TRANSLATIONAL CONTROL IN STRESS AND APOPTOSIS

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Abstract | Cells respond to stress stimuli through coordinated changes in gene expression. The regulation of translation is often used under these circumstances because it allows immediate and selective changes in protein levels. There are many examples of translational control in response to stress. Here we examine two representative models, the regulation of eukaryotic initiation factor-2α by phosphorylation and internal ribosome initiation through the internal ribosome-entry site, which illustrate the importance of translational control in the cellular stress response and apoptosis.

ENDOPLASMIC RETICULUM (ER) STRESS
Perturbations of the ER function that are caused by the accumulation of misfolded proteins, oxidative stress, inhibition of glycosylation or alteration in Ca^{2+} homeostasis.

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The regulation of gene expression at the level of translation is an important, but still not completely understood, control mechanism. However, it is becoming increasingly evident that the regulation of translation provides the cell with the plasticity that is needed to respond to rapid changes in the environment. Many recent studies using comparative genomic and proteomic profiling of cells have documented a lack of correlation between the mRNA and protein levels of numerous genes[1–8]. This indicates that post-transcriptional control is more important in the regulation of gene expression than is often assumed. Translation is the final step in the flow of the genetic information, and regulation at this level allows for an immediate and rapid response to changes in physiological conditions. Such regulation is of increased importance under certain conditions — such as cellular stress (for example, heat shock, hypoxia, nutrient deprivation and ENDOPLASMIC RETICULUM (ER) STRESS) or apoptosis — that require immediate changes in protein levels. Given the considerable time lag that is associated with the synthesis, processing and export of *de novo* synthesized mRNA, the use of existing mRNAs by a controlled selective-translation mechanism is uniquely suited for this purpose.

Initiation of translation in eukaryotes

Translation is divided into three distinct phases: initiation, elongation and termination[9]. Although all three phases are subject to regulatory mechanisms, under most circumstances the rate-limiting step in translation (that is, initiation) is regulated. This mechanism presumably evolved because it is more effective to control the onset of a given biological process than to interrupt it later and, in the case of translation, to have to deal with the consequences of aberrant protein synthesis[9]. Translation initiation is a complex process that begins with the interaction of the cap-binding protein complex, eukaryotic initiation factor-4F (eIF4F), with the mRNA 5′-end cap structure (m^7GpppN; where N is any nucleotide). eIF4F comprises three subunits: eIF4E, which is the cap-binding protein; eIF4A, which is an RNA helicase; and the scaffolding protein eIF4G that bridges the mRNA and the ribosome through eIF3, which binds the ribosome directly[10] (BOX 1). The 40S ribosomal subunit with its associated initiation factors is thought to scan the 5′ untranslated region (UTR) until it recognizes the initiation codon AUG. Following the assembly of the 80S ribosome on the mRNA at the initiation codon, elongation of the polypeptide chain commences (for a detailed review on translation initiation, see REFS 10,11).

IRES-mediated translation initiation

A sizeable proportion of cellular mRNAs, perhaps as much as 3–5%, was shown to be translated by a cap-independent mechanism[12]. It is likely that most of these mRNAs contain an INTERNAL RIBOSOME-ENTRY SITE (IRES) in their 5′ UTR, as IRES-mediated translation is the only validated cap-independent translational mechanism[12–14].

Box 1 | **Initiation of translation**

The translation of eukaryotic mRNAs involves the recognition and recruitment of mRNAs by the translation-initiation machinery, and the assembly of the 80S ribosome on the mRNA. This process is mediated by proteins that are known as eukaryotic initiation factors (eIFs).

The formation of the 43S pre-initiation complex is predicated on the availability of a pool of dissociated ribosomal subunits, which is maintained with the aid of eIF3 and eIF1A factors (see figure). The 40S subunit, which associates with eIF3 and eIF1A, is then further bound by the ternary complex — consisting of eIF2, methionyl-initiator tRNA ($Met\text{-}tRNA_i^{Met}$) and GTP — to form a 43S pre-initiation complex. The assembly of the ternary complex is regulated by eIF2B[10].

The recognition of the m^7G cap structure at the 5′ end of the mRNA is mediated by the cap-binding protein eIF4E, which is part of the cap-binding complex eIF4F. This complex, which is responsible for the selection of mRNAs for translation, consists of eIF4E, eIF4A (an RNA helicase) and eIF4G (a scaffold protein). eIF4B and eIF4H promote the RNA helicase activity of eIF4A. The binding of the 43S pre-initiation complex to mRNA is assisted in mammals by the interaction between eIF3 and eIF4G. eIF3 is thought to recruit the 40S ribosome. Once the 40S ribosomal subunit is bound to the mRNA, it is thought to scan the mRNA in the 5′→3′ direction until it locates an initiation codon (most often AUG) in a favourable sequence context, where it is joined by the 60S ribosomal subunit to form an 80S initiation complex. The initiation factors that participate in translation are released after the formation of the 48S initiation complex and are recycled for another round of initiation. The release of eIFs is assisted by eIF5, which facilitates the hydrolysis of GTP carried by eIF2 and, hence, the dissociation of the 48S complex. eIF5B is required for the joining of the 60S subunit , at which point the polypeptide-elongation step of translation commences[10,21].

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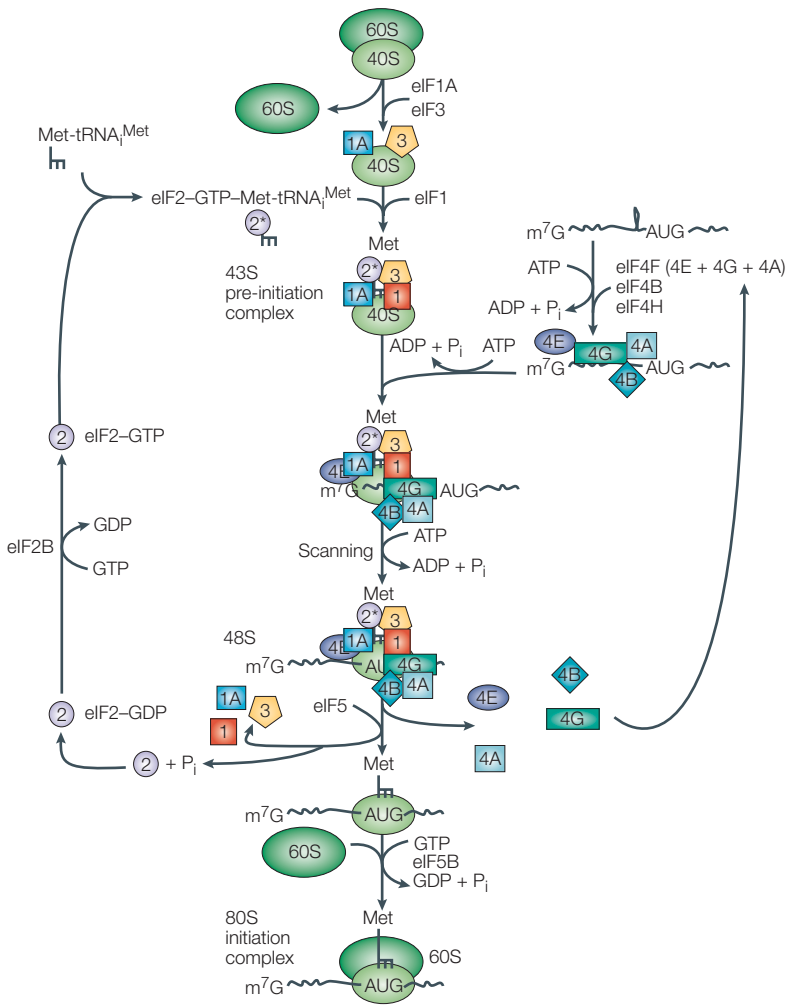


The IRES directly recruits ribosomes, thereby bypassing the requirement for the mRNA 5′ cap structure and eIF4E (FIG. 1). IRESs were initially discovered in picornaviruses, where they initiate the translation of viral RNAs that are naturally uncapped (and yet efficiently translated)[15,16]. Cellular IRESs have been described in a limited, but growing, number of mRNAs (for a list of cellular mRNAs with a reported IRES, see the IRES Database in Online links box).

Although the existence of IRESs is well documented and accepted for viral mRNAs, our understanding of the function and regulation of cellular IRESs is still evolving. They have been reported mostly in mRNAs that harbour long 5′ UTRs with a high GC content and an extensive predicted secondary structure. However, there are no common discernable features in a 5′ UTR that indicate the presence of an IRES. The detection of IRESs remains largely empirical and relies on the identification of IRES activity using several assays, including model BICISTRONIC mRNA constructs[13,15]. This approach, however, has been claimed to be compromised by the possible occurrence of cryptic promoters[17,18], splicing[19] or the composition and arrangement of reporter genes in the bicistronic construct[20]. Despite these limitations, there is growing evidence to support the existence of IRESs in cellular mRNAs (see below and also REF. 21).

Interestingly, many mRNAs that contain IRESs encode proteins that have important roles in cell growth and proliferation, differentiation and the regulation of apoptosis. This is, perhaps, not surprising given that these cellular processes require the strict control of gene expression. IRES-mediated translation provides a means for escaping the global decline in protein synthesis and allows the selective translation of specific mRNAs. This led to the idea that the selective regulation of IRES-mediated translation is important for the regulation of cell death and survival[22].

The precise molecular mechanism of cellular IRES-directed translation is not completely understood. Cellular IRESs are often found in long and structured 5′ UTRs, and are relatively inefficient in directing translation under physiological conditions that favour cap-dependent translation[23]. However, IRESs continue to function when cap-dependent translation is compromised[23]. This is because IRES translation is independent of the presence or integrity of several canonical initiation factors (primarily eIF4E)[13]. Efficient IRES-dependent translation requires auxiliary cellular proteins that are known as IRES *trans*-acting factors (ITAFs). Several ITAFs have been implicated in IRES-mediated translation, although the requirement for these proteins is not absolute and seems to be IRES specific[24]. The cellular ITAFs include polypyrimidine tract-binding protein (PTB)[25], La autoantigen[26,27], upstream of N-ras (UNR)[25], heterogeneous nuclear riboproteins C1 and C2 (hnRNPC1/C2)[28,29], p97/DAP5/NAT1 (a distant homologue of eIF4G)[30–32] and embryonic-lethal abnormal vision (ELAV/Hu)[33]. The mechanism of ITAF function is not fully understood, but it is generally believed that many ITAFs function as RNA CHAPERONES. For example, the La autoantigen possesses RNA chaperone activity[34]. The

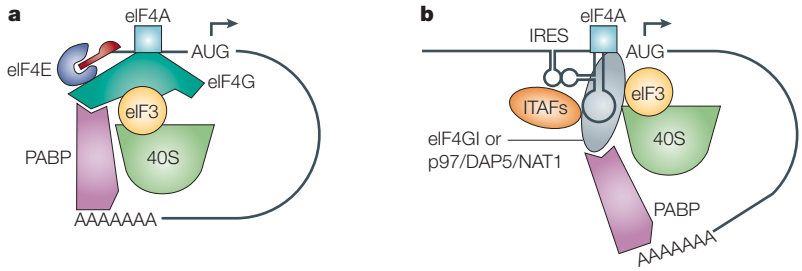


Figure 1 | **Cap-dependent versus internal ribosome-entry site-dependent translation initiation. a** | The cap-binding protein, eukaryotic initiation factor-4E (eIF4E; blue), binds to the 5′ m^7GpppN cap structure (red). The capped end of the mRNA is bridged to the 40S ribosomal subunit (green) by an adapter molecule eIF4G (dark green), which binds to eIF3 (yellow). eIF4A (cyan) is an RNA-dependent ATPase and RNA helicase, which is thought to be involved in the unwinding of the secondary structure of the mRNA 5′ untranslated region (UTR). Poly(A)-binding protein (PABP; pink) circularizes the mRNA through its interaction with both the 3′ UTR (through the poly(A) tail) and the 5′ UTR (through the interaction with eIF4G). **b** | Internal ribosome-entry site (IRES) *trans*-acting factors (ITAFs; orange) and proteolytic fragments of eIF4GI or p97/DAP5/NAT1, a distant homologue of eIF4G, (light grey) stimulate IRES translation. Only eIFs that are pertinent to this process are indicated, and individual components of the translation machinery are not drawn to scale. Adapted with permission from REF. 22 © (2000) Elsevier Science.

fact that various IRES elements require different ITAFs for their translation might explain the observed cell-type and tissue specificity of IRES translation[30,35], and indicates that distinct pathways might control IRES translation through the modulation of the activity or the amount of ITAFs.

Recently, a possible connection between eIF2α phosphorylation and IRES translation has been proposed (see below). The activity of several IRESs — such as those in the cationic amino-acid transporter (CAT1)[36], platelet-derived growth factor-2 (PDGF2), vascular endothelial growth factor (VEGF) and c-Myc[37] — was shown to increase during differentiation or various cellular stresses that increase the phosphorylation of eIF2α and reduce global protein synthesis. However, eIF2α phosphorylation did not correlate with increased activity of other IRESs, such as those in immunoglobulin-binding protein (BiP)/glucose-regulated protein of 78 kDa (GRP78), proto-oncogene serine/threonine-protein kinase (PIM1) or human inhibitor of apoptosis protein 2 (HIAP2) (REFS 36,38). The mechanism of the suggested regulation of IRES translation by eIF2α phosphorylation is not clear, as the phosphorylation of eIF2α inhibits the formation of the ternary complex that is required for initiation on all cellular mRNAs. Therefore, the increase in eIF2α phosphorylation is expected to reduce both cap-dependent and IRES-dependent translation.

Translational response to stress

Cells encounter a range of physiological and environmental stresses that require adaptive changes in gene expression. Stress conditions include ultraviolet (UV) irradiation, temperature changes, nutrient limitation, oxidative stress, hypoxia and exposure to various drugs or toxins. Exposure of cells to stress elicits adaptive responses that require the coordinated expression of stress-response genes, which affect cell survival, apoptosis, cell-cycle progression and differentiation[39].

Global translation is reduced in response to most, if not all, types of cellular stress. This results in a notable saving of cellular energy, which is mainly consumed in the process of translation (estimated as up to 50% of the cellular energy, depending on the organism[9,40,41]). The reduction in translation would also prevent the synthesis of unwanted proteins that could interfere with the cellular stress response. Remarkably, the stress-induced attenuation of global translation is often accompanied by a switch to the selective translation of proteins that are required for cell survival under stress[42,43]. Extensive research has been devoted to the identification of the mechanisms by which mRNAs translate under stress conditions. Much of this research has focused on the regulation of translation initiation at two steps: the formation or regeneration of the eIF2–methionyl-initiator tRNA (Met-$tRNA_i^{Met}$)–GTP ternary complex, and ribosome recruitment to the mRNA. Specific examples of both types of translational control are discussed below.

Availability of the ternary complex

The binding of Met-$tRNA_i^{Met}$ to the 40S ribosomal subunit is mediated by the ternary complex, which consists of eIF2–GTP and Met-$tRNA_i^{Met}$. The binding of GTP to eIF2 is the rate-limiting step in the assembly of the ternary complex. Many different types of stress — such as hypoxia, viral infection, amino-acid starvation and heat shock — reduce global translation by triggering the phosphorylation of the α-subunit of eIF2 at residue Ser51. This inhibits the exchange of GDP for GTP on the eIF2 complex (FIG. 2), which is catalysed by eIF2B, and thereby prevents the formation of the ternary complex[44–46]. As eIF2α is present in excess over eIF2B, even small changes in the phosphorylation of eIF2α have a notable effect on the formation of the ternary complex and translation. The phosphorylation of eIF2α reduces the translation of most mRNAs (as the ternary complex is absolutely required for most mRNAs with few exceptions). Paradoxically, however, eIF2α phosphorylation enhances the translation of a few selected mRNAs, which encode proteins that function in the adaptation to stress and the recovery of translation (see below)[44].

The phosphorylation of eIF2α is mediated by four distinct protein kinases — haem-regulated inhibitor kinase (HRI), protein kinase RNA (PKR), PKR-like endoplasmic reticulum (ER) kinase (PERK)) and general control non-derepressible-2 (GCN2) — which integrate diverse stress signals into a common pathway (FIG. 2). These kinases share homology in their kinase catalytic domains, but their effector domains are distinct and are subject to different regulatory mechanisms. HRI is activated under conditions of low haem or treatment with arsenite, osmotic or heat shock[47]. GCN2 is activated in response to amino-acid starvation[48] and UV irradiation[49,50]. PKR is activated by double-stranded RNA[51], whereas PERK is activated in response to ER stress[52]. Regardless of the type of stimulus, the phosphorylation of eIF2α causes identical effects on translation — the inhibition of translation, but the activation of selective translation (as discussed below).

INTERNAL RIBOSOME-ENTRY SITE
(IRES). A ribosome-binding site that is found in the 5′ UTR or in a coding region of a few cellular and viral RNAs. The IRES facilitates translation by recruiting ribosomes directly to the mRNA independently of the cap structure.

BICISTRONIC mRNA
Allows two different proteins to be translated from the same mRNA strand; the first protein is usually translated by a cap-dependent mechanism, whereas the second protein is translated through an IRES.

RNA CHAPERONE
An RNA-binding protein that aids the correct folding of a given RNA.

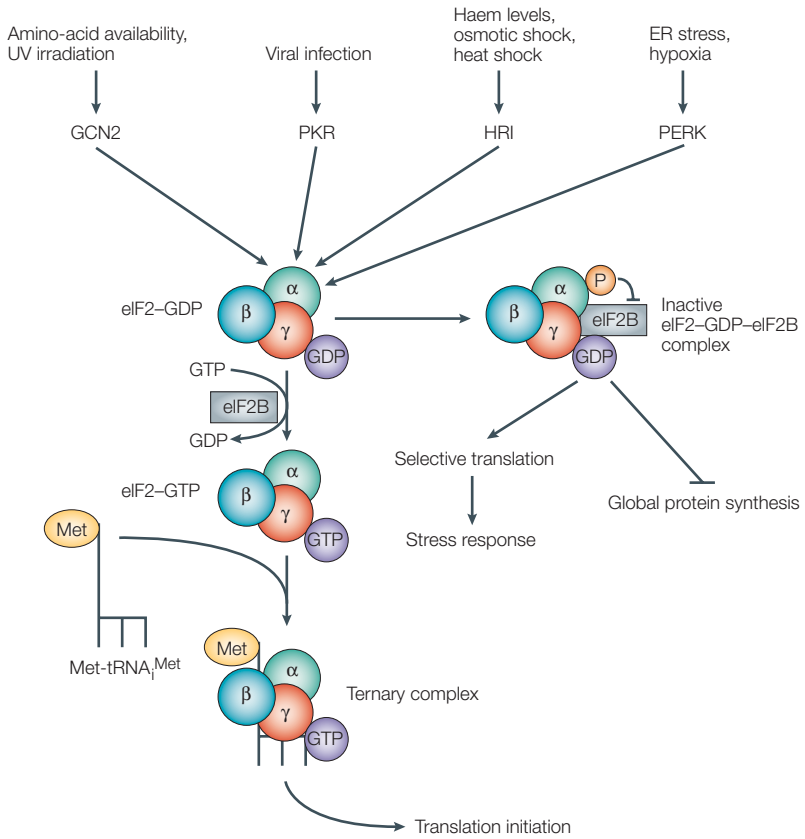


Figure 2 | **Integration of stress responses by the phosphorylation of eukaryotic initiation factor-2α.** Many stress conditions result in the phosphorylation of eukaryotic initiation factor-2α (eIF2α), which is accomplished by four distinct protein kinases: general control non-derepressible-2 (GCN2), protein kinase RNA (PKR), haem-regulated inhibitor kinase (HRI) and PKR-like ER kinase (PERK). eIF2α is a subunit of eIF2 (together with eIF2β and eIF2γ) that is part of the ternary complex. The ternary complex consists of eIF2, GTP and methionyl-initiator tRNA (Met-tRNA$_i^{Met}$), and delivers the initiator tRNA to the ribosome. As GTP is hydrolysed during translation initiation, eIF2 needs to be recharged following each round of initiation. This recharging (activation) is accomplished by GDP–GTP exchange, which is catalysed by eIF2B. Phosphorylation of eIF2α inhibits the GDP–GTP exchange by reducing the dissociation rate of eIF2B. Ultimately, this results in the inhibition of global translation. Selective translation of a subset of mRNAs continues, however, which allows cells to adapt to stress conditions. ER, endoplasmic reticulum UV, ultraviolet.

Selective translation mediated by eIF2α

GCN4 translation. The first and best-studied example of selective translation by the phosphorylation of eIF2α is that of the yeast transcriptional activator GCN4. The translational control of GCN4 was initially described in *Saccharomyces cerevisiae* in response to amino-acid starvation and serves as the model for this type of control[45,53,54] (FIG. 3a). Amino-acid starvation activates GCN2, which phosphorylates eIF2α and results in the inhibition of global translation. By contrast, the translation of *GCN4* mRNA is increased[44]. The 5′ UTR of GCN4 contains four UPSTREAM OPEN READING FRAMES (uORFs), which have an important role in the translational control of *GCN4* mRNA. When the level of phosphorylated eIF2α is low, the ribosome initiates at uORF1 and then reinitiates at uORF2, 3 or 4, but cannot reinitiate efficiently after termination at these latter ORFs. Consequently, ribosomes fail to reach the GCN4 start codon. However, at high levels of phosphorylated eIF2α many 40S ribosomal subunits fail to initiate at uORF2, 3 or 4, and instead scan through the leader to reach the GCN4 start codon. This is because a competent 43S ribosomal complex is formed more slowly after termination at the first uORF, as the concentration of the ternary complex is low. Therefore, the ability of the ribosome to reinitiate at either uORF2–4 or the GCN4 start codon is dictated by the level of phosphorylated eIF2α[44].

The unfolded protein response. In mammalian cells, the phosphorylation of eIF2α is important in the regulation of selective translation during ER stress and the UNFOLDED PROTEIN RESPONSE (UPR)[55]. The ER is the site of protein folding, as well as some post-translational modifications of proteins that are destined for the plasma membrane and organelles. As such, the ER is sensitive to perturbations in cellular homeostasis that are triggered by different types of stress, including Ca^{2+} depletion, glucose deprivation, hypoxia or protein misfolding, which result in the induction of the UPR[52]. The UPR is manifested by a reduction in global protein synthesis and a specific upregulation of stress-response proteins, such as activating transcription factor-4 (ATF4) and BiP/GRP78 (REF. 55).

An important function of the UPR is to reduce the demand on the protein-folding machinery to protect cells from ER stress. Indeed, the failure to alleviate ER stress leads to the activation of apoptotic pathways and, ultimately, cell death[55]. In fact, the ER has now been recognized as a third subcellular compartment (in addition to mitochondria and membrane-bound death receptors) in the control of apoptosis[56]. A recent report documented an ER-specific apoptotic pathway in which caspase-12 is activated in response to ER stress[57]. The activation of caspase-12 leads to the activation of downstream caspase-9 and caspase-3, which form a caspase cascade that is analogous to the mitochondria-induced pathway[58] (BOX 2). It is not clear, however, which caspase functions as an initiator during ER stress in human cells, as caspase-12 is nonfunctional in most, but not all, humans[59].

Activation of the UPR by eIF2α phosphorylation is mediated by the ER-transmembrane kinase PERK[60]. Generally, the activation of PERK is blocked by its interaction with the chaperone protein BiP. An increase in misfolded proteins in the ER causes the dissociation of BiP from PERK, which allows PERK oligomerization and autophosphorylation, followed by the phosphorylation of eIF2α[61]. The phosphorylation of eIF2α by PERK as part of the UPR attenuates the translation of most mRNAs. However, the translation of transcription factor *ATF4* mRNA is enhanced under these conditions[62]. This upregulation is crucial for the UPR, in that ATF4 transcriptionally activates UPR-responsive genes, which encode proteins that ameliorate the ER stress[43,63]. The 5′ UTR of mammalian ATF4 contains two uORFs, one of which (uORF2) overlaps with the ATF4 ORF and so inhibits the translation of ATF4. When the ternary complex is abundant (that is, when levels of eIF2α phosphorylation are low), the ribosome translates uORF2. By contrast, at reduced levels of the ternary complex

UPSTREAM OPEN READING FRAME (uORF). A short reading frame that is located in the 5′ UTR of some mRNAs. Certain uORFs code for short polypeptides, whereas others are non-coding.

UNFOLDED PROTEIN RESPONSE (UPR). A coordinated adaptive programme that is triggered by ER stress. The UPR leads to the inhibition of global protein synthesis, and the selective transcription and translation of proteins, which helps the cell to deal with ER stress.

(which is due to high levels of eIF2α phosphorylation) the ribosome scans through uORF2 and initiates at the ATF4 initiation codon[64,65] (FIG. 3b).

eIF2α phosphorylation and human disease

The physiological relevance of eIF2α phosphorylation for the control of translation has been shown using *eif2α*-mutated[66] and *Perk*-deleted[67,68] mice. These studies showed that the phosphorylation of eIF2α influences glucose metabolism *in vivo* and implied that several eIF2α kinases might be involved in this process. *Perk*-knockout animals had defects in the ER of pancreatic acinar cells, developed hyperglycaemia and suffered a loss of pancreatic β cells[67,68]. They also had skeletal dysplasias and postnatal growth retardation, which was probably caused by defective ER function of the osteoblasts[68]. Similarly, mice that harboured an *S51A* mutation in *eif2α* had a severely reduced number of pancreatic β cells and, in contrast to *Perk*-null animals, developed fatal hypoglycaemia shortly after birth[66]. The fact that *Perk*-null mice develop less severe symptoms indicates that other eIF2α kinases can, at least partially, compensate for the absence of PERK.

It was proposed that blood glucose levels could influence the folding status of proteins in the ER and signal, through PERK and eIF2α, to cause a reduction in protein synthesis[66,69]. A reduction in the amount of available glucose would result in a decrease in energy supply and a subsequent decrease in protein folding. The accumulation of misfolded proteins would then lead to the activation of PERK, the phosphorylation of eIF2α and the reduction of protein synthesis[69]. It is possible that the selective translation of specific genes that encode survival proteins under conditions of elevated eIF2α phosphorylation is necessary for the survival of pancreatic β cells and osteoblasts that otherwise would die by apoptosis. However, the downstream targets of this pathway remain to be defined. Also, why pancreatic β cells and osteoblasts are sensitive to the loss of PERK or eIF2α phosphorylation remains to be determined. Importantly, several human families have been identified that suffer from an autosomal-recessive condition that is known as Wolcott–Rallison syndrome, which is characterized by infancy-onset diabetes accompanied by skeletal defects and growth retardation, and is caused by a loss-of-function mutation in the *PERK* gene[70].

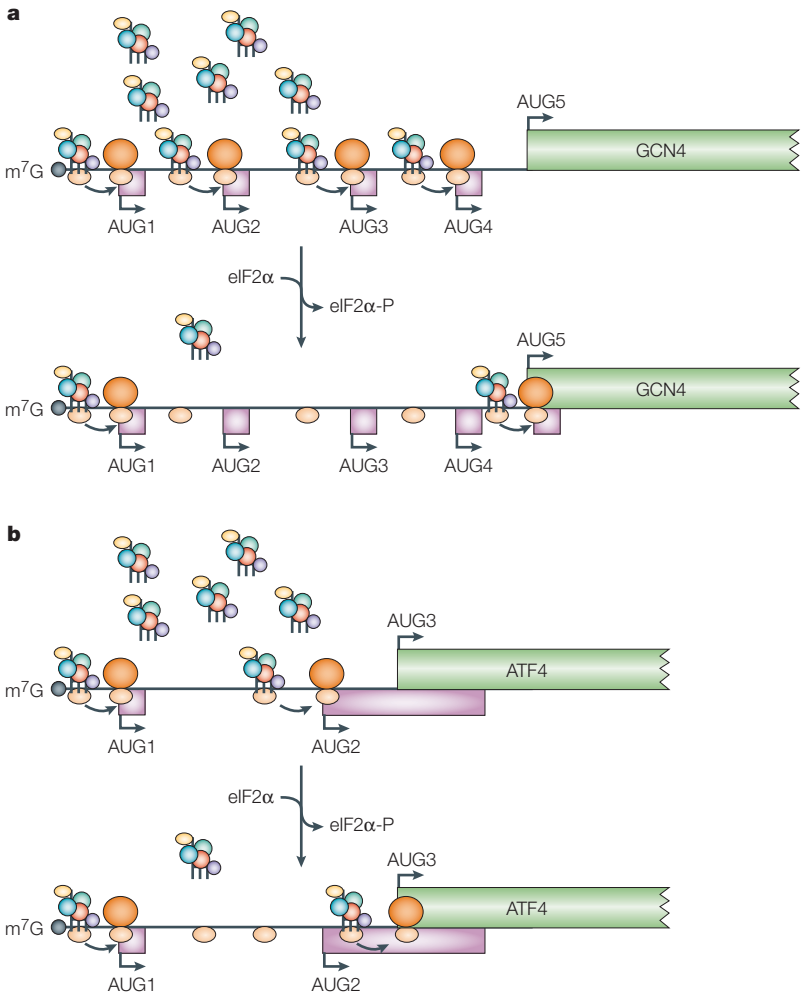


Figure 3 | **RNA regulatory elements in the 5′ untranslated regions of mRNAs that are involved in selective translation. a** | Translation of the yeast transcriptional activator GCN4 is regulated by four upstream open reading frames (uORFs). At low levels of eukaryotic initiation factor-2α (eIF2α) phosphorylation, when the ternary complex is abundant, ribosomes initiate at uORF1 and resume scanning to reinitiate at uORF2, uORF3 or uORF4. However, ribosomes that terminate at these latter uORFs cannot resume scanning, thereby decreasing the probability of initiation at the GCN4 ORF. By contrast, during amino-acid starvation, increased levels of eIF2α phosphorylation lower the abundance of the ternary complex and reinitiation at uORF2–4 becomes less frequent, which allows scanning ribosomes to reach the GCN4 ORF. **b** | Translation of the mammalian activating transcription factor-4 (ATF4) is regulated by two uORFs. Similar to the regulation of GCN4, when the ternary complex is abundant (in the presence of low levels of eIF2α phosphorylation), the ribosomes initiate at uORF1 and frequently reinitiate at uORF2. As uORF2 overlaps with the ATF4 ORF, the translation of uORF2 suppresses the translation of ATF4. During endoplasmic reticulum (ER) stress, when the level of the ternary complex is reduced, the ribosome scans through uORF2 and initiates at the ATF4 initiation codon. Coding regions are shown as green rectangles, uORFs are shown as pink rectangles, 5′ untranslated regions (UTRs) are shown as thin lines, initiation codons (AUG) are indicated with arrows and ribosomes are shown in orange (60S subunit, dark orange; 40S subunit, light orange). The ternary complex is represented as in Fig. 2. GCN4, general control non-derepressible; m^7G, cap structure.

Internal translation initiation

Ribosomes can selectively recruit mRNAs for translation. This mRNA-specific regulation is controlled by discrete sequence elements that are found in the 5′ and 3′ UTRs of many mRNAs, and by proteins that bind to these sequences[11]. An important mode of translational regulation during stress is the selective recruitment of mRNAs through the IRES. Importantly, the translation initiation of several IRES-containing mRNAs occurs predominantly during stress and apoptosis.

Apoptosis is accomplished by the activation of caspases that cleave key cellular substrates, which results in an orderly dismantling of the affected cell[71]. Components of the cellular translation machinery, including eIF4G, are targets of caspase-mediated cleavage during apoptosis, which explains the inhibition of protein synthesis that accompanies apoptosis[72]. Paradoxically, however, protein synthesis is required for apoptosis to occur in different experimental settings. Two important regulators of apoptosis, the pro-apoptotic protease-activating factor-1 (APAF1)[73] and the anti-apoptotic X-chromosome-linked inhibitor

of apoptosis (XIAP)[74], are translated by an IRES-mediated mechanism. Interestingly, APAF1 and XIAP have opposite functions in the regulation of apoptosis: the former is essential for the activation of the initiator caspase-9 (REF. 75), whereas the latter is a member of the IAP family of proteins that block caspase activity[76].

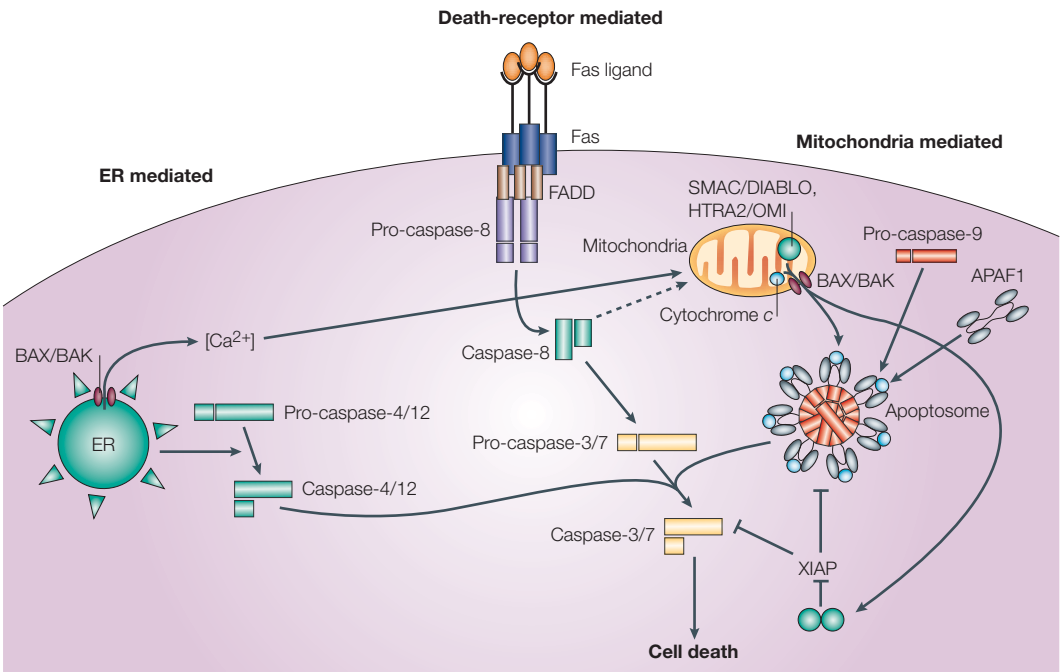
Various cellular stresses initiate apoptosis by the mitochondrial (or intrinsic) pathway. This results in the release of cytochrome *c* from the mitochondria into the cytoplasm[77], where it binds to APAF1 (REF. 78), which results in the ATP-dependent oligomerization of APAF1 into the so-called apoptosome complex[79,80] (BOX 2). The apoptosome further recruits and activates caspase-9, which, in turn, activates caspase-3. XIAP is considered to be the most potent intrinsic inhibitor of caspases, as it binds directly to activated caspase-9 or caspase-3 to render them inactive[81]. The relative levels of XIAP and APAF1 are therefore important for the progression of the apoptotic pathway, and for the life/death decision of the cell. The IRES-mediated translation of these proteins seems to be important in regulating the levels of both proteins, as described below (FIG. 4).

IRES-mediated translation of XIAP

The IRES of XIAP resides in a 162-nucleotide stretch of the 5′ UTR upstream of the initiation AUG codon and functions during normal proliferative conditions[26,74]. The 5′ UTR of XIAP shows IRES activity in bicistronic reporter plasmids and RNA[19,31,74]. There is disagreement about the strength of this IRES. Van Eden and colleagues suggested that the strong translational activity of the XIAP 5′ UTR could not be attributed to IRES activity, because spurious splicing of the bicistronic RNA eliminated the first cistron[19]. However, splicing of the bicistronic mRNA was observed only in the dual luciferase (Rluc–Fluc) reporter system[19] and not in the β-galactosidase–chloramphenicol acetyl transferase (β-gal–CAT) reporter system[28].

Box 2 | **Apoptotic pathways and their regulation**

All known apoptotic signals converge on caspases that engender a self-amplifying cascade (see figure). Apoptotic triggers can be either external or internal, and evoke distinct cellular responses. Intracellular stress (such as DNA damage) results in the activation of the mitochondrial (or intrinsic) pathway. This pathway is characterized by cytochrome *c* release, formation of the apoptosome (consisting of cytochrome *c*, the pro-apoptotic protease-activating factor-1 (APAF1) and pro-caspase-9) and caspase-9 activation. Endoplasmic reticulum (ER) stress results in the activation of an ER-specific pathway as well as the mitochondria-dependent pathway. The ER-specific pathway activates caspase-12 in mice (or a similar caspase in humans) and/or caspase-4, and, subsequently, caspase-3 or caspase-7. Extracellular ligand binding to death receptors triggers the extrinsic pathways that either directly result in caspase activation, or require further amplification through the mitochondrial pathway (dashed arrow), depending on the cell type. All apoptotic signalling pathways converge at the level of effector caspases, such as caspase-3 and caspase-7. There are many control points along these pathways, some of which regulate the release of cytochrome *c* and other apoptogenic factors from the mitochondria by BCL2 family proteins, such as BAX and BAK. Others regulate the levels or activity of caspase inhibitors, or inhibitors of apoptosis (IAPs), such as X-chromosome-linked inhibitor of apoptosis (XIAP), through their antagonists (such as SMAC/DIABLO or serine protease HTRA2/OMI) or through other regulatory mechanisms[76]. BCL2, β-cell leukaemia/lymphoma-2; FADD, Fas-associated death domain. Modified with permission from REF. 111 © (2004) Ashley Publications Ltd.



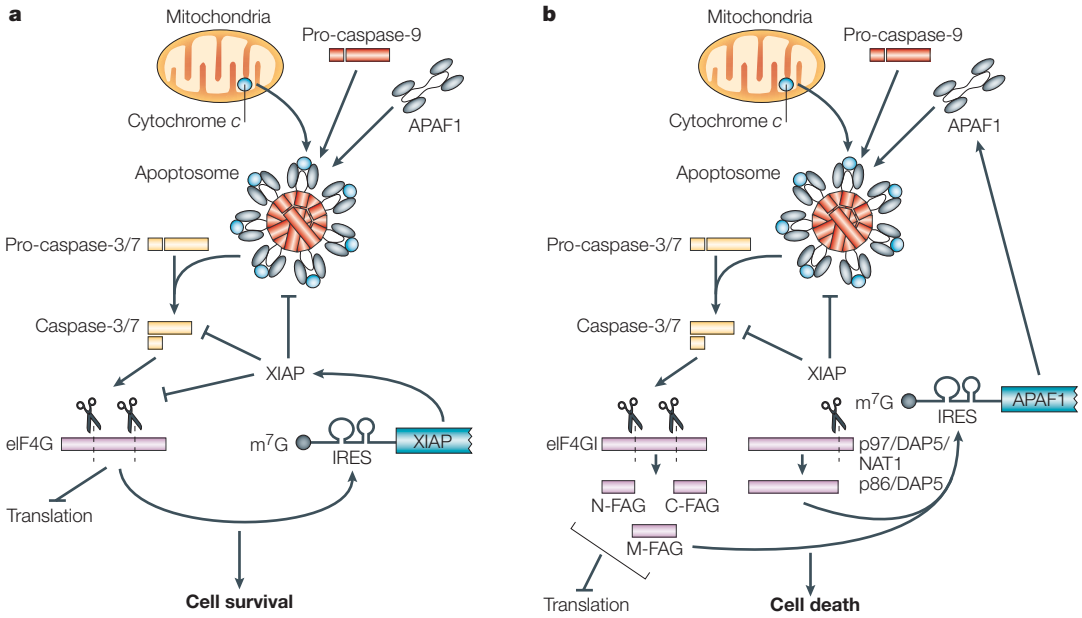


Figure 4 | **Translational regulation of XIAP and APAF1 in apoptosis.** The triggering of apoptosis results in the activation of caspases (BOX 2) that cleave numerous cellular substrates. Among the caspase targets are several initiation factors, including the members of the eukaryotic initiation factor-4G (eIF4G) family, and their proteolytic cleavage leads to the inhibition of cap-dependent protein synthesis that often accompanies apoptosis. **a** | By contrast, the translation of caspase inhibitor, X-chromosome-linked inhibitor of apoptosis (XIAP), is mediated by an internal ribosome-entry site (IRES) that is induced by certain apoptotic conditions, such as serum starvation or low-dose γ-irradiation, which results in the suppression of caspases to delay the commitment of cells to apoptosis. Once the stress trigger is removed, the cells can restore their metabolic activity and resume growth. The precise molecular mechanism of the XIAP IRES induction by stress is not known. **b** | Other types of stress, such as Fas- or etoposide-induced apoptosis, favour the IRES-mediated translation of pro-death proteins, such as the pro-apoptotic protease-activating factor-1 (APAF1). The caspase-mediated processing of eIF4GI and the distant homologue of the eIF4G eukaryotic initiation factor p97/DAP5/NAT1 produces specific cleavage products (middle terminal fragment of apoptotic cleavage of eIF4G (M-FAG) and p86/DAP5) that selectively enhance the translation of the APAF1 IRES, which results in increased levels of APAF1 protein to maintain and further accelerate cell death[30,31]. C-FAG, carboxy-terminal fragment of apoptotic cleavage of eIF4G; m^7G, cap structure; N-FAG, amino-terminal fragment of apoptotic cleavage of eIF4G.

Importantly, XIAP IRES activity and protein levels increase under conditions of cellular stress, such as serum starvation, low-dose γ-irradiation or treatment of cells with interleukin-6 (REFS 42,74,82), despite a reduction in overall protein synthesis. Similarly, treatment of small cell lung cancer cells with fibroblast growth factor-2 (FGF2) resulted in an increase in the translation of *XIAP* mRNA[83]. In all cases, the increase in XIAP levels resulted in enhanced survival of cells following exposure to various apoptotic triggers[42,74,82,83]. Furthermore, antisense- or small-interfering RNA (siRNA)-mediated downregulation of XIAP restored the sensitivity of the cells to apoptotic triggers, which confirms that XIAP levels are crucial for cell survival and that the increase in the translation of *XIAP* mRNA delays or reverses the commitment to death[38,42,83].

The precise mechanism of the IRES-mediated regulation of XIAP translation is not fully understood. A sequence-specific protein complex that consists of at least four distinct proteins binds to the IRES[26]. Two of these proteins were identified as La[26] and hnRNPC1/C2 (REF. 28), which are both generic RNA-binding proteins and ITAFs that are known to interact with several viral and cellular RNAs, and are therefore unlikely to specifically regulate only the IRES of XIAP. Interestingly, both La and hnRNPC1/C2 are proteolytically processed during Fas- or anti-immunoglobulin M (IgM)-induced apoptosis[84,85]. It should be noted that La cleavage was not observed when apoptosis was induced by the CK2 inhibitor 4,5,6,7-tetrabromo-2-azabenzimidazole (TBB)[86], which indicates that the cleavage of La might be apoptotic-trigger specific. Nevertheless, it is possible that modifications of RNA-binding proteins, including those that bind to the XIAP IRES, might be required during apoptosis to attenuate the anti-apoptotic response.

Not all apoptotic conditions enhance the IRES-mediated translation of XIAP. For example, treatment of cells with etoposide (which triggers the mitochondrial apoptotic pathway) or thapsigargin (which induces ER stress) does not cause activation of the XIAP IRES or an increase in XIAP protein levels[30,38]. Translational upregulation of XIAP is likely to function as a survival reflex in response to acute, but transient, stress conditions, such as growth-factor deprivation, γ-irradiation or hypoxia[30,74,87]. Elevated levels of XIAP might therefore delay the onset of apoptosis and allow the cell to deal with the stress conditions (FIG. 4a).

IRES-mediated translation of APAF1

The translation of APAF1 is mediated by an IRES that supports low levels of translation[73]. There is no sequence similarity between the IRESs of XIAP and APAF1, and distinct sets of proteins bind to them. The activity of the APAF1 IRES requires at least two ITAFs: PTB and UNR[25]. Both are thought to function as RNA chaperones, which allow the APAF1 IRES to attain the correct conformation, which is amenable to ribosome loading[88].

As APAF1 is essential for the progression of the intrinsic apoptotic pathway[89], it has been argued that the IRES-mediated translation of APAF1 is required to maintain sufficient levels of APAF1 protein during apoptosis to propagate caspase-9 activation[73]. Indeed, treatment of cells with etoposide results in an increase in APAF1 IRES activity by a mechanism that involves members of the eIF4G family (see below)[30]. Interestingly, however, the APAF1 IRES does not seem to function efficiently during TRAIL- (tumour necrosis factor-related apoptosis-inducing ligand) or staurosporine-induced apoptosis, which indicates that it might be subject to differential regulation depending on the apoptotic trigger[23].

The mammalian family of eIF4G proteins consists of two isoforms, eIF4GI and eIF4GII, and two more-distantly related proteins p97/DAP5/NAT1 and poly(A) binding protein-interacting protein-1 (PAIP1)[90]. Several family members (eIF4GI, eIF4GII and p97) are cleaved by caspases during apoptosis to suppress protein synthesis and to attenuate the anti-apoptotic response[91]. The caspase-cleaved fragments of two family members, eIF4GI and p97/DAP5/NAT1, enhance translation from the APAF1 IRES[30,31]. This situation is reminiscent of cells that are infected with certain picornaviruses, in which eIF4G is specifically cleaved to block host protein synthesis, whereas the translation of viral mRNAs continues unhindered. In fact, the cleaved C-terminal two-thirds of eIF4GI stimulates translation from some viral IRESs[92,93]. Therefore, specific proteolytic fragments of eIF4G family members might selectively enhance the translation of target mRNA by an IRES mechanism (FIG. 4b).

It is notable that the translation of p97/DAP5/NAT1 is also driven by an IRES[32]. Importantly, the activity of the p97/DAP5/NAT1 IRES is enhanced in etoposide-treated cells, similar to APAF1. This activation is mediated by caspase-cleaved fragments of eIF4GI and p97/DAP5/NAT1 itself[30–32]. Therefore, the truncated form of p97/DAP5/NAT1 could function as an apoptosis-specific translation-initiation factor.

IRESs in cell survival

The following examples illustrate three divergent physiological conditions in which IRES-mediated translation is implicated in cell survival.

Genotoxic stress. c-Myc, which is a mammalian transcription factor of the BASIC-HELIX-LOOP-HELIX (bHLH)–LEUCINE-ZIPPER (ZIP) FAMILY and a potent proto-oncogene, is translated through an IRES[94,95]. The IRES translation of c-Myc is activated following the induction of apoptosis by the death-receptor pathway (by the Fas ligand) in HeLa cells[96] and in response to genotoxic stress[97].

ER stress. Prolonged ER stress leads to the induction of apoptosis. HIAP2, which is a member of the IAP family, is upregulated during ER stress. The 5′ UTR of HIAP2 contains a uORF[98] and an ER-stress-inducible IRES[38]. The uORF in the HIAP2 5′ UTR severely inhibits translation of the downstream gene[98]. The HIAP2 IRES is not active in proliferating cells, but becomes activated in response to ER stress. In turn, elevated levels of HIAP2 protein delay the onset of ER-stress-induced apoptosis[38]. The activation of the HIAP2 IRES is brought about by the caspase-cleaved fragment of p97/DAP5/NAT1 (REF. 38). This is similar to the induction of the APAF1 IRES during etoposide-induced apoptosis[30]. The HIAP2 IRES, however, is not activated by etoposide treatment, which indicates that, in addition to caspase activation, a further ER-stress-specific event is required for the induction of HIAP2. Alternatively, the cleavage of p97/DAP5/NAT1 might occur by an ER-specific caspase to activate the HIAP2 IRES. Importantly, the cleavage of p97/DAP5/NAT1 in ER-stress-induced apoptosis occurs before caspase-3 activation. This shows that it is a specific event and not a consequence of generalized protein degradation in the final stages of apoptosis[38].

Hypoxia. Conditions of low oxygen levels (hypoxia) are common in many human diseases, including stroke, heart disease and cancer[99]. In particular, solid tumours show hypoxic regions that are often aggressive and non-responsive to chemotherapy. The cellular response to hypoxia is mediated primarily by the hypoxia-inducible factor-1 (HIF1) transcription factor, which coordinates the transcriptional induction of hypoxia-inducible genes[99]. In addition, hypoxia results in a rapid inhibition of translation as a consequence of eIF2α phosphorylation by PERK[100,101] and the dephosphorylation of 4E-BP1, which is a repressor of eIF4E[102]. In this respect, it is notable that the translation of the α-subunit of HIF1 has been reported to be mediated by an IRES element in response to hypoxia[103]. Significantly, one of the most prominent hypoxia-inducible genes is VEGF, the expression of which is regulated at various levels, including translation[104]. Although VEGF itself is not an oncogene, it is upregulated in tumorigenesis and is important in blood-vessel formation in solid tumours[105]. Hypoxia-induced VEGF enhances tumour survival by the inhibition of apoptosis[106]. Importantly, the translational upregulation of VEGF in response to hypoxia is IRES dependent[107,108]. Therefore, at least two important hypoxia-related proteins (HIF1α and VEGF) are synthesized by IRES-dependent translation. Microarray profiling of POLYSOME-bound RNA in normoxic and hypoxic HeLa cells identified a subset of cellular mRNAs that are preferentially translated during hypoxia[101]. Some of these are translated by an IRES-dependent mechanism (for example, VEGF[107], FGF2 (REF. 109) and BiP[110]), whereas others, such as ATF4 (REF. 101), are upregulated as a consequence of eIF2α phosphorylation.

Concluding remarks and outlook

The ability of cells to adapt to stress is crucial for their survival. Disparate adaptation strategies have evolved to

BASIC-HELIX-LOOP-HELIX (bHLH)–LEUCINE-ZIPPER (ZIP) FAMILY
Transcription factors that have a bHLH DNA-binding motif and a zip dimerization motif, which regulate the expression of their target genes as hetero- or homodimers.

POLYSOME
An mRNA with more than one associated translating 80S ribosome.

allow organisms to respond to environmental and physiological stress. Regulation of global translation combined with selective translation is one such instrument, and is ideally suited for this purpose as it provides rapid and reversible regulation of gene expression. Although some mechanisms of selective translation, such as regulation by eIF2α phosphorylation, were described some time ago, others, such as IRES-mediated translation, are only recently attracting attention and remain largely unexplored. The investigation of how selective translation works promises to be an exciting scientific journey, as the data so far indicate that selective translation commonly occurs during cellular stress and apoptosis. However, although this idea is tantalizing, many questions remain unanswered. What are the RNA regulatory sequences that allow the selective recruitment of mRNA to the translation machinery under stress? Which ITAFs are involved in IRES translation and are these proteins shared by many, or only by specific subsets of, IRES-containing mRNAs? Which are the signalling pathways that affect IRES-dependent translation and how are these regulated?

The biological implications of selective translation are clearly important. The stress conditions that cells must respond to — such as hypoxia, heat shock, toxins or drug exposure — are often an underlying cause of human diseases, including diabetes, heart disease and stroke. Similarly, dysregulated apoptosis has been associated with many human disorders, ranging from autoimmune disease to neurodegeneration to cancer. Understanding how translational control determines the cellular response to stress will provide a better insight into many human disorders, and might ultimately lead to the development of new therapeutic modalities.

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Competing interests statement

The authors declare no competing financial interests.

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