990; 4: 1560–72.

106. **Schepens B, Tinton SA, Bruynooghe Y, *et al.*** The polypyrimidine tract-binding protein stimulates HIF-1alpha IRES-mediated

translation during hypoxia. *Nucleic Acids Res*. 2005; 33: 6884–94.

107. **Coles LC, Bartley MA, Bert A, *et al.*** A multi-protein complex containing cold shock domain (Y-box) and polypyrimidine tract binding proteins forms on the VEGF mRNA. *Eur J Biochem*. 2004; 271: 648–60.

108. **Huez I, Créancier L, Audigier S, *et al.*** Two independent internal ribosome entry sites are involved in translation initiation of vascular endothelial growth factor mRNA. *Mol Cell Biol*. 1998; 18: 6178–90.

109. **Song Y, Tzima E, Ochs K, *et al.*** Evidence for an RNA chaperone function of polypyrimidine tract-binding protein in picornavirus translation. *RNA*. 2005; 11: 1809–24.

110. **Tillmar L, Carlsson C, Welsh N.** Control of insulin mRNA stability in rat pancreatic islets: regulatory role of a 3′-UTR pyrimidine-rich sequence. *J Biol Chem*. 2002; 277: 1099–106.

111. **Welsh M, Nielsen DA, MacKrell AJ, *et al.*** Control of insulin gene expression in pancreatic beta cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. *J Biol Chem*. 1985; 260: 13590–4.

112. **Wicksteed B, Herbert TP, Alarcon C, *et al.*** Cooperativity between the preproinsulin mRNA untranslated regions is necessary for glucose-stimulated translation. *J Biol Chem*. 2001; 276: 22553–8.

113. **Tillmar L, Welsh N.** Hypoxia may increase rat insulin mRNA levels by promoting binding of the polypyrimidine tract-binding protein (PTB) to the pyrimidine-rich insulin mRNA 3′-untranslated region. *Mol Med*. 2002; 8: 263–72.

114. **Tillmar L, Welsh N.** Glucose-induced binding of the polypyrimidine tract-binding protein (PTB) to the 3′-untranslated region of the insulin mRNA (ins-PRS) is inhibited by rapamycin. *Mol Cell Biochem*. 2004; 260: 85–90.

115. **Fred RG, Welsh N.** Increased expression of polypyrimidine tract binding protein results in higher insulin mRNA levels. *Biochem Biophys Res Commun*. 2005; 328: 38–42.

116. **Knoch KP, Meisterfeld R, Bergert H, *et al.*** Regulation of PTB nucleocytoplasmic translocation and expression of secretory granule proteins in INS-1 cells. *Diabetologia*. 2004; 47: A153.

117. **Knoch KP, Schneider H, Ehehalt F, *et al.*** PTB1 binds to the 5′-UTR of mRNAs encoding proteins of the insulin secretory granules. *Diabetologia*. 2006; 49: 156–7.

118. **Knoch KP, Meisterfeld R, Kersting S, *et al.*** cAMP-dependent phosphorylation of

PTB1 promotes the expression of insulin secretory granule proteins in β-cells. *Cell Metab*. 2006; 3: 123–34.

119. **Knoch KP, Bergert H, Borgonovo B, *et al.*** Polypyrimidine tract-binding protein promotes insulin secretory granule biogenesis. *Nat Cell Biol*. 2004; 6: 207–14.

120. **Sanchez M, Galy B, Muckenthaler MU, et al.** Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. *Nat Struct Mol Biol*. 2007; 14: 420–6.

121. **Zimmer M, Ebert BL, Neil C, *et al.*** Small-molecule inhibitors of HIF-2a translation link its 5′UTR iron-responsive element to oxygen sensing. *Mol Cell*. 2008; 32: 838–48.

122. **Yang C, Carrier F.** The UV-inducible RNA-binding protein A18 (A18 hnRNP) plays a protective role in the genotoxic stress response. *J Biol Chem*. 2001; 276: 47277–84.

123. **Jin K, Li W, Nagayama T, *et al.*** Expression of the RNA-binding protein TIAR is increased in neurons after ischemic cerebral injury. *J Neurosci Res*. 2000; 59: 767–74.

124. **Osborne NN, Nash MS, Wood JP.** Melatonin counteracts ischemia-induced apoptosis in human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 1998; 39: 2374–83.

125. **Jud MC, Czerwinski MJ, Wood MP, *et al.*** Large P body-like RNPs form in *C. elegans* oocytes in response to arrested ovulation, heat shock, osmotic stress, and anoxia and are regulated by the major sperm protein pathway. *Dev Biol*. 2008; 318: 38–51.

126. **Anderson P, Kedersha N.** Stress granules: the Tao of RNA triage. *Trends Biochem Sci*. 2008; 33: 141–50.

127. **Galban S, Gorospe M.** Factors interacting with HIF-1α mRNA: novel therapeutic targets. *Current Pharmaceutical Design*. 2009, In Press.

128. **Brahimi-Horn MC, Pouysségur J.** Harnessing the hypoxia-inducible factor in cancer and ischemic disease. *Biochem Pharmacol*. 2007; 73: 450–7.

129. **Ziello JE, Jovin IS, Huang Y.** Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale J Biol Med*. 2007; 80: 51–60.

130. **Vogelstein B, Kinzler KW.** Cancer genes and the pathways they control. *Nat Med*. 2004; 10: 789–99.

131. **Chan DA, Krieg AJ, Turcotte S, *et al.*** HIF gene expression in cancer therapy. *Methods Enzymol*. 2007; 435: 323–45.

132. **Liao D, Johnson RS.** Hypoxia: a key regulator of angiogenesis in cancer. *Cancer Metastasis Rev*. 2007; 26: 281–90.

133. **Melillo G.** Targeting hypoxia cell signaling for cancer therapy. *Cancer Metastasis Rev*. 2007; 26: 341–52.

134. **Norrby K.** *In vivo* models of angiogenesis. *J Cell Mol Med*. 2006; 10: 588–612.

135. **Silvestre JS, Lévy BI.** Angiogenesis therapy in ischemic disease. *Arch Mal Coeur Vaiss*. 2002; 95: 189–96.

136. **Fraisl P, Aragonés J, Carmeliet P.** Inhibition of oxygen sensors as a therapeutic strategy for ischaemic and inflammatory disease. *Nat Rev Drug Discov*. 2009; 8: 139–52.