Hypoxia Review Series

J. Cell. Mol. Med. Vol 13, No 9A, 2009 pp. 2770-2779

Guest Editors: W.Y. Kim & M. Ivan

Surviving hypoxia by modulation of mRNA translation rate

Michael Fähling *

Charité, Universitätsmedizin Berlin, Institut für Vegetative Physiologie, Berlin, Germany

Received: July 15, 2009; Accepted: July 31, 2009

Abstract

Cells can survive hypoxia/anoxia by metabolic rate depression, which involves lowering of mRNA translation rates in an ATP-dependent manner. By activating anaerobic ATP production (glycolysis), the inhibitory influence on mRNA translation in hypoxia can be abolished. In severe hypoxia, glycolysis cannot fully restore the ATP demand, thus causing a long-lasting inhibition of global protein synthesis. During moderate hypoxia, fermentative ATP production may maintain normal ATP levels. However, an activation of hypoxia tolerance mechanisms, including specific mRNA translation, also takes place. The latter may be attributed to oxygen-dependent (but not ATP dependent) processes such as the activation of the hypoxia-inducible factor cascade. In summary, hypoxia-induced decline in cellular ATP level can be counteracted by suppression of global mRNA translation rate. Sustained protein synthesis seems to be attributed to the activation of specific mRNA translation under long-term hypoxic conditions.

Keywords: hypoxia • post-transcriptional control • mRNA translation • mTOR • PERK • eIF • eEF • IRES • AMPK • PI3K/Akt

Oxygen in eukaryotic metabolism

Eukaryotic organisms use oxygen as the final electron acceptor in the respiratory chain reaction. Under normoxic conditions, oxidative phosphorylation constitutes the major source of the cellular energy intermediate ATP. High-rate energy production is a prerequisite for cells and tissues to achieve a high metabolic rate. Increased oxygen consumption or disturbance in oxygen supply results in decreased oxygen levels. Thus, hypoxia is characterized by inadequate oxygen availability and occurs if oxygen demand exceeds supply, leading to lowered intracellular tensions as compared to normal. Hypoxia plays a central role in various environments such as in rapidly proliferating cells and embryonic development. Hypoxia may also result from pathological changes like genetic disorders leading to abnormal haemoglobin forms or disabled enzymes involved in oxygen utilization and ATP production, impaired gas exchange (*e.g.* as a result of lung oedema), inadequate pulmonary ventilation (*e.g.* in obstructive pulmonary diseases or respiratory arrest), decreased oxygen saturation of the blood (*e.g.* caused by hypopnoea or sleep apnoea), diffusion barriers (*e.g.* in fibrosis), intoxication (*e.g.* carbonic oxide), wounding (due to the disruption of blood vessels), ischaemia or anaemia [1]. Moreover, a lack of oxygen is critically involved in the pathogenesis of stroke, myocardial infarction, chronic lung disease and cancer [2–4]. Accordingly, hypoxia-inducible responses are highly regulated in normal embryonic development and are dysregulated in a number of disease states [1, 5].

Adaptation to hypoxic conditions depends on several factors such as duration and severity, oxygen sensing mechanisms and the tissue affected. One key mechanism is the suppression of metabolic rate that lowers tissue energy demand to a level that can be supplied by pathways of fermentative ATP production alone [6]. Metabolic rate depression is a conserved mechanism and represents an early adaptation to hypoxia in general. It can be seen as a physiological means to establish hypoxia tolerance [7]. However, this strategy alone cannot ensure survival because of the need to produce red blood cells, to form blood vessels and to transform energy supply to glycolysis. Gene expression is a critical feature in the cell's adaptation to hypoxia, but again, gene expression itself is an energy-consuming process. The question, which tissues and

*Correspondence to: Michael FÄHLING,
Charité, Universitätsmedizin Berlin,
Institut für Vegetative Physiologie,
Tucholskystr. 2,
D-10117 Berlin, Germany.
Tel.: 0049-30-450 528268
Fax: 0049-30-450 528972
E-mail: michael.faehling@charite.de

doi:10.1111/j.1582-4934.2009.00875.x

processes are mainly affected by an inadequate oxygen availability is addressed by estimating energy consumption rates.

Oxygen consumption of tissues and processes

During aerobic metabolism, glucose, other carbohydrates, fats and proteins can be used as substrates in energy production. If oxygen tension is low, however, nicotinamide adenine dinucleotide (NADH) accumulates and blocks the Krebs cycle. As a consequence, only glucose serves as an energy-rich substrate for anaerobic glycolysis and ATP yields are much lower. The body oxygen utilization at standard metabolic rate is highest in brain (20%) and skeletal muscle (20%), followed by the liver (17%), heart (11%), gastrointestinal tract (10%), kidney (6%) and lung (4%) [8]. High oxygen consumption is correlated with a high density of mitochondria. It has been estimated that ~90% of mammalian oxygen consumption is mitochondrial, of which ~20% is uncoupled by the mitochondrial proton leak and ~80% is coupled to ATP synthesis. Of the total ATP synthesized, ~25–30% is used for protein synthesis, 19–28% by the Na^+/K^+-ATPase, 4–8% by the Ca^{2+}-ATPase, 2–8% by the actinomyosin ATPase, 7–10% for gluconeogenesis and 3% for ureagenesis, with mRNA synthesis and substrate cycling contributing significantly [8]. The same authors stated that the ATP consumption by proteolysis is difficult to estimate; however, ubiquitin-dependent proteolysis requires ~4 ATP/protein, thus proteolysis does not significantly contribute. Other authors stated that proteolysis accounts for 11% of total ATP consumption in turtle hepatocytes [9]. The impact of transcriptional processes is similarly difficult to assess. The contribution of RNA-synthesis is estimated to be ~1–5%, whereas the contribution of DNA synthesis being much smaller than that of RNA, since its turnover rates are much lower. Notably, these data may differ for the tissues considered and their individual activity states. For instance, maximal stimulation of adenylate cyclase can completely deplete adipocytes of their intracellular ATP [10]. Nearly 10% of the oxygen consumed is needed for enzymatic reactions, *e.g.* by oxidases, oxygenases and hydroxylases [8], which themselves are part of crucial cellular pathways like detoxification of xenobiotica, hormone syntheses or oxygen sensing and activation of hypoxia tolerance pathways. Thus, oxygen is essential for the metabolism of eukaryotic organisms in general. Consequently, the nervous system (with a high activity of the Na^+/K^+-ATPase) and a primary oxidative ATP-production, mainly depend on a proper oxygen supply. Protein synthesis, or mRNA translation, belongs to the most energy consuming processes, thus one would expect that during metabolic rate depression resulting from hypoxia, mRNA translation rate would be suppressed. It is well known that the alteration of gene expression in hypoxia is a result of a complex regulatory network with multiple divergences and convergences. Central to this are transcription factors like the hypoxia-inducible factor (HIF), activating transcription factor 4 and 6 (ATF4, −6), activator protein-1 (AP-1), cAMP-response element-binding factor, nuclear factor of activated T-cells (NFAT), nuclear factor κB (NF-κB), c-fos and jun-B. They are activated by, *e.g.* prolyl hydroxylases, reactive oxygen/nitrogen species (ROS/RNS), calcium (Ca^{2+})- or adenosine signalling, as well as kinases like p38-mitogen-activated protein kinase (MAPK), adenosine mono-phosphate (AMP)-activated protein kinase (AMPK) or the phosphatidylinositol 3-kinase (PI3K)-Akt pathway [1]. Protein synthesis is the final step in gene expression and is thought to be rate limiting [11]. To control the expression of proteins at the level of translation, rather than transcription, has the great advantage of a much faster response. It is likely that translational control mechanisms have evolved to allow for a rapid gene expression control of factors involved in responses to stress or other environmental changes [12], such as hypoxia.

Metabolic rate depression

Investigating metabolic rate depression started with the question of how anoxia/hypoxia-tolerant organism can survive periods where only low oxygen is available (*e.g.* interstitial mussels or mammals during diving), or can suppress their metabolism (*e.g.* during hibernation) [13–20]. Further results were obtained in cellular models, *e.g.* brain cortical cells, or hepatocytes [9, 21]. In most cases the metabolic rate in hypoxia is lowered to 5–40% of the resting rates [22]. For instance, severe hypoxia causes a rapid drop in protein synthesis to ~7% compared to control [23]. During hibernation, metabolic rate can drop to $<$5% [24]. As a result of these studies, a unifying theory of hypoxic tolerance was developed [9]: Cells respond to acute hypoxia with a global decline in protein biosynthesis ('translational arrest') and a generalized decline in membrane permeabilities ('channel arrest') or firing frequencies ('spike arrest') in the case of neuronal tissues. As a consequence, most energy-consuming events (translation and the Na^+/K^+-ATPase) are inhibited in order to spare energy for other essential processes. This early adaptation to acute hypoxia was termed the 'defence phase'. Under prolonged hypoxia a more complex organized reaction occurs, which has been summarized as the 'rescue phase'. It involves the suppression of genes for less required enzymes (*e.g.* Krebs cycle and gluconeogenesis), favouring the activation of genes for sustained survival (*e.g.* HIF1 and its target genes). Thus, in hypoxia the inhibition of multiple metabolic processes must be coordinated to achieve a net suppression that balances the rates of ATP production and ATP-consuming processes at a new lower net rate of ATP turnover [6]. Indeed, metabolic reprogramming that allows maintaining cellular energetics in homeostasis, albeit at a much depressed level, represents an ultimate survival response, by coordinating declining ATP production with suppressed energy demands [7]. Reduced oxygen consumption by translational arrest is an established mechanism for reducing cellular injury during hypoxia [6, 25]. Consistently, it has been shown that the magnitude of hypoxia resistance inversely correlated with relative mRNA translation rates [26].

The activation of anaerobic metabolism in hypoxic cells has been described as an effort to maintain energy equilibrium and viability [27, 28]. Activated glycolysis causes extracellular acidosis as lactate molecules are coupled to hydrogen ions and released as lactic acid. It was shown that production of H^+ ions in hypoxia promotes interactions between von Hippel–Lindau tumour suppressor protein and rRNA genes to reduce rRNA synthesis. This silencing program restricts ribosomal biogenesis to preserve energy equilibrium and viability, thus explaining the protective effect of acidosis in hypoxic settings [29]. Since ribosomes have their own turnover rates, the suppression of ribosomal biogenesis can be seen as an energy-saving mechanism during prolonged hypoxia. However, it does not explain how hundreds of hypoxia sensitive genes can be effectively translated under prolonged conditions of oxygen deprivation. At least, a prominent decrease in the number of ribosomes has not been described yet. Moreover, re-oxygenation enhances protein synthesis rates above normal, which has been attributed to the combined action of still activated glycolysis and a re-activation of the respiratory chain reaction in mitochondria that causes ATP synthesis rates above normal. Thus, mRNA translation may be controlled at further 'checkpoints'.

mRNA translation in hypoxia: the down and up and down

Generally, influences on mRNA translation may be global, in which the translation of most mRNAs is regulated, or mRNA specific, wherein the translation of defined subsets of mRNAs is modulated. A global regulation of protein synthesis mainly occurs by the modification of translation initiation factors. Control of mRNA-specific translation is often attributed to regulatory RNA-binding proteins (RNA-BPs), or micro RNAs (miRNAs) as *trans*-factors that recognize particular *cis*-elements, which are usually present in the mRNA's 5′- or 3′-untranslated regions (UTRs) [30]. In hypoxia, the lack of ATP directly affects the global translational machinery to a degree dependent on its severity and duration [31, 32].

The time-dependent, multi-phase response in metabolic reprogramming indicates that different processes participate in the cellular adaptation to hypoxia. At the global level a rapid inhibition in the rate of protein synthesis can be observed in anoxia, with maximum inhibition at 1–2 hrs [33]. Short-term inhibition of mRNA translation seems to be crucial, because cells normally do not synthesize ATP in excess. Even activation of 'stored energy' in the phosphotransferase reaction, catalysed by creatine kinase, sustains energy demand only for minutes. Therefore, a rapid inhibition of mRNA translation compensates the decrease in ATP production, without dramatic consequences for general protein levels, due to the delay achieved by relatively long protein half-life times. Furthermore, it has been shown that, at least in anoxia, protein degradation rates are suppressed as well [34, 35]. However, suppression of both, protein synthesis and decay, would cause a steady state, which is insufficient for active adaptation. Interestingly, the rapid inhibition of global protein synthesis is partially restored after 8 hrs of anoxia and different mechanisms of translational inhibition have been suggested for short-term and prolonged conditions [33].

In moderate hypoxia the short-term inhibition of protein synthesis is less pronounced, and correlates well with the kinetics of decreased ATP levels. This short-term inhibition is followed by a compensatory phase, and a second inhibitory phase under prolonged conditions [1]. The compensatory phase may be important for the expression of genes involved in the organization of prolonged adaptation to hypoxia. It has been suggested that an adaptive increase in glycolysis may account for this mechanism.

Suppression of mRNA translation in prolonged moderate hypoxia can be rescued by either supplementation of glucose, or re-oxygenation [36]. Glucose supplementation would allow continued ATP production by glycolysis to compensate for reduced ATP levels from oxidative phosphorylation, and re-oxygenation would permit the cell to use alternative substrates for ATP production, since glycolysis is strictly glucose dependent, while oxidative ATP production is not. Activation of glycolysis causes an accelerated rate of glucose consumption [36]. The finding that glucose alone is sufficient to restore mRNA translation in prolonged hypoxia, indicates that global mRNA translation mainly depends on ATP availability. On the other hand, the development of cellular quiescence is not simply a consequence of a lack of energy [7]. As shown by sucrose gradient analysis the cellular amount of disaggregated polysomes (as a matter of inhibited translational initiation), is moderate and relatively constant from 1–3 hrs up to 16–18 hrs [37] (Fig. 1). According to an elevated ATP production by oxygen independent glycolysis, several studies showed a partial recovery of polysomal assembling for up to 16–18 hrs [33, 38], or an abolished inhibition of the expression rate of short-lived reporter proteins [1]. In moderate hypoxia, a massive loss in ribosomes assembled at mRNAs is only seen under prolonged conditions, when glucose has been mainly consumed. These data indicate that a switch in energy metabolism is relatively rapid and can compensate for the energy demand of mRNA translation if glucose as the energy-rich substrate for glycolysis is present. Importantly, it has to be taken into account that during moderate hypoxia oxygen is still available, even if at reduced levels, and can be used for ATP generation. This may explain differences observed under severe hypoxia ($O_2 < 0.1\%$), or strict anoxic conditions, as it was shown that an adaptive increase in glycolysis alone is not sufficient to meet the ATP demand of most cells [39]. Thus, rapid activation of anaerobic ATP generation together with a protein half-life time, which normally takes about 2 hrs, may explain why no dramatic alteration in most protein levels can be observed during up to 18 hrs of moderate hypoxia. However, adaptive processes, mediated by inhibition of oxygen-dependent enzymes (which account for 10% of the consumed oxygen, as mentioned above), are rapidly activated and modulate the gene expression response in prolonged hypoxia. For instance, it has been implicated that HIF controls candidate hypoxia tolerance factors, and thus participates in the down-regulation of ATP-costly activities in hypoxia [7]. Moreover,

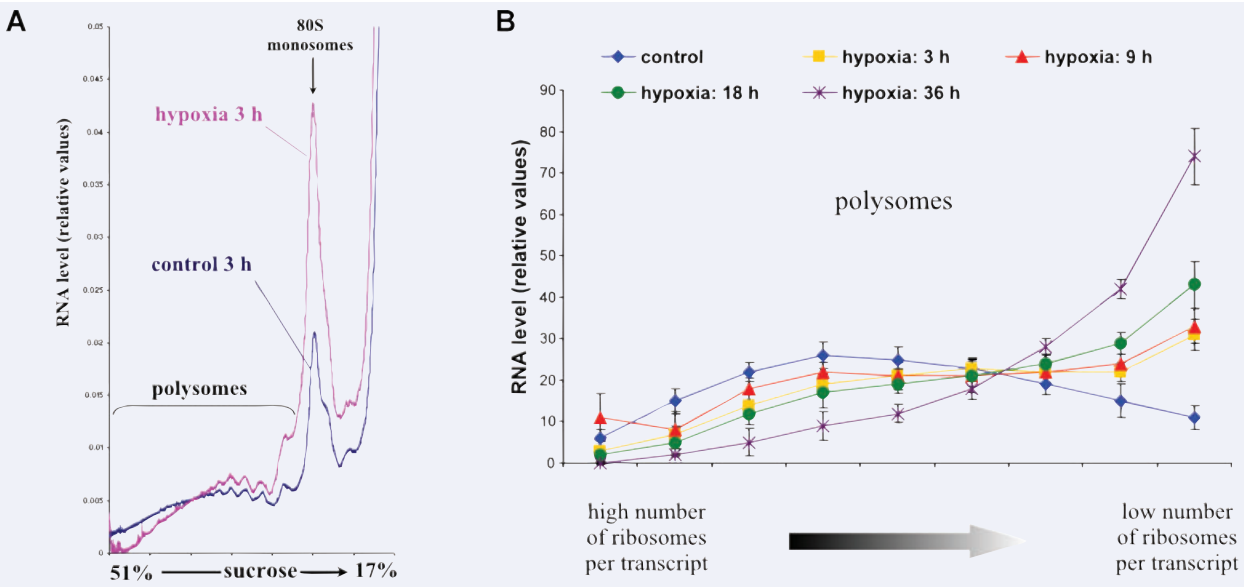

Fig. 1 Analysis of polysomal gradient analysis. HEK293 cells were incubated under control (21% oxygen) or hypoxic (1% oxygen) conditions for up to 36 hrs. (**A**) Typical ribosomal profiles after sucrose gradient ultra-centrifugation monitored at 254 nm absorbance from the bottom (51% sucrose) to the top (17% sucrose). For further technical details see: [73]. A description of this method and discussion of the suitability to analyse hypoxic conditions can be found in [1]. (**B**) Quantification of rRNA levels in polysomal fractions. Note: Only the translationally active part of the gradient (polysomes), as indicated in (**A**), is shown. The data indicate that in the hypoxic response a decrease in high-density polysomes (more than six ribosomes per transcript) in favour of an increase in low-density polysomes occurs. The disaggregation of polysomes (as a result of suppressed translational initiation) can be observed as early as after 3 hrs, with a partial recovery at 9 hrs. Prolonged hypoxic conditions (36 hrs) cause a dramatic decrease in high density polysomes.

HIF induces up-regulation of glucose transporters (*e.g.* GLUT1), glycolytic enzymes, as well as factors that prevent intracellular acidification [40].

The data indicate that the observed long-term inhibition of mRNA translation in moderate hypoxia is different compared to short-term conditions. It starts when glycolysis can no longer satisfy energy demand, *e.g.* due to the lack of glucose. Furthermore, the inhibition of oxygen-dependent enzymes leads to cellular adaptations such as the activation of HIF pathways. Thus, the expression and accumulation of survival factors seem to influence the translational machinery. Notably, cells may have a primary oxidative metabolism, such as neurons, and thus cannot, or even less, compensate the lack in ATP during hypoxia by an activation of glycolysis.

Regulation of global mRNA translation in hypoxia

The process of mRNA translation (for details see *e.g.*: [41–47]) can be divided into three main steps: initiation, elongation and termination. Each step requires translation factors, termed eukaryotic translation initiation, elongation and termination/release factors (eIFs, eEFs and eRFs) that transiently associate with the ribosome [45]. The translational initiation is thought to be the major site of control of mRNA translation [48, 49].

The initiation stage of mRNA translation involves the recruitment of the 40S ribosomal subunit to the mRNA and its recognition of the initiation site. This involves the formation of a 43S pre-initiation complex, which consists of the Met-tRNAi, bound to eIF2-GTP (but not eIF2-GDP), as well as eIF1, eIF1A, eIF3 and the 40S ribosomal subunit. Furthermore, it involves formation of the mRNA 5′-cap-binding complex eIF4F, which consists of eIF4G, eIF4E and eIF4A. Binding to the mRNA 5′-cap is mediated by eIF4E, which also binds eIF4G, a multi-domain scaffold protein that interacts with further components of the translational machinery, *e.g.* eIF4A, a RNA helicase, but also *e.g.* the poly-A BP. eIF4G, another scaffolding protein, interacts with eIF3, thus providing a link to the 43S pre-initiation complex. The assembly of both ribosomal subunits to form the 80S ribosome occurs at the initiation site, causing release of eIFs and terminating the initiation process. Together, over 25 essential factors are involved in this event [50]. To our knowledge, the initiation of mRNA translation is the most crucial point in the regulation of protein syntheses in hypoxia. However, it should be noted that the elongation process consumes nearly 99% of the energy needed for translation [45].

The coordination of metabolic rate suppression takes place at multiple metabolic loci, and is mainly attributed to reversible protein phosphorylation. Short-term inhibition of mRNA translation under both, severe and moderate hypoxia has been attributed to eIF2-α phosphorylation [31, 33, 51]. Phosphorylation of the eIF2-α subunit results in an inhibition of the GDP-GTP exchange catalysed by eIF2B, which is essential to release the initiation factors from the ribosome. As a consequence, eIF2B remains bound to the phosphorylated and inactive eIF2 complex, leading to inhibition of translational initiation. At least four kinases have been shown to phophorylate eIF2-α [52]. Among them is the endoplasmatic reticulum-resident eIF2-α kinase (PERK). PERK is part of the unfolded protein response pathway [53, 54], and becomes hyperphosphorylated upon hypoxic stress [55]. Thus, the rapid activation of PERK and subsequent phosphorylation of eIF2-α prevents the formation of an active ternary complex and suppresses the global initiation of mRNA translation in hypoxia. Consistent with the data mentioned before, eIF2-α phosphorylation shows a partial recovery after 4–8 hrs [33, 55].

Under prolonged hypoxic conditions, a disruption of the eIF4F complex can be observed. This is mediated by the sequestration of eIF4E by 4E-BPs, as well as translocation of eIF4E into the nucleus and processing (P-) bodies by the 4E-T transporter protein [33]. The activity of 4E-BP can be modulated by its phosphorylation status, which is controlled by the mammalian target of rapamycin (mTOR). The serine/threonine kinase mTOR is central to the control of translation in response to stress and nutrient deprivation [56, 57]. Hypoxia has been demonstrated to inhibit mTOR and thus to promote increased binding of 4E-BP to eIF4E, thereby preventing its association with eIF4G, resulting in an inhibition of cap-dependent translation [58, 59]. Knock-down of 4E-BP1 by short hairpin interfering RNA in U87 cancer cells was associated with a three-fold drop in steady state ATP concentrations [60]. Thus, regulating the rates of protein synthesis *via* the control of cap-dependent, eIF4F-driven mRNA translation is required to facilitate energy conservation and to gain hypoxia tolerance [7]. It was demonstrated that 4E-BP1 knock-down does not affect the inhibition of global mRNA translation. However, during both normoxic and hypoxic conditions, the absence of 4E-BP1 stimulated the synthesis of specific proteins that are involved *e.g.* in cytoskeletal organization [61].

Besides 4E-BP1, mTOR triggers phosphorylation of the ribosomal protein S6-kinase (S6K). Activation of S6K by mTOR under normal conditions leads to an increased activity of the elongation factor 2 [62] and the ribosomal protein S6 [56], thus enhancing translational efficiency. Several stressors are known to affect mTOR activity [63]. Among them is a loss in ATP, activation of the PI3K-Akt (alias protein kinase B) pathway, or the cytosolic enzyme AMPK [64]. In hypoxia, however, mTOR activity does not seem to be dominantly affected by decreased ATP levels, or the PI3K/Akt pathway [58, 59]. However, a downstream target of the PI3K/Akt pathway, the tuberous sclerosis complex 1 and 2 (TSC1 [hamartin], TSC2 [tuberin]), seems to be crucial. Currently at least two effectors have been described to affect mTOR activity in hypoxia: (1) Phosphorylation of TSC2 by AMPK [31, 65, 66], or raptor, which in turn influences TSC1 [67]. (2) It was shown that down-regulation of mTOR activity by hypoxia requires *de novo* mRNA synthesis and correlates with increased expression of the hypoxia-inducible gene REDD1 (for regulated in development and DNA damage responses) [59]. The latter finding supports the assumption that oxygen-dependent/ATP-independent alteration of the gene expression program modulates mRNA translation in prolonged hypoxia. Moreover, this is in line with several studies linking AMPK and PI3K/Akt pathways to changes in the hypoxic transcriptional response [1]. AMPK is further involved in eEF2 phosphorylation and thus in translational inhibition at the level of elongation [68].

However, alterations in mTOR activity alone are not sufficient to assume a measurable change in overall mRNA translation [54], thus probably additional mechanisms are required for the activation of specific mRNA translation. The global inhibition of protein synthesis under prolonged hypoxic conditions may be further linked to a PERK-independent phosphorylation of eIF2-α. It was shown that in moderate hypoxia eIF2-α phosphorylation is required for translational repression under long-term conditions, which, however, was PERK independent [36]. Thus, eIF2-α phosphorylation appears to be crucial for the short term as well as long-term suppression of global mRNA translation, whereas mTOR-mediated disruption of the eIF4F complex is likely to cause activation of mRNA specific translation (Fig. 2). In conclusion, hypoxia inhibits mRNA translation at different target points. Under short-term conditions, hypoxia affects translational initiation (*via* PERK $>$ eIF2-α), while under long-term conditions at least three processes are involved: (1) REDD1 $>$ mTOR $>$ S6K/4E-BP; (2) AMPK $>$ mTOR $>$ S6K/4E-BP; (3) PERK independent $>$ eIF2-α, as well as translational elongation (*via* AMPK $>$ eEF2K $>$ eEF2).

Regulation of specific mRNA translation in hypoxia

In the past, the protein synthesis machinery was viewed as a static entity, but it has become clear that its activity is highly regulated. The view of a constant 1:1 ratio of induced/repressed mRNA to protein levels is simply wrong, as shown in diverse studies (*e.g.* [69–75]).

Post-transcriptional control is mainly attributed to the mRNA 5′ and 3′ UTRs. *Trans*-factors like RNA-BPs interact with specific mRNA *cis*-elements to form ribonucleoprotein-complexes (RNPs). This occurs co-transcriptionally (mediating mRNA processing and nuclear export), and shows a high dynamic by factor exchange in the cytoplasm. Another group of *trans*-factors are miRNAs. miRNAs are 21–23 oligo-nucleotide RNAs that regulate the function of mRNAs, mainly by mRNA destabilization or by inhibiting their translation [76–80]. Both, RNA-BPs and miRNAs, modulate the fate of the transcripts at the level of mRNA stability, localization or translational efficiency [1].

Subgroups of genes involved in similar cellular processes are supposed to be regulated by similar *cis*-element/*trans*-factor

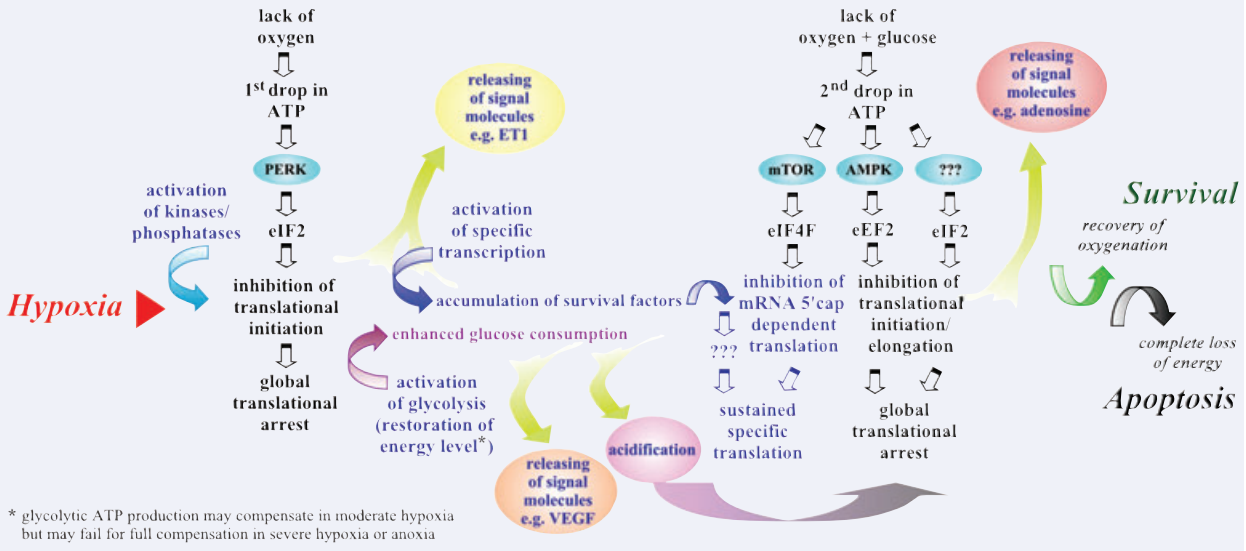

Fig. 2 Simplified model of cellular adaptation in moderate hypoxia and its influence on mRNA translation. Upon rapid suppression of global protein synthesis by PERK-mediated inhibition of translational initiation, cells respond to hypoxia by activation of glycolysis. Restored ATP levels ensure the expression of survival factors, which may be released such as signal molecules (*e.g.* endothelin-1, vascular endothelial growth factor or adenosine). Furthermore, newly synthesized factors can be involved in glucose uptake, glycolysis, preventing acidosis, or represent factors affecting mRNA specific translation. Under prolonged hypoxic conditions glucose may be consumed to a large extent, which causes a second drop in ATP levels. A combined action of AMPK and mTOR, as well as unknown factors cause a global translational arrest accompanied by sustained specific mRNA translation.

combinations. This is also the case for post-transcriptional control of gene expression during the hypoxic response. For instance, a hypoxia-inducible RNA-BP was found to interact with a number of genes, which show an increased expression rate under hypometabolic conditions, including erythropoietin, inducible nitric oxide synthases, tyrosine hydroxylase and tumour necrosis factor-α [81]. Furthermore, a specific spectrum of miRNAs is induced or down-regulated during the hypoxic response [82–85]. This was designated as a miRNA signature of hypoxia [86].

Accordingly, a discrepancy was observed when comparing expression rates of short-lived reporter proteins [1], and polysomal gradient analysis (see Fig. 1), indicating the activation of further, probably mRNA-specific mechanisms of mRNA translation. The data indicate that although ribosomal assembling at mRNAs is impaired at 'free' cytosolic polysomes, the overall gene expression rate may revert to normal in moderate hypoxia. This implies an alternative route of mRNA translation, which, obviously, does not take place in the cytoplasm. As it was shown that distinct areas of mRNA translation are not negatively influenced by hypoxia [87], it has been suggested that a subset of mRNAs can undergo regulation of local mRNA translation [1]. For instance, it has been proposed that the endoplasmatic reticulum can undergo functional specialization contributing to a higher order spatial compartmentalization and organization of mRNA translation [88]. Membrane-bound polysomes are highly structured, which could promote alternative routes of translational initiation and/or ribosome recycling [89]. Therefore, membrane-bound ribosomes may be a 'privileged' site for protein synthesis during cell stress [90]. Accordingly, it has been suggested that inhibition of eIF2-α function, *via* induction of the unfolded protein response, also results in a compartmentalization of protein synthesis to the endoplasmatic reticulum [91]. However, a direct analysis of local mRNA translation in hypoxia has not yet been achieved.

Another way of how specific subsets of mRNAs may be selected for translation is by sorting at RNA-granules like stress granules (SGs). SGs are rapidly induced in response to environmental stress [92]. Selective recruitment of specific mRNAs into SGs is thought to regulate their stability and rate of translation [93]. It was shown that phosphorylation of eIF2-α is essential for SG assembly [94, 95]. Although a direct role of SGs in hypoxia is less well investigated, these findings would provide an option for mRNA selection for sustained translation.

One would expect that either specific *cis*-elements (mRNA secondary structures, binding motifs) or *trans*-factors can 'label' hypoxia-sensitive mRNAs as such. Moreover, since pre-mRNAs are co-transcriptionally assembled with RNA-BPs, these factors in turn may modulate mRNA secondary structures or occupy *cis*-elements. Although several *cis*-elements and *trans*-factors have been identified to participate in mRNA-specific translation under hypoxic conditions (see: [1]), no common mechanism, which would explain the selective translation of hypoxia-sensitive genes has yet been identified. However, as shown above, inhibition of mTOR may cause activation of mRNA-specific translation. In this context, one hypothesis is that under conditions of cap-dependent inhibition of mRNA translation, the recruitment of the ribosomal subunits occurs at internal ribosome entry sites (IRES). The

existence/function of IRES elements is currently under debate (see: [1]). Briefly, not all mRNAs, which contain IRES elements within their mRNA 5′UTRs are translationally inducible in hypoxia, and not all mRNAs inducible in hypoxia contain IRES elements. This, however, may be attributed to the fact that at present, IRES elements are defined solely through functional criteria and cannot yet be predicted by the presence of characteristic RNA sequences or structural motifs [96]. On the other hand, it has been proposed that RNA-BPs, designated as IRES *trans*-acting factors, can function as RNA chaperones, allowing for direct binding of the small ribosomal subunit through modification of mRNA secondary and/or tertiary structure. Furthermore, they may function as adaptor proteins that facilitate RNA-ribosome interaction [97]. Together with other findings, linking both an alteration in the binding behaviour of RNA-BPs/miRNAs to UTRs with subsequent activation of mRNA translation in hypoxia, a *trans*-factor mediated change in the ribosomal assembling at the mRNA translation initiation site can be proposed. This would be in line with the 'cumulative specificity hypothesis', postulating that the correct translation initiation site is recognized by the ribosome through its unique accessibility [98, 99]. In the view that mRNPs are substrates, recognized by the enzymatic complex, the ribosome, translational initiation may attribute to the accessibility of the initiation site. Thus, specific *trans*-factor/*cis*-element interactions, as a result of newly synthesized factors in prolonged hypoxia, may provide differences in the accessibility of mRNAs for the ribosome. This is supported by findings that poly-A leader sequences in mRNA constructs promote the recruitment of the 40S ribosomal subunit and the efficient formation of initiation complexes at cognate AUG initiator codons in the absence *e.g.* of eIF4F [100]. At least poly-A leader sequences as well as IRES elements are located in close proximity to the translation initiation site and promote cap-independent mRNA translation. This is in line with the view that translational initiation can be controlled by elements and factors surrounding the initiation site and making it accessible. In this context, an alteration of the translational machinery during the hypoxic response may be taken into account. This in turn would cause an increased selectivity for mRNA subgroups [1].

Can an alteration of the translational machinery cause a specific change in gene expression?

Generally spoken, yes it can, although only few examples have so far been described in hypoxia. One example is the mTOR-mediated hypophosphorylation of S6K in hypoxia, which in turn causes hypoxic deactivation of the ribosomal protein S6 (rpS6) and the elongation factor 2 (as mentioned above). It should be noted that the functional role of S6K remains somewhat unclear [45]. As shown in a knock-out model, neither a clear phenotype (except reduced size), nor the previously assumed role in the regulation of 5′ terminal oligopyrimidine sequence containing mRNAs (mainly present in genes encoding ribosomal proteins) could be verified [101]. But, accumulating evidence suggests that S6K modulates the functions of translation initiation factors during protein synthesis. S6K is thought to coordinate the regulation of ribosome biogenesis as well, which in turn drives efficient translation [57, 102].

Beside hypoxia, alterations in the translational machinery are currently being investigated in tumours, which usually contain poor oxygenation [103]. It was shown that an altered ribosome biogenesis is associated with increased tumour susceptibility. This might be explained by intrinsic ribosomal defects, leading to alterations in the rate of translation of specific mRNAs important for tumorigenesis [104]. In turn, several proto-oncogenes and tumour suppressors have been shown to directly regulate ribosome production or the initiation of mRNA translation, or both [105]. Furthermore, it is evident that gene mutations encoding components of the translational machinery can give rise to a wide spectrum of diseases, including X-linked dyskeratosis, Diamond–Blackfan anaemia, Cartilage-hair hypoplasia, Shwachman–Diamond syndrome Treacher–Collins syndrome or myopathy [12, 104]. This includes mutations in specific mRNAs that influence their rate of translation, mutations that affect components of the translational machinery and alterations in translation factors. Interestingly, there is no 'single' translational phenotype. However, some organs seem to be affected more frequently than others. For example, brain and muscle are commonly affected in diseases with defects in mitochondrial translation, an observation that has been attributed to the high energy requirements of muscle cells and neurons, although a diverse range of tissues and organs are affected in these diseases as a whole [12]. Furthermore, defects in the overall fidelity of protein synthesis led to severe neurodegeneration and ataxia in mice [106]. Moreover, accelerated protein synthesis causes cardiac hypertrophy [107, 108], and translational deregulation is involved in the development of diabetic nephropathy [109]. Finally, in yeast, a very large diversity of ribosomes was found, showing functional specificity [110]. In turn, this may lead to calibrated translation of specific mRNAs, and offers a new level of control of gene expression. Thus, ribosomes are not simply translation machines, but may also be regulatory elements that can selectively influence or 'filter' the translation of various mRNAs [111]. The potential role of an alteration in ribosomal biogenesis during hypoxia remains to be elucidated.

Altogether, many open questions remain on how selective gene expression can take place in both, severe and moderate hypoxia. But there are also many ideas on how it could work, which need to be verified.

Acknowledgements

I thank Bernd J. Thiele, Holger Scholz, Bert Flemming, Andreas Steege, Andreas Patzak, Pontus B. Persson and Anja Bondke for stimulating discussion. I apologize to all authors whose excellent papers could not be cited due to space limitation. I acknowledge financial support by Deutsche Forschungsgemeinschaft (DFG) (FA 845/2–1).

References

1. **Fähling M.** Cellular oxygen sensing, signalling and how to survive translational arrest in hypoxia. *Acta Physiol*. 2009; 195: 205–30.
2. **Harris AL.** Hypoxia – a key regulatory factor in tumour growth. *Nat Rev Cancer*. 2002; 2: 38–47.
3. **Lopez-Barneo J, del Toro R, Levitsky KL, *et al.*** Regulation of oxygen sensing by ion channels. *J Appl Physiol*. 2004; 96: 1187–95.
4. **Vaupel P, Hockel M, Mayer A.** Detection and characterization of tumor hypoxia using pO2 histography. *Antioxid Redox Signal*. 2007; 9: 1221–35.
5. **Giaccia AJ, Simon MC, Johnson R.** The biology of hypoxia: the role of oxygen sensing in development, normal function, and disease. *Genes Dev*. 2004; 18: 2183–94.
6. **Storey KB, Storey JM.** Metabolic rate depression in animals: transcriptional and translational controls. *Biol Rev Camb Philos Soc*. 2004; 79: 207–33.
7. **Ebbesen P, Pettersen EO, Gorr TA, *et al.*** Taking advantage of tumor cell adaptations to hypoxia for developing new tumor markers and treatment strategies. *J Enzyme Inhib Med Chem*. 2009; 24: 1–39.
8. **Rolfe DF, Brown GC.** Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev*. 1997; 77: 731–58.
9. **Hochachka PW, Buck LT, Doll CJ, *et al.*** Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proc Natl Acad Sci USA*. 1996; 93: 9493–8.
10. **Chung FZ, Weber HW, Appleman MM.** Extensive but reversible depletion of ATP via adenylate cyclase in rat adipocytes. *Proc Natl Acad Sci USA*. 1985; 82: 1614–7.
11. **Holz MK, Ballif BA, Gygi SP, *et al.*** mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell*. 2005; 123: 569–80.
12. **Scheper GC, van der Knaap MS, Proud CG.** Translation matters: protein synthesis defects in inherited disease. *Nat Rev Genet*. 2007; 8: 711–23.
13. **Storey KB, Hochachka PW.** Enzymes of energy metabolism from a vertebrate facultative anaerobe, Pseudemys scripta. Turtle heart phosphofructokinase. *J Biol Chem*. 1974; 249: 1417–22.
14. **Hochachka PW.** Defense strategies against hypoxia and hypothermia. *Science*. 1986; 231: 234–41.
15. **Lutz PL.** Mechanisms for anoxic survival in the vertebrate brain. *Annu Rev Physiol*. 1992; 54: 601–18.
16. **Storey KB.** Metabolic adaptations supporting anoxia tolerance in reptiles: recent advances. *Comp Biochem Physiol B Biochem Mol Biol*. 1996; 113: 23–35.
17. **Hand SC.** Oxygen, pHi and arrest of biosynthesis in brine shrimp embryos. *Acta Physiol Scand*. 1997; 161: 543–51.
18. **Ibarguren I, Diaz-Enrich MJ, Cao J, *et al.*** Regulation of the futile cycle of fructose phosphate in sea mussel. *Comp Biochem Physiol B Biochem Mol Biol*. 2000; 126: 495–501.
19. **Boutilier RG.** Mechanisms of metabolic defense against hypoxia in hibernating frogs. *Respir Physiol*. 2001; 128: 365–77.
20. **Larade K, Storey KB.** Reversible suppression of protein synthesis in concert with polysome disaggregation during anoxia exposure in Littorina littorea. *Mol Cell Biochem*. 2002; 232: 121–7.
21. **Tinton S, Calderon PB.** Role of protein phosphorylation in the inhibition of protein synthesis caused by hypoxia in rat hepatocytes. *Int J Toxicol*. 2001; 20: 21–7.
22. **Guppy M, Withers P.** Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol Rev Camb Philos Soc*. 1999; 74: 1–40.
23. **Pettersen EO, Juul NO, Ronning OW.** Regulation of protein metabolism of human cells during and after acute hypoxia. *Cancer Res*. 1986; 46: 4346–51.
24. **Storey KB.** Mammalian hibernation. Transcriptional and translational controls. *Adv Exp Med Biol*. 2003; 543: 21–38.
25. **DeGracia DJ, Kumar R, Owen CR, *et al.*** Molecular pathways of protein synthesis inhibition during brain reperfusion: implications for neuronal survival or death. *J Cereb Blood Flow Metab*. 2002; 22: 127–41.
26. **Anderson LL, Mao X, Scott BA, *et al.*** Survival from hypoxia in C. elegans by inactivation of aminoacyl-tRNA synthetases. *Science*. 2009; 323: 630–3.
27. **Gladden LB.** Lactate metabolism: a new paradigm for the third millennium. *J Physiol*. 2004; 558: 5–30.
28. **Kuznetsov AV, Janakiraman M, Margreiter R, *et al.*** Regulating cell survival by controlling cellular energy production: novel functions for ancient signaling pathways? *FEBS Lett*. 2004; 577: 1–4.
29. **Mekhail K, Rivero-Lopez L, Khacho M, *et al.*** Restriction of rRNA synthesis by VHL maintains energy equilibrium under hypoxia. *Cell Cycle*. 2006; 5: 2401–13.
30. **Gebauer F, Hentze MW.** Molecular mechanisms of translational control. *Nat Rev Mol Cell Biol*. 2004; 5: 827–35.
31. **Liu L, Cash TP, Jones RG, *et al.*** Hypoxia-induced energy stress regulates mRNA translation and cell growth. *Mol Cell*. 2006; 21: 521–31.
32. **Thomas JD, Johannes GJ.** Identification of mRNAs that continue to associate with polysomes during hypoxia. *RNA*. 2007; 13: 1116–31.
33. **Koritzinsky M, Magagnin MG, van den Beucken T, *et al.*** Gene expression during acute and prolonged hypoxia is regulated by distinct mechanisms of translational control. *EMBO J*. 2006; 25: 1114–25.
34. **Land SC, Hochachka PW.** Protein turnover during metabolic arrest in turtle hepatocytes: role and energy dependence of proteolysis. *Am J Physiol*. 1994; 266: C1028–36.
35. **Hand SC.** Quiescence in Artemia fran*cis*cana embryos: reversible arrest of metabolism and gene expression at low oxygen levels. *J Exp Biol*. 1998; 201: 1233–42.
36. **Thomas JD, Dias LM, Johannes GJ.** Translational repression during chronic hypoxia is dependent on glucose levels. *RNA*. 2008; 14: 771–81.
37. **Fähling M, Steege A, Mrowka R, *et al.*** Rate of protein synthesis under hypometabolic conditions: the down and up and down. *FASEB J*. 2008; 22: 1174.
38. **van den Beucken T, Koritzinsky M, Wouters BG.** Translational control of gene expression during hypoxia. *Cancer Biol Ther*. 2006; 5: 749–55.
39. **Ingwall JS.** Energy metabolism in heart failure and remodelling. *Cardiovasc Res*. 2009; 81: 412–9.
40. **Iyer NV, Kotch LE, Agani F, *et al.*** Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev*. 1998; 12: 149–62.
41. **Clemens MJ, Bommer UA.** Translational control: the cancer connection. *Int J Biochem Cell Biol*. 1999; 31: 1–23.
42. **Preiss T, Hentze MW.** From factors to mechanisms: translation and translational

control in eukaryotes. *Curr Opin Genet Dev*. 1999; 9: 515–21.
43. **Dever TE.** Gene-specific regulation by general translation factors. *Cell*. 2002; 108: 545–56.
44. **Ramakrishnan V.** Ribosome structure and the mechanism of translation. *Cell*. 2002; 108: 557–72.
45. **Proud CG.** Signalling to translation: how signal transduction pathways control the protein synthetic machinery. *Biochem J*. 2007; 403: 217–34.
46. **Steitz TA.** A structural understanding of the dynamic ribosome machine. *Nat Rev Mol Cell Biol*. 2008; 9: 242–53.
47. **Zaher HS, Green R.** Fidelity at the molecular level: lessons from protein synthesis. *Cell*. 2009; 136: 746–62.
48. **Kozak M.** Initiation of translation in prokaryotes and eukaryotes. *Gene*. 1999; 234: 187–208.
49. **Sonenberg N, Hinnebusch AG.** New modes of translational control in development, behavior, and disease. *Mol Cell*. 2007; 28: 721–9.
50. **Preiss T, Hentze W.** Starting the protein synthesis machine: eukaryotic translation initiation. *Bioessays*. 2003; 25: 1201–11.
51. **Blais JD, Filipenko V, Bi M, *et al.*** Activating transcription factor 4 is translationally regulated by hypoxic stress. *Mol Cell Biol*. 2004; 24: 7469–82.
52. **Kimball SR.** Eukaryotic initiation factor eIF2. *Int J Biochem Cell Biol*. 1999; 31: 25–9.
53. **Harding HP, Zhang Y, Ron D.** Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature*. 1999; 397: 271–4.
54. **Koumenis C, Wouters BG.** "Translating" tumor hypoxia: unfolded protein response (UPR)-dependent and UPR-independent pathways. *Mol Cancer Res*. 2006; 4: 423–36.
55. **Koumenis C, Naczki C, Koritzinsky M, *et al.*** Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2alpha. *Mol Cell Biol*. 2002; 22: 7405–16.
56. **Gingras AC, Raught B, Sonenberg N.** Regulation of translation initiation by FRAP/mTOR. *Genes Dev*. 2001; 15: 807–26.
57. **Ma XM, Blenis J.** Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol*. 2009; 10: 307–18.
58. **Arsham AM, Howell JJ, Simon MC.** A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. *J Biol Chem*. 2003; 278: 29655–60.
59. **Brugarolas J, Lei K, Hurley RL, *et al.*** Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev*. 2004; 18: 2893–904.
60. **Dubois L, Magagnin MG, Cleven AH, *et al.*** Inhibition of 4E-BP1 sensitizes U87 glioblastoma xenograft tumors to irradiation by decreasing hypoxia tolerance. *Int J Radiat Oncol Biol Phys*. 2009; 73: 1219–27.
61. **Magagnin MG, van den Beucken T, Sergeant K, *et al.*** The mTOR target 4E-BP1 contributes to differential protein expression during normoxia and hypoxia through changes in mRNA translation efficiency. *Proteomics*. 2008; 8: 1019–28.
62. **Wang X, Li W, Williams M, *et al.*** Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. *EMBO J*. 2001; 20: 4370–9.
63. **Reiling JH, Sabatini DM.** Stress and mTORture signaling. *Oncogene*. 2006; 25: 6373–83.
64. **Wouters BG, Koritzinsky M.** Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nat Rev Cancer*. 2008; 8: 851–64.
65. **Inoki K, Li Y, Xu T, Guan KL.** Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev*. 2003; 17: 1829–34.
66. **Borger DR, Gavrilescu LC, Bucur MC, *et al.*** AMP-activated protein kinase is essential for survival in chronic hypoxia. *Biochem Biophys Res Commun*. 2008; 370: 230–4.
67. **Gwinn DM, Shackelford DB, Egan DF, *et al.*** AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*. 2008; 30: 214–26.
68. **Horman S, Browne G, Krause U, *et al.*** Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. *Curr Biol*. 2002; 12: 1419–23.
69. **Chen G, Gharib TG, Huang CC, *et al.*** Discordant protein and mRNA expression in lung adenocarcinomas. *Mol Cell Proteomics*. 2002; 1: 304–13.
70. **Gygi SP, Rochon Y, Franza BR, Aebersold R.** Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol*. 1999; 19: 1720–30.
71. **Fähling M, Steege A, Perlewitz A, *et al.*** Role of nucleolin in posttranscriptional control of MMP-9 expression. *Biochim Biophys Acta*. 2005; 1731: 32–40.
72. **Fähling M, Mrowka R, Steege A, *et al.*** Translational control of collagen prolyl 4-hydroxylase-alpha(I) gene expression under hypoxia. *J Biol Chem*. 2006; 281: 26089–101.
73. **Fähling M, Mrowka R, Steege A, *et al.*** Translational regulation of the human achaete-scute homologue-1 by fragile X mental retardation protein. *J Biol Chem*. 2009; 284: 4255–66.
74. **Ufer C, Wang CC, Fähling M, *et al.*** Translational regulation of glutathione peroxidase 4 expression through guanine-rich sequence-binding factor 1 is essential for embryonic brain development. *Genes Dev*. 2008; 22: 1838–50.
75. **Koritzinsky M, Seigneuric R, Magagnin MG, *et al.*** The hypoxic proteome is influenced by gene-specific changes in mRNA translation. *Radiother Oncol*. 2005; 76: 177–86.
76. **Bartel DP.** MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004; 116: 281–97.
77. **Dalmay T, Edwards DR.** MicroRNAs and the hallmarks of cancer. *Oncogene*. 2006; 25: 6170–5.
78. **Chu CY, Rana TM.** Small RNAs: regulators and guardians of the genome. *J Cell Physiol*. 2007; 213: 412–9.
79. **Meister G.** miRNAs get an early start on translational silencing. *Cell*. 2007; 131: 25–8.
80. **Filipowicz W, Bhattacharyya SN, Sonenberg N.** Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet*. 2008; 9: 102–14.
81. **Czyzyk-Krzeska MF, Beresh JE.** Characterization of the hypoxia-inducible protein binding site within the pyrimidine-rich tract in the 3′-untranslated region of the tyrosine hydroxylase mRNA. *J Biol Chem*. 1996; 271: 3293–9.
82. **Donker RB, Mouillet JF, Nelson DM, Sadovsky Y.** The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. *Mol Hum Reprod*. 2007; 13: 273–9.
83. **Kulshreshtha R, Ferracin M, Negrini M, *et al.*** Regulation of microRNA expression: the hypoxic component. *Cell Cycle*. 2007; 6: 1426–31.
84. **Fasanaro P, D'Alessandra Y, Di S, *et al.*** MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. *J Biol Chem*. 2008; 283: 15878–83.

85. **Giannakakis A, Sandaltzopoulos R, Greshock J, *et al.*** miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. *Cancer Biol Ther*. 2008; 7: 255–64.
86. **Kulshreshtha R, Ferracin M, Wojcik SE, *et al.*** A microRNA signature of hypoxia. *Mol Cell Biol*. 2007; 27: 1859–67.
87. **Oh N, Kim KM, Choe J, Kim YK.** Pioneer round of translation mediated by nuclear cap-binding proteins CBP80/20 occurs during prolonged hypoxia. *FEBS Lett*. 2007; 581: 5158–64.
88. **Levine T, Rabouille C.** Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. *Curr Opin Cell Biol*. 2005; 17: 362–8.
89. **Christensen AK, Kahn LE, Bourne CM.** Circular polysomes predominate on the rough endoplasmic reticulum of somatotropes and mammotropes in the rat anterior pituitary. *Am J Anat*. 1987; 178: 1–10.
90. **Lerner RS, Nicchitta CV.** mRNA translation is compartmentalized to the endoplasmic reticulum following physiological inhibition of cap-dependent translation. *RNA*. 2006; 12: 775–89.
91. **Stephens SB, Dodd RD, Brewer JW, *et al.*** Stable ribosome binding to the endoplasmic reticulum enables compartment-specific regulation of mRNA translation. *Mol Biol Cell*. 2005; 16: 5819–31.
92. **Anderson P, Kedersha N.** RNA granules. *J Cell Biol*. 2006; 172: 803–8.
93. **Anderson P, Kedersha N.** Stressful initiations. *J Cell Sci*. 2002; 115: 3227–34.
94. **Kedersha NL, Gupta M, Li W, *et al.*** RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. *J Cell Biol*. 1999; 147: 1431–42.
95. **McEwen E, Kedersha N, Song B, *et al.*** Heme-regulated inhibitor kinase-mediated phosphorylation of eukaryotic translation initiation factor 2 inhibits translation, induces stress granule formation, and mediates survival upon arsenite exposure. *J Biol Chem*. 2005; 280: 16925–33.
96. **Lopez-Lastra M, Rivas A, Barria MI.** Protein synthesis in eukaryotes: the growing biological relevance of cap-independent translation initiation. *Biol Res*. 2005; 38: 121–46.
97. **Graber TE, Holcik M.** Cap-independent regulation of gene expression in apoptosis. *Mol Biosyst*. 2007; 3: 825–34.
98. **Nakamoto T.** The initiation of eukaryotic and prokaryotic protein synthesis: a selective accessibility and multisubstrate enzyme reaction. *Gene*. 2007; 403: 1–5.
99. **Nakamoto T.** Evolution and the universality of the mechanism of initiation of protein synthesis. *Gene*. 2009; 432: 1–6.
100. **Shirokikh NE, Spirin AS.** Poly(A) leader of eukaryotic mRNA bypasses the dependence of translation on initiation factors. *Proc Natl Acad Sci USA*. 2008; 105: 10738–43.
101. **Pende M, Um SH, Mieulet V, *et al.*** S6K1(−/−)/S6K2(−/−) mice exhibit perinatal lethality and rapamycin-sensitive 5′-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. *Mol Cell Biol*. 2004; 24: 3112–24.
102. **Jastrzebski K, Hannan KM, Tchoubrieva EB, *et al.*** Coordinate regulation of ribosome biogenesis and function by the ribosomal protein S6 kinase, a key mediator of mTOR function. *Growth Factors*. 2007; 25: 209–26.
103. **Brown JM, Wilson WR.** Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer*. 2004; 4: 437–47.
104. **Montanaro L, Trere D, Derenzini M.** Nucleolus, ribosomes, and cancer. *Am J Pathol*. 2008; 173: 301–10.
105. **Ruggero D, Pandolfi PP.** Does the ribosome translate cancer? *Nat Rev Cancer*. 2003; 3: 179–92.
106. **Lee JW, Beebe K, Nangle LA, *et al.*** Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature*. 2006; 443: 50–55.
107. **Nagatomo Y, Carabello BA, Hamawaki M, *et al.*** Translational mechanisms accelerate the rate of protein synthesis during canine pressure-overload hypertrophy. *Am J Physiol*. 1999; 277: H2176–84.
108. **Hannan RD, Jenkins A, Jenkins AK, *et al.*** Cardiac hypertrophy: a matter of translation. *Clin Exp Pharmacol Physiol*. 2003; 30: 517–27.
109. **Kasinath BS, Mariappan MM, Sataranatarajan K, *et al.*** Novel mechanisms of protein synthesis in diabetic nephropathy–role of mRNA translation. *Rev Endocr Metab Disord*. 2008; 9: 255–66.
110. **Komili S, Farny NG, Roth FP, *et al.*** Functional specificity among ribosomal proteins regulates gene expression. *Cell*. 2007; 131: 557–71.
111. **Mauro VP, Edelman GM.** The ribosome filter hypothesis. *Proc Natl Acad Sci USA*. 2002; 99: 12031–6.