6 Alternative Mechanisms of mRNA Translation Initiation in Cellular Stress Response and Cancer

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Abstract

Throughout evolution, eukaryotic cells have devised different mechanisms to cope with stressful environments. When eukaryotic cells are exposed to stress stimuli, they activate adaptive pathways that allow them to restore cellular homeostasis. Most types of stress stimuli have been reported to induce a decrease in overall protein synthesis accompanied by induction of alternative mechanisms of mRNA translation initiation. Here, we present well-studied and recent examples of such stress responses and the alternative translation initiation mechanisms they induce, and discuss the consequences of such regulation for cell homeostasis and oncogenic transformation.

Keywords

mRNA translation · Alternative translation initiation · Cellular stress · Cancer · IRES · Non-AUG

6.1 Introduction

Most eukaryotic mRNAs are translated into proteins through a 5′-end m^7G cap-dependent translation mechanism. Translation initiation is marked by the formation of a ternary complex (TC) composed of eukaryotic initiation factor 2 (eIF2) bound to Met–$tRNA_i^{Met}$ and GTP [1]. eIF2B, a guanine nucleotide exchange factor (GEF), controls TC assembly by converting eIF2–GDP into the active eIF2–GTP complex before each round of translation [2]. Once properly assembled and active, the TC binds the 40S ribosomal subunit, forming the 43S pre-initiation complex (43S PIC). Several initiation factors, such as eIF1, eIF1A, eIF3 and eIF5, help this binding [3–8]. Separately, eIF4E binds the 5′-end m^7G cap of the mRNA and recruits eIF4G and eIF4A forming the eIF4F complex [8] and stimulating eIF4A's helicase activity, which promotes mRNA restructuring [9, 10]. eIF4G is the scaffold for the eIF4F components and binds to poly(A)-binding protein (PABP) and eIF3 at subunits c, d and e, helping recruit the 43S PIC to the transcript [11]. Following recruitment, 43S PIC will often require scanning downstream in order

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to find the initiation codon [12, 13], unless this one is within close reach, in which case scanning is unnecessary and instead a specific Kozak sequence termed TISU can help prevent "leakage" to a downstream AUG [14, 15]. In most cases, however, the AUG is relatively far from the 5′-end and the 5′ untranslated region (UTR) is at least mildly structured, so scanning of – or jumping over, during a phenomenon named ribosomal shunting [16] – the 5′ UTR by the 43S PIC is often a requisite for translation initiation and typically entails the hydrolysis of ATP, eIF1, eIF1A, and DHX29 [3, 17]. ATP hydrolysis can be used by eIF4A to unwind secondary structures in the mRNA, and actively displace the ribosome in a 5′ to 3′ direction. At the same time, the ribosome is prevented from backsliding, because the unwound structures behind it resume their initial winding conformation [18, 19]. Scanning, when necessary, usually stops when the 43S PIC reaches the first AUG codon positioned in a favourable Kozak context (a purine, usually adenine, in position −3 and a guanine in position +4) [12]. At this point the 48S pre-initiation complex (48S PIC) is formed, with pairing of all three nucleotides of the anticodon and release of eIFs from the small subunit. Upon recognition of the initiation site, eIF5 and eIF5B promote the hydrolysis of eIF2–bound GTP [20, 21]. eIF5B–GTP binds to the 40S subunit and stimulates the 60S subunit joining, requiring a second step of GTP hydrolysis in order to make the 80S ribosome, which will be ready to start decoding the message [22, 23]. When this happens, eIF2 is completely released from the ribosome in its GDP-bound form that latter must be reverted to GTP-bound again to allow reassembly of the TC for another round of translation. The formed eIF2–GTP is only stable when Met–$tRNA_i^{Met}$ joins in to form the TC [2].

Although the translation process may be hindered at several stages, the translation initiation phase is the rate-limiting step and involves more factors and sub-steps that are prone to error, and many of them may be inactivated, modified or adjusted under adverse cell conditions [24]. In fact, translation initiation is globally impaired under stress conditions and overall protein synthesis is reduced, a response that has been termed Integrated Stress Response (ISR). Such reduction can happen due to eIF4E's or eIF2's inability to bind eIF4G/5′-end cap or integrate the TC, respectively. The first occurs for example during stress conditions that inhibit the mTOR proliferation and survival pathway; as inactivated mTOR kinase is no longer able to phosphorylate 4E-binding proteins (4E-BP) and the resulting hypophosphorylated 4E-BP binds to eIF4E preventing its association with eIF4G and the formation of eIF4F. The core event of the ISR however, is the second mechanism, involving phosphorylation of serine 51 of the eIF2α subunit by any of several protein kinases activated by a wide range of different stress conditions. This phosphorylation stabilizes the interaction between eIF2B and eIF2–GDP and prevents the formation of the eIF2–GTP–Met–$tRNA_i^{Met}$ complex (TC).

Repression of global protein synthesis is often accompanied by selective translation of mRNA encoding crucial stress-responsive proteins that can lead to either stress recovery and survival or cell death. This selective translation involves alternative initiation elements, often RNA structures or modifications, and may not require some of the more common elements such as the 5′-end m^7G cap. Genes that can maintain their expression under stress conditions may contribute to modify cell fate if they operate on repair, survival or programmed death pathways. Many cancer cells take advantage of this ability in transcripts such as XIAP, HIF1α or VEGF to escape apoptosis, resist to hypoxic conditions, or vascularise the tumour surroundings, respectively. It is well reported that many of these stress-response transcripts can maintain expression because of the alternative mechanisms of initiation that mediate their translation. Out of these mechanisms, internal ribosome entry at internal ribosome entry sites (IRES) has been the most widely studied and is accepted as a backup mechanism for cells to cope with conditions when canonical translation is shut down.

In this chapter, we aim to compare the different mechanisms of translation initiation involved in stress-response. We will also briefly consider to what extent these mechanisms may create an

adaptive advantage to the eukaryotic cell under stress conditions or how sometimes this advantage turns into a burden to the organism when coupled with processes of cellular transformation.

6.2 Translation Initiation in Stress Response

Throughout evolution, eukaryotic cells have devised different mechanisms to cope with stressful environments. When eukaryotic cells are exposed to stress stimuli, they activate a common adaptive pathway that allows them to restore cellular homeostasis: the integrated stress response (ISR) [25]. Most types of stress stimuli have been reported to activate the ISR [26, 27], including hypoxia [28], nutrient deprivation [29], oxidative stress, heat shock [30], viral infection (dsRNA) [31], endoplasmic reticulum (ER) stress / unfolded protein response (UPR) [32], UV irradiation [33], proteasome inhibition [34] and oncogene activation [35]. They all lead to the phosphorylation of eIF2α and consequent inhibition of TC formation, by activating kinase haem-regulated inhibitor (HRI), protein kinase activated by double-stranded RNA (PKR), general control non-derepressible-2 (GCN2) or PKR-like endoplasmic reticulum kinase (PERK) [26, 36]. As a consequence, there is a decrease in overall protein synthesis. However, several selected genes that participate in cellular stress response are still translated under such conditions [26, 37]. Below, we discuss some well-studied examples of stress stimuli, the inhibition they induce and the alternative mechanisms they activate, as well as the consequences of such regulation for cell homeostasis and transformation.

6.2.1 Endoplasmic Reticulum Stress

The endoplasmic reticulum (ER) is a central organelle in which proteins are translated and properly folded, may undergo some post-translational modification and from there are sent to the Golgi complex and sites of action. When perturbations in ER homeostasis occur, causing an accumulation of unfolded proteins in the ER, these perturbations are sensed and transduced to the whole cell and an evolutionarily conserved response is activated—the unfolded protein response (UPR) [38–40]. The UPR consists of three branches, each of which can be distinguished by the action of a different stress sensor protein: inositol-requiring protein-1α (IRE1α), protein kinase RNA (PKR)-like ER kinase (PERK) and activating transcription factor 6 (ATF6) [39, 40]. In normal conditions, the UPR modulators IRE1, PERK and ATF6 interact with molecular chaperone Binding immunoglobulin protein (Bip)/GRP78 and remain inactive [39–42]. However, when unfolded proteins accumulate in the ER, BiP is required to assist with the folding and the sensor proteins are liberated and activated. Other proteins have also been shown to regulate IRE1α and the other sensors [43]. Upon activation, all three stress sensors induce signal transduction in order to deal with the stress and reduce the amount of misfolded proteins. This includes a tight reprogramming of transcription and translation to ensure less and more specific gene expression [44]. The key regulatory pathway for this response is the PERK/eIF2α/ATF4 pathway in which the kinase PERK phosphorylates eIF2α (eIF2α-P). This signal then induces overall translational impairment, but it also enables translation of the transcription factor ATF4 mRNA [44]. ATF4 is a transcription factor whose translation has been shown to be regulated through reinitiation at upstream open reading frames (uORF) [45] and also by an Internal ribosome entry site (IRES) stimulated by eIF2α phosphorylation [46] (see also below section "Translation initiation by internal ribosome entry" and Fig. 6.1).

Translation of HIAP2 (a member of the inhibitor of apoptosis protein family) is also mediated via an inducible IRES element during ER stress [47]. HIAP2 IRES activity is enhanced during ER stress through the caspase-mediated proteolytic processing of eukaryotic initiation factor p97/DAP5/eIF4G2/NAT1 (DAP5), which produces

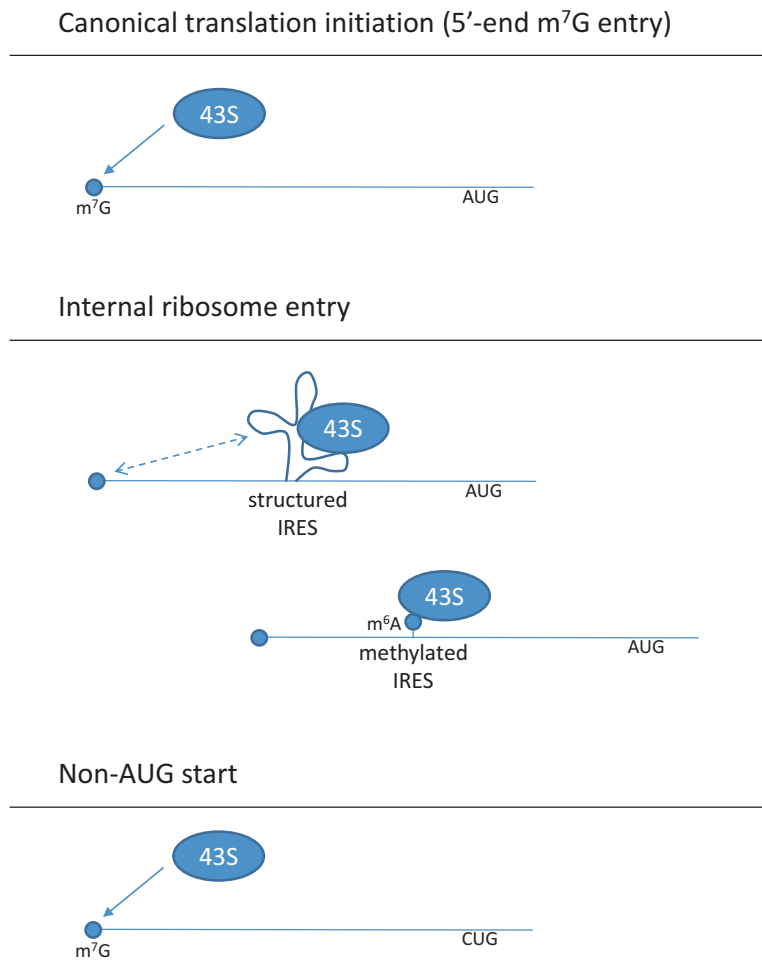

Fig. 6.1 During canonical translation initiation the ribosome is recruited to the 5′-terminal m^7G cap (top). Cell stress and cancer induce alternative methods of translation initiation such as internal ribosome entry at a structured internal ribosome entry site (IRES) or at a methylated site (center). The 5′-end may sometimes still be required for this initiation (indicated by the dashed arrow). Another type of alternative initiation involves the use of non-AUG start codons (bottom)

a fragment that specifically activates IRES [47]. In fact, DAP5 is a translation initiation factor that can regulate the expression of a selected group of mRNAs during ER stress via internal ribosome entry [48, 49]. Other DAP5 targets include IRES in c-Myc, Apaf-1, XIAP and HMGN3 mRNA. In this regard, DAP5-mediated translation seems to be crucial in cell differentiation and death, stressing the role of IRES translation in deciding cell fate [49]. Furthermore, DAP5 translation itself is mediated by an IRES element within its 5′ UTR, thus generating a positive self-regulatory loop that allows its continuous translation under conditions impairing 5′ end ribosome entry–dependent translation [49, 50].

6.2.2 Hypoxia

Hypoxia results from the decrease in the oxygen available to reach the different organs and cells. Under hypoxic stress, a group of transcription factors known as hypoxia inducible factors (HIF) are stabilized and initiate a cascade of cell signals by activating target genes in the nucleus [51]. Inhibition of protein synthesis and consequent energy saving is an advantage for hypoxic cells, so, in order to achieve this, canonical translation initiation is drastically reduced under such conditions. However, hypoxic cells need to translate mRNAs critical for an adaptive response to low oxygen levels [52]. To accomplish this selective

translation, cells use non-canonical mechanisms of translation initiation, such as IRES-mediated translation. IRES elements present within the 5 UTRs of several transcripts have been proved to mediate translation of stress-regulated mRNAs, such as vascular endothelial growth factor (VEGF), HIF-1α and -2α, glucose transporter-like protein 1, p57(Kip2), La, BiP, and triose phosphate isomerase (TPI) transcripts [52]. Many proteins share such characteristics. Phosphofructokinase 1 (PFK1), the major regulatory enzyme of the glycolytic pathway, converts fructose-6-phosphate to fructose-1,6-bisphosphate. This pathway is highly dynamic and may be affected by different stress conditions, such as hypoxia, which is known to significantly influence the glycolytic pathway [53, 54]. Ismail and colleagues showed that PFK1's 5′ UTR includes a hypoxia-responsive IRES element, which was established to be the possible mechanism responsible for PFK1 protein upregulation [55]. These authors found that, after 48 h of chemically induced hypoxia in C6 glioma cells, PFK1 protein levels were upregulated with no significant change in the counterpart mRNA levels; this may explain the astrocytes' increased glycolytic capacity upon brain hypoxia [55].

6.2.3 Starvation

In the absence of nutrients, amino acids, growth factors and cytokines, which contribute to the activation of signal pathways related to cell survival and proliferation, the ISR facilitates cellular adaptation to stress conditions through the common target eIF2α [56]. Under starvation, there have been examples of leaky scanning and reinitiation events through which cells can make alternative protein products by selecting downstream initiation codons to better respond to the stress [56]. Reinitiation in ATF4, for example, is governed by the eIF2α pathway and also subjected to regulation by mRNA m^6A methylation. Zhou et al. demonstrated that m^6A in the 5′ UTR controls ribosome scanning and start codon selection [56].

IRES-mediated translation initiation is the most common alternative to canonical translation under starvation. One of the most widely used models to understand this mechanism in eukaryotes is the X-linked inhibitor of apoptosis (XIAP) mRNA [57]. When ternary complex (TC) is available, XIAP mRNA translation is maintained in a 5′-end m^7G cap-dependent mode; however, under serum deprivation, the XIAP IRES can initiate translation in an alternative eIF5B-dependent manner circumventing low TC numbers due to eIF2α phosphorylation [58]. Notably, not all cellular IRES use eIF5B-dependent mode of tRNA delivery during serum deprivation [27].

6.2.4 DNA Damage

Apaf-1 has a central role in DNA damage-induced apoptosis and its depletion contributes to malignant transformation [59]. As such Apaf-1 provides a good example of specialized DNA damage translation since the human Apaf-1 mRNA can initiate translation through an alternative mechanism, possibly involving an IRES [60, 61]. This internal ribosome entry site has been reported to require the assistance from a free 5′-end *in cis* [62], though it does not need m^7G cap recognition by eIF4E [60]. Under DNA damage conditions m^7G cap-binding factor eIF4E is often suppressed, but structured 5′ UTR regions such as IRES may mediate m^7G cap-independent, 5′-end-dependent translation initiation, which leads to preferential translation of some mRNAs like Apaf-1 [60]. A group of mRNAs including 53BP1, HIF1α, BRCA-1, and GADD45a, has also been shown to be more actively translated in response to DNA damage in breast cancer cells, through a selective eIF4G1-dependent process and with reduced dependence on eIF4E [63].

6.2.5 Heat Shock

Although we can find living organisms in a wide range of temperatures (from the freezing point of water, or below, to 113 °C) [64], each of them has

adapted to a certain optimal growth temperature. Heat above such temperatures becomes a major stressor, with often temperatures only moderately above the optimum growth temperature already causing a significant barrier to survival [65]. Heat stress can cause protein unfolding, entanglement, and unspecific aggregation [65]. During cellular heat shock response, a class of molecular chaperones, the heat shock proteins (Hsp), is upregulated in response to protein misfolding [65]. Heat shock goes beyond the unfolding of individual proteins, causing deleterious effects on the internal organisation of the cell, such as defects of the cytoskeleton [65].

Translation of Hsp70, a stress-induced molecular chaperone that also modulates tumour cell responses to cytotoxic agents and inhibits apoptosis [66], is mediated through internal ribosome entry, most likely due to increased m^6A modifications in its mRNA [67]. The increasing number of m^6A-containing transcripts results from the exposure to different cellular stresses that drive a widespread redistribution of m^6A [67]. A recent study by Coots et al. established the effect of m^6A in the 5′ UTR translation initiation [68]. They showed that when eIF4F-dependent translation is impaired, cells use the m^6A-dependent mode of translation initiation. They identified the ATP-binding cassette subfamily F member 1 (ABCF1) as a critical mediator of m^6A-dependent translation. This protein acts as an alternative to recruit the ternary complex during non-canonical translation as it can interact with eIF2 and ribosomes, thus playing a critical role in mRNA translation initiation under stress conditions [69, 70]. The HSP70 5′ UTR has also been shown to drive m^7G cap-independent translation via an IRES structure [71]; however, it is not yet known whether/how both features cooperate to enhance translation of heat shock-responsive proteins. Translation of the BiP protein was also found to be enhanced by continuous heat stress that activates an IRES-dependent translation [72]. This suggests that the IRES-dependent mechanism of translation initiation can be used by cells subjected to heat shock, being critical to cell survival and proliferation under stress [72].

Recently, the switch to activate a specialized ribosome for alternative translation of stress response genes was shown to be regulated by an alternative translation initiation process itself [73]: Mitochondrial ribosome protein-encoding MRPL18 mRNA was shown to translate into a shorter isoform from a downstream non-canonical CUG initiation codon following exposure to heat shock and phosphorylation of eIF2α. The shorter isoform is translated in frame but lacks the mitochondrial targeting signal in the N-terminus and is localized to the cytoplasm where it integrates – not the usual mitoribosome but now – the cytosolic 80S ribosomes promoting the specific synthesis of stress-proteins such as Hsp70.

6.2.6 Oxidative Stress

Under oxidative stress, NRF2, a master regulator of the oxidative stress response, is also translationally induced through an IRES [74–76]. NRF2 IRES-dependent translation is enhanced due to stimulation of an IRES element present within its 5′ UTR by La autoantigen IRES transacting factor (ITAF) binding [76]. Translation of some transcription factors is also mediated by IRES elements upon oxidative and genotoxic stress, such as p53, the octamer-binding protein 4 (OCT4) whose translation is stimulated by H_2O_2 treatment in breast cancer and liver carcinoma cells [77], and runt-related transcription factor 2 (RUNX2), whose translation is stimulated by mitomycin C [78]. BiP is also induced during oxidative stress through a mechanism that involves an alternative, less usual procedure for translation initiation, the usage of a non-canonical initiation codon, UUG, in a uORF [79]. The UUG-initiated uORF in the 5′ UTR of BiP and eIF2A were shown to be necessary for BiP expression during oxidative stress. At the same time, the uORFs generate peptides that could serve as major histocompatibility complex class I ligands.

6.3 Mechanisms of Alternative Translation Initiation

6.3.1 Translation Initiation by Internal Ribosome Entry

Internal ribosome entry site (IRES)-mediated translation is an additional and alternative mode of translation initiation used by some transcripts, which can be regulated independently of the canonical system. IRES-mediated translation has been extensively investigated. It has been estimated that at least 10–15% of cellular mRNAs can be translated by an IRES-dependent mechanism, in which the 40S ribosomal subunits bind the transcript internally, not at the 5′-end, often in a cap-independent manner and with a different requisite of translation factors [80]. We subdivided here this alternative internally initiated translation into two large groups: one more comprehensively studied, involving a structured RNA region usually just referred to as IRES; and a second more recently identified mechanism for internal entry that involves a methylated RNA site (Fig. 6.1). To note that it is still currently unknown how often actually these two mechanisms might overlap.

6.3.1.1 Internal Ribosome Entry @ Structured RNA Regions

According to a recent systematic screen for IRES-mediated translation activity, about 10% of all human 5′ UTRs have the potential to be IRES-translated [81], and these can be present in the coding region as well [82]. This translation initiation mechanism allows cells to cope with environmental changes affecting their viability, and thus must be essential for cellular life itself. In order to understand how central and elementary the mechanisms that govern IRES function are, Colussi et al. investigated if IRES could initiate translation in bacteria and saw that the IRES element could bind directly to both eukaryotic and bacterial ribosomes by occupying the space normally used by tRNAs [83]. Some IRES use dynamic RNA structures to target core and conserved, ancient domains of the translation machinery while circumventing organism-specific regulations to effectively initiate mRNA translation of specific transcripts in a large variety of cell types and cell conditions and with few requirements [83]. This is important because proteins with crucial roles in main cellular processes need backup regulation, their expression levels must be adjusted in response to external cues that impair the canonical mechanism of translation initiation. Indeed, alterations in their expression levels may account for many types of human diseases that arise in human population, including different types of cancer, and IRES-dependent translation initiation may play a decisive role in such processes.

From our current knowledge, most structured IRES described so far were identified in transcription factor mRNAs (21%), growth factor transcripts (15%), and in messages encoding transporters, receptors and channels (22%) [9]. FGF and VEGF families of proteins—growth factors of crucial importance to the development of specific tissues that play a significant role in promoting cell proliferation and differentiation, and in regulating cell survival—are translated via IRES elements present in the corresponding mRNAs [84–88]. As for transporters, receptors and channels, such as CAT-1, voltage-gated potassium channel and oestrogen receptor α, among others, play a critical role in signal transduction as they are main vehicles in cell-cell communication, which turns them into key elements to maintain cell homeostasis following environmental changes. Thus, alterations in their expression associate with changes in cellular function, which may lead to disease development and progression [84]. That is why transcripts encoding such proteins can be translated through an IRES-dependent mechanism that acts as a back-up tool when canonical translation initiation is impaired by environmental stress conditions, such as ER stress. Regarding transcription factors, they are fundamental in gene expression regulation, as they respond to quick changes in the environment in order to adapt their expression levels to a given context—c-MYC, HIF1α and p53 are good examples of transcription factors whose translation initiation is mediated by IRES elements [85, 86, 89].

6.3.1.2 Internal Ribosome Entry @ Methylated Regions

There are about 3–5 m^6A modifications per transcript [90]. Most m^6A modifications are located on the coding region and the 3′ UTR [91, 92]; however, Meyer et al. found recently that, when located in the 5′ UTR, such modifications could mediate translation initiation through internal ribosome entry [93] (Fig. 6.1). These authors have shown that m^6A modifications in the 5′ UTR act as ribosome engagement sites (MIRES) [93]. The ability of m^6A in the 5′ UTR to bind eIF3 is enough to recruit the 40S ribosomal subunit to initiate translation when eIF4E is not available to bind the 5′-end cap structure [93]. Although the mechanism that permits m^6A recognition by the translation machinery for subsequent m^7G-independent initiation is not yet completely understood, the significance of 5′ UTR m^6A residues has been observed in both ribosome profiling datasets and individual cellular mRNA analyses, such as the heat-shock protein 70 (Hsp70) [93, 94].

6.3.2 Non-AUG Translation

Another less common alternative translation process is the initiation at non-AUG codons (for a recent review see [95]) (Fig. 6.1). These translation starts are not errors but regulated events, leading to the production of stress response proteins as well as proteins involved in development. Impairment of this specialized translation process may lead to diseases such as cancer and neurodegeneration. It is increasingly clear that non-AUG translation is highly regulated by stress, signalling, translation factors and RNA structures and sequences. The most commonly used non-AUG codons are the near-cognate that differ by only one nucleotide, with CUG being most frequently used. Some proteins, like DAP5, are exclusively translated from a non-AUG (GUG) codon [96]. Interestingly, several proteins involved in activating alternative translation seem to be first translated from an alternative initiation codon: DAP5 regulates IRES-translation [97] and a CUG-translated shorter version of MRPL18 protein activates specialized translation of stress response genes by integrating the cytoplasmic 80S ribosome [73]. Non-AUG translation can be influenced by eIF2α phosphorylation during ISR, Kozak sequence, mRNA structures upstream or downstream or the expression of specific stress-induced translation factors like eIF2A.

6.4 Translation Initiation in Cancer

Oncogene activation and tumour suppressor gene inhibition are key events to the onset and development of cancer. Additionally, coding-independent mutations in regulatory elements, UTRs, splice sites and non-coding RNAs and synonymous mutations may also affect gene expression (reviewed in [98]). As any other stress situation, tumorigenesis includes backup mechanisms that allow tumour cells to cope with stress, such as those involved in stress-adaptive protein synthesis [48, 99–103]. Many transcripts relevant to cancer can initiate translation through non-canonical translation initiation mechanisms. Below we will briefly present a few well-known examples.

6.4.1 Non-AUG Translation in Cancer

Though IRES-dependent translation is the most widely studied, we will start by discussing initiation at non-AUG sites in cancer. Some of these sites have been shown to be regulated by eIF2A, which stimulates translation from non-AUG uORFs in cancer-related mRNAs that act to positively regulate the expression of their downstream ORFs [104]. eIF2A can initiate Leu-encoding codons at CUG and UUG by promoting the recruitment of LeutRNACUG for initiation [79, 105, 106]. Interestingly, mutation and inactivation (decreased expression) of the RNA helicase DDX3 in cancer leads to the formation of RNA structures in 5′ UTR downstream of non-AUG initiation codons, inducing their usage [107].

PTEN, a tumour suppressor gene that is frequently mutated in cancer, generates an alternative protein isoform, PTENβ, by using an upstream non-canonical AUU start codon [108]. PTENβ translation requires a favourable Kozak sequence and an evolutionarily conserved hairpin that is present 18 nucleotides downstream from the AUU. PTENβ negatively regulates rDNA transcription and cell proliferation and may have a role in tumour suppression [108].

FGF2 stimulates the growth and development of new blood vessels (angiogenesis) that contribute to the pathogenesis of cancer. In FGF2 mRNA, at least four upstream in-frame CUG codons were shown to generate longer isoforms that localized to the nucleus due to a nuclear localization signal present between the CUG and AUG codons [109, 110]. These isoforms could possibly affect cell growth and differentiation and one of them of 34 KDa enhanced survival in low serum conditions.

c-myc, a well-known protooncogene, uses an upstream in-frame CUG codon resulting in the production of a larger isoform with a distinct N-terminus that may contribute to the oncogenicity of c-myc, particularly in Burkitt's lymphoma [111]. As cell density increases, as during tumour formation and growth, the availability of amino acids, specifically methionine, becomes limiting, and translation initiation from the CUG is promoted [112].

6.4.2 Internal Ribosome Entry in Cancer

IRES elements within the 5′ UTR or coding region of transcripts encoding oncogenes, growth factors and proteins involved in the regulation of cell-cycle and programmed cell death, can mediate translation under stress situations triggered by the tumour's microenvironment, contributing to the survival of cancer cells [9, 99, 103, 113]. A common feature of these environments is the difficult access to oxygen. Indeed, cancer cells activate 4E-binding proteins (4E-BP) and inhibit the mTORC1 pathway in response to hypoxic conditions, but at the same time promote a switch to IRES-mediated translation thus maintaining tumour growth and angiogenesis [100, 102]. Large and advanced breast cancers were shown to overexpress 4E-BP and eIF4G and trigger m^7G-independent mRNA translation [102]. In inflammatory breast cancer, cells have adapted to a state of prolonged hypoxia and optimised the production of proteins required for tumour embolus survival and dissemination, a state promoted by high levels of eIF4GI protein coupled with a constitutively active 4E-BP1 [114]. This leads to higher rates of translation in IRES-containing mRNAs, namely VEGF and p120 catenin, which maintain high rates of angiogenesis, and membrane associated E-cadherin, respectively [115].

FGF (Fibroblast growth factors), such as *FGF1* and *FGF2*, are crucial for proliferation and differentiation of a wide variety of cells, and hence their translation has to be tightly regulated—some of them contain IRES elements within their 5′ UTRs, which allow cap-independent translation initiation [116, 117]. IRES-mediated regulation of FGF2 translation is considered a critical step in tumorigenesis, not only in solid tumours but also in multiple myeloma, which turns the FGF2 IRES into the non-cytotoxic primary molecular target of thalidomide, and therefore the preferred target of immunomodulatory drugs in multiple myeloma [114].

c-Myc IRES is also activated in multiple myeloma cells under thapsigangin- or tunicamycin-induced ER stress, or bortezomib (a myeloma therapeutic) treatment, thus maintaining c-Myc protein levels [118].

Sp1 (Specificity protein-1), a protein that is accumulated under hypoxic conditions in an IRES-dependent manner in lung tumour tissue, is another case of a protein whose expression is upregulated during tumorigenesis by activation of IRES-mediated translation, suggesting that translational regulation might contribute to the accumulation of Sp1 during tumorigenesis [119].

CAT-1 synthesis and sodium-coupled neutral amino acid transporter 2 (SNAT2), two amino acid transporters, is controlled by IRES under amino acid or glucose starvation [120, 121].

CAT-1 IRES-dependent translation is induced in tumour cells under glucose deprivation through phosphorylation of eIF2α by the transmembrane endoplasmic reticulum kinase (PERK) [121].

XIAP and sterol regulatory element-binding transcription factor 1 (SREBP-1) are translated via IRES during deprivation of growth factors in tumour cells, protecting them from apoptosis [122, 123]. IRES-dependent translation of these proteins allows the cell to survive under nutritional stress, which is an advantage for cancer cell continued existence [124]. Adding to this, XIAP expression is up-regulated under γ-irradiation through IRES-dependent translation, causing tumour cells to be resistant to radiotherapy [125, 126]. This agrees with the study by Holcik et al., in which they used RNA interference to inhibit XIAP, and saw that it enhances chemotherapeutic drug sensitivity and decreases myeloma cell survival [126].

β-catenin is also translated through internal ribosome entry in human ovarian cancer cells treated with paclitaxel (PTX), a chemotherapeutic drug used in the treatment of ovarian cancer—this then regulates the expression of downstream factors (c-Myc and cyclin D1), reducing PTX sensitivity [127].

c-Myc oncogenic transcription factor and Bcl2-associated athanogene 1 (BAG-1) are also regulated by IRES under oxidative and genotoxic stress and increase tumour cells' resistance to DNA damage-inducing drugs [128–131]. Bcl-2's expression in turn, and cIAP's are again enhanced by etoposide as well as arsenite *via* IRES-mediated translation [132, 133].

p53—a tumour suppressor, protooncogene [134] and transcriptional master regulator of the oxidative and genotoxic stress responses—is also translated through IRES-mediated processes [82, 135–138]. The p53 transcript contains IRES structures that control translation of the full-length (FL) p53 and the N-terminally truncated isoform Δ40p53 from the same mRNA [82, 135]. Several stress conditions that induce DNA damage as well as ER stress induce FLp53 or Δ40p53, respectively, *via* two different IRES structures, one in the 5′ UTR – for FL – and another in the 5′ coding region – controlling mostly Δ40p53 [138]. Furthermore, in response to doxorubicin, IRES-mediated translation of both p53 isoforms is stimulated by the ITAF polypyrimidine tract-binding protein (PTB), following PTB relocation from the nucleus to the cytoplasm [139]. Other ITAFs such as DAP5, Annexin A2, and PTB-associated Splicing Factor (PSF), have also been reported to control p53 IRES activity [140, 141]. Besides, identification of two other p53 ITAFs [translational control protein 80 (TCP80) and RNA helicase A (RHA)] that positively regulate p53 IRES activity, established a connection between IRES-mediated p53 translation and p53 tumour suppressive function in two breast cancer cell lines. Following DNA damage, the levels of TCP80 and RHA are extremely low and these two cell lines exhibited defective p53 induction and synthesis, since expression of both proteins was required to significantly increase p53 IRES activity [142, 143]. Cells devised a critical cellular response that counteracts cellular transformation—the oncogene-induced senescence (OIS)—which is characterized by cell cycle arrest and induction of p53, which prevents the proliferative potential of preneoplasic clones [144]. During OIS, there is a switch from canonical translation initiation to IRES-mediated translation, during which p53 IRES-dependent translation is promoted, providing a molecular barrier for cellular transformation [145].

In conclusion, and considering the aforementioned examples, it seems clear that the IRES-mediated translation of key regulators and pro-survival factors grant tumour cells enough tools for attaining resistance to chemotherapy and radiation [146]. On the other hand, the presence of IRES within transcripts coding tumour suppressor proteins can prevent cancer outbreak by maintaining the protein levels. Expression of some proteins is crucial to determine the cell fate under stress conditions—apoptosis or survival and proliferation. Thus, IRES-mediated translation is of key importance in the process of tumorigenesis. Furthermore, the IRES structures themselves and the cooperating ITAFs are vital targets for cancer treatment.

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