Chapter 3

Translational Control in Tumour Progression and Drug Resistance

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http://dx.doi.org/10.5772/54625

1. Introduction

Protein biosynthesis is a multi-step process that starts with the transcription of nuclear DNA, depository of genetic information, into messenger RNA (mRNA) that is used as template for the following polypeptide chain synthesis, also known as translation. Each step of this essential process is highly controlled in order to modulate any specific protein requirement of the cell in response to different stimuli and cellular events. This regulatory process is called translational control. Deregulation of the core signaling network in translational control, the phosphatidyl inositol trisphosphate kinase (PI3K), Protein Kinase B (PKB or Akt), mammalian target of rapamycin (mTOR) and RAS mitogen-activated protein kinase (MAPK)/MAPK-interacting Kinases (MNK) pathways, frequently occurs in human cancers and leads to aberrant modulation of mRNA translation. However, investigations on the contribution of these two pathways to translational regulation led to the interesting finding that translation factors are also substrate of signaling molecules. Post-translational modifications, including cleavage and phosphorylation, usually affect translational factors activity in protein biosynthesis; on the other hand, direct interaction of translational components with signaling mediators can either activate the pathway in which the mediator is involved or redirect translation factors to other activities, such as cytoskeletal rearrangements. These findings shed light on new functions of translation factors, different from their canonical role in protein synthesis. Taken together, these new functions are an intriguing step forward to the discovery of molecular mechanisms at the base of cellular response during “special” conditions such as cancer and drug resistance.

2. Translational machinery

Protein biosynthesis is a process present in all organisms, eukaryotes and prokaryotes, sharing similar mechanisms. In particular, translation starts at the ribosome and involves
four different stages: initiation, elongation, termination and recycling [1]. All of these stages are tightly controlled by specific translation factors. Many of these factors are GTPases that are activated upon binding to the ribosome on a site called the GTPase-activating centre (GAC) [2]. In eukaryotes, at the initiation point, the 40S ribosomal subunit, carrying the eukaryotic initiation factor 3 (eIF3), is bound by a ternary complex consisting of the eukaryotic initiation factor 2 (eIF2), GTP and methionyl initiator tRNA (Met-tRNAi), to form the 43S preinitiation complex (Fig. 1). The recruitment of mRNA is due to the the eukaryotic initiation factor 4F (eIF4F) complex formed by the cap-binding protein eukaryotic initiation factor 4E (eIF4E), the scaffold protein eukaryotic initiation factor 4G (eIF4G) and the RNA helicase eukaryotic initiation factor 4A (eIF4A), stimulated by the accessory factor eukaryotic initiation factor 4B (eIF4B). 4E binding proteins (4E-BPs) can compete with eIF4G for binding eIF4E thus inhibiting the association with 5’ mRNA cap structures. The eIF4F complex, together with the poly(A)-binding protein (PABP), is able to recognise the 5’-terminal cap or the 3’-terminal poly(A) tract of mRNA and to transfer it to the 43S complex, resulting now in the 48S complex. Once the first AUG has been recognized, the pre-initiation complex formed by the initiation factors enables the binding of the 60S ribosomal subunit to the 40S ribosomal subunit to form the 80S initiation complex, via GTP hydrolysis of eIF2-GTP mediated by the eukaryotic initiation factor 5A (eIF5A) [3]. Many virus infections and stresses can induce a switch from a cap-dependent to a cap-independent initiation of translation. In this case the eIF2 ternary complex binds to an internal ribosome entry site (IRES) present on the mRNA 5’-untranslated region (5’ UTR), driving the translation directly to the 60S association phase [4]. In both cases the complex is now ready to receive the first elongator tRNA and to start with the elongation stage of the biosynthesis.

**Figure 1.** Eukaryotic translation initiation pathway. The initiation of translation in eukaryotes starts with the dissociation of the 40S ribosomal subunit from the 80S subunit probably promoted by eIF3 and eIF1A. Met-tRNA, eIF2 and GTP form a ternary complex that binds to the 40S ribosomal subunit to form the 43S preinitiation complex. The eIF4 factors plus poly(A)-binding protein (PABP) recognize the mRNA [5’-terminal cap or 3’-terminal poly(A)] and transfer it to the 43S initiation complex to form the 48S initiation complex. After the recognition of the first initiation codon by eIF4A, in the presence of eIF1 and eIF1A, eIF5 stimulates GTP hydrolysis by eIF2 and the subsequent replacement of the initiation factors bound to 43S by the 60S subunit to form the 80S initiation complex. The released eIF2-GDP is recycled to eIF2-GTP by the GEF eIF2B. Adapted from Rhoads R.E. et al. [3].
The elongation phase of protein synthesis is a cyclic process consisting of basic steps repeated until the entire coding sequence of the mRNA is translated. In higher eukaryotes the elongation factors work always as a complex; for instance, the elongation factor 1 complex consists of eEF1A and the three subunit (β, γ, δ) of the elongation factor 1B (eEF1B) [5]. During elongation, a GTP-bound eEF1A transports the new aminoacyl-tRNA (aa-tRNA), as a ternary complex (Fig.2), to the empty A site of the 80S initiation complex [6]. In particular, eEF1A•GTP protects the aa-tRNA against hydrolysis and assists the ribosome in making a correct interaction between the current codon on the mRNA and the anticodon of the transported aa-tRNA [7]. Such a decoding event triggers the ribosome to induce GTP hydrolysis on eEF1A [8] and leads to its major conformational change that causes the release of aa-tRNA and the accommodation of the 3' end in the peptidyl transferase (PT) centre on the 60S subunit, followed by peptide-bond formation [9]. In parallel, the inactive GDP-bound eEF1A (eEF1A•GDP) is released from the ribosome. This inactive form of eEF1A, unable to bind another aa-tRNA, is recycled to the active form (eEF1A•GTP) by exchange factor eEF1B. eEF1B consists of three subunits and works as a guanine nucleotide-exchange factor (GEF) for eEF1A [10]. In the following step of elongation, elongation factor 2 (eEF2) catalyzes the translocation of A and P site tRNAs to the P and E sites respectively, as well as movement of the mRNA by exactly one codon to allow a new round of elongation [11]. A-site-bound aa-tRNA reacts with P-site-bound pept-tRNA (peptidyl-tRNA) to form a peptide bond, resulting in deacylated tRNA in the P site and pept-tRNA prolonged by one amino acid in the A site.

Figure 2. Elongation cycle in eukaryotes. Crucial step in the elongation stage is the selection of the correct aa-tRNA, based on codon-anticodon interaction. The ribosome supervises this point during an initial selection of the ternary complex and during proofreading of the aa-tRNA. The initial selection utilizes the ability of cognate tRNA to stimulate the GTPase activity of eEF1A much faster than non-cognate and near-cognate tRNA.

When the ribosome come across one of the stop codons, UAA, UAG or UGA, eukaryoyic release factor 1 (eRF1) is recruited to the ribosome to promote the release of the newly synthesized polypeptide. After termination, the ribosome dissociates into its constituent subunits and the mRNA and deacylated tRNA is released thus allowing the ribosome to be
recycled by eukaryotic release factor 3 (eRF3) in cooperation with the eukaryotic elongation factor 2 (eEF2) [1].

3. Signalling transduction and translational control

Since regulatory mechanisms of protein biosynthesis are essential for maintaining a proper cellular metabolism, it is not surprising that the translation process is closely regulated by the coordinated activity of multiple intracellular signalling pathways acting at the centre of translational control. In eukaryotes, the most important pathways regulating the translation apparatus are the PI3K/Akt/mTOR and the Ras-MAPK signalling cascades [5] that can be stimulated by nutrient, insulin, growth factors and energy status (Fig. 3). Activation of these pathways mediates modifications such as changes in the phosphorylation states of translation factors and specific RNA-binding proteins, resulting in a translational machinery’s activation or inhibition. Given the complexity of these two pathways, already well reviewed by several groups (we highly recommend Sonenberg N. et al. [5] and Proud C.G. [12]), in this paragraph we will just give a brief introduction (summarized in Table 1) of the main regulatory proteins participating in translational control and their influence on protein synthesis.

The lipid kinase PI3K is an important signalling mediator and its activation produces an increase of phosphatidyl inositol 3,4-biphosphate that activates downstream effectors such as the protein kinase B (PKB) also named Akt. The action of PI3K is antagonized by the lipid phosphatase and tensin homologue deleted on chromosome 10 (PTEN) [13]. Mutations in PTEN, present in many human tumours, lead to a constitutive activated Akt and mTOR signalling [14]. In translational control, PI3K plays its regulatory role by the activation of Akt and the consequent suppression of glycogen synthetase kinase 3 (GSK3) and its inhibitory activity on eIF2B [15]. Moreover PI3K can modulate mTOR signalling through Akt.

mTOR is a member of the phosphoinositide 3-kinase-related kinase (PIKK) family and exhibits protein kinase activity; some but not all mTOR functions are specifically repressed by rapamycin [16]. Rapamycin forms a complex with the immunophilin FK506 binding protein-12 (FKBP12) that binds to the FKBP12-rapamycin binding (FRB) domain of mTOR and inhibits its kinase activity. In mammalian cells, two functionally distinct mTOR complexes exist: mTOR complex 1 (mTORC1), containing mTOR, Raptor, and LST8; and mTOR complex 2 (mTORC2), containing Rictor, LST8, and Sin1. mTORC2 regulation and function remain largely unknown, although this complex has been linked to cytoskeletal rearrangements and cell survival through Akt [17]. mTORC1, among its many functions, promotes protein translation through activation of the S6 kinases (S6ks) and inhibition of the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) [18]. mTORC1 signalling can be modulated by PI3K through Akt. In particular Akt negatively regulates the tuberous sclerosis complex 2 (TSC2) GAP activity on the mTORC1 complex. TSC2 is a GTPase-activating protein (GAP) for the small G-protein Rheb [19] and forms a dimeric complex with TSC1. Rheb is a G-protein that stimulates mTOR activity [20]. Akt-mediated
phosphorylation of TSC2 leads to the inhibition of its GAP activity towards Rheb, allowing Rheb to accumulate in its GTP-bound state and leading to the activation of mTORC1. mTORC1 signalling, through activation of Akt, is also stimulated in case of loss of PTEN function. Interestingly, stimulation of mTORC1 resulting from constitutive Akt activation leads to a transformed phenotype. In fact, the proliferation of some tumour-derived cell lines (e.g. those lacking the tumour suppressor PTEN) can be inhibited by rapamycin, confirming a key role for signalling through mTORC1 [21]. mTORC1 is also linked to a range of other oncogenes or proto-oncogenes, including Ras, nuclear factor (NF) and the liver kinase B1 (LKB1) [22]. mTOR pathway can also positively modulates eEF2 activity by phosphorylating and suppressing the Ca2+/calmodulin-dependent kinase III (CaMKIII) ability to bind to calmodulin [23]. CaMKIII, also known as calcium/calmodulin-dependent eukaryotic elongation factor 2 kinase (eEF2K) is a specific calcium/calmodulin-dependent enzyme that regulates protein synthesis [24]. The only known substrate of this kinase is eEF2. eEF2K phosphorylates eEF2 on T56 and stops peptide elongation by decreasing the affinity of eEF2 for the ribosome. Thus, eEF2K-mediated phosphorylation acts as an internal negative regulator of eEF2 translational activity [25].

**Figure 3.** Translational control by signal transduction pathways. Activated signalling pathways modulate translation factors activity and mRNA-specific binding proteins regulating the rates of protein synthesis and translation and/or the stability of specific mRNAs. Adapted from Proud C.G. [12].
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Table 1. Signalling transduction in protein biosynthesis. Overview of the kinases involved in post-translational modifications on translation factors and related proteins and their effects on translational control. For more details and references see the text.

Classical MAPKs extracellular signal regulated kinase 1 and 2 (ERK1/2) and p38 MAPK a/b and c-Jun N-terminal kinase (JNK) pathways are the best understood MAPK signalling cascades in mammalian cells. The regulation of translational machinery through the MAPK signalling cascades is clearly connected with extraordinary events such as cancer and transformation. Each cascade involves downstream kinases that phosphorylate components of the translational machinery. In particular, ERK1/2 signalling activates p90 ribosomal s6 kinases (p90rsk) that phosphorylate several translation factors or their regulators, including TSC2 [26], creating a link between ERK1/2 cascade and mTOR signalling [27]. Moreover
p90\textsuperscript{rsk} phosphorylates eEF2 kinase, inhibiting eEF2 kinase activity [28], and eIF4B, promoting its association with eIF3 [29]. p38 MAPK a/b regulates the activation of the MAPK-activated protein kinase 2 (MK2) that mediates phosphorylation of TSC2 too [30]. Interestingly, MK2 is also able to control both the stability and the translation of some mRNAs, such as the tumour necrosis factor α (TNF-α) mRNA, containing at their 3'UTR a particular sequence called adenine/uridine-rich element (ARE), important for modulating the expression of specific proteins. It is likely that this regulating mechanism involves MK2-mediated modification of ARE binding proteins (ARE-BPs) [31]. MAPK signal-interacting kinases 1 and 2 (Mnk1 and 2) are both substrates for either ERK1/2 or p38 MAPKs a/b. During translational control, their activation leads to the phosphorylation of eIF4E [32] and eIF4G [5]. Phosphorylation of the former decreases eIF4E ability to bind 5'cap structure while phosphorylation of the latter has unknown consequences.

Ribosomal S6 kinase (RSK) is involved at different levels in signal transduction. There are two subfamilies of RSK: p90\textsuperscript{rsk}, also known as MAPK-activated protein kinase-1 (MAPKAP-K1), and p70\textsuperscript{rsk}, also known as S6 Kinases (S6Ks). There are four variants of p90\textsuperscript{rsk} in humans (RSK 1-4) and two known mammalian homologues of S6Ks: S6K1 and S6K2. S6Ks are implicated in the positive regulation of cell growth and proliferation [33]. Once activated, S6Ks phosphorylate the S6 protein of the 40S ribosomal subunit and the translation initiation factor eIF4B to promote translation. S6Ks regulate mTOR through a negative feedback signalling pathway that affects insulin receptor substrate-1 (IRS-1). S6Ks was shown to directly phosphorylate IRS-1 to inhibit PI3K and Akt activation [34]. S6Ks activation decreases IRS-1 expression while rapamycin treatment restores IRS-1 expression [35]. Due to their major function in regulating translation, S6Ks are required also for cell growth and G1 cell cycle progression. Interestingly, S6K1 activation correlates with enhanced translation of a subset of mRNAs that contain a 5'-tract of oligopyrimidine (TOP mRNAs). These mRNAs encode for ribosomal proteins, elongation factors, the poly-A binding protein and other components of the translational machinery that become selectively translated by their TOP sequences in response to growth factors. However, S6Ks are not essential for the regulation of TOP mRNA translation [36].

AMP activated protein kinase (AMPK) is sensitive to the reduction of cellular content of AMP directly connected to that of ATP. In fact, in case of ATP decrease, cells need to slowdown protein synthesis in order to save energy; AMPK quickly reacts to this impair and negatively regulates mTORC1 via TSC2 phosphorylation [37]. Moreover AMPK can also modulate eEF2 activity by phosphorylating and activating eEF2K. The eEF2 phosphorylation mediated by eEF2K down-regulates eEF2 translational activity [38].

As above described, the best known mechanisms of translational control involve the initiation factors (2, 2B, 4B, 4E e 4G), the elongation factor 2 and the S6Ks. Less is known about the regulation of eEF1A and eEF1B even though several groups have studied the phosphorylation and regulation of these two factors [39]. Hereafter are summarized the current knowledge. The casein kinase 2 (CK2) phosphorylates the β subunit of eEF1B in vitro [40] leading to a decreased affinity of eEF1B toward eEF1A. Insulin or phorbol esters are
able to enhance the phosphorylation of eEF1A and eEF1B in vivo [41]. Experimental results, including phosphopeptide-mapping, showed that insulin-stimulated multipotential S6 kinase was able to highly phosphorylate eEF1A and two subunits of the eEF1B complex (EF-1β and EF-1δ) from rabbit reticulocytes. However, phosphorylation of these proteins by S6 kinase in vitro resulted in a modest stimulation of their activity [42]. eEF1A and eEF1B are also substrates of protein kinase C (PKC) in vitro and this may explain the ability of phorbol esters (which activate several PKC isoforms) to increase the phosphorylation of these proteins in vivo. More precisely, PKCδ phosphorylates eEF1A at Threonine 431 (based on murine sequence) [43]. Phorbol esters also increase the phosphorylation of the valyl-tRNA synthetase that associates with eEF1A/B. The available evidence suggests that phosphorylation of eEF1A/B and of valyl-tRNA synthetase by PKC increases their activities in translation elongation and aminoacylation, respectively. The increased activity of eEF1A/B appears to result from enhanced GEF activity [44].

3.1. Translational control in apoptosis and tumour therapy

Generally, decrease in protein synthesis is an important adaptive mechanism that allows the cell to conserve or direct energy to other cellular functions. For example, upon induction of apoptosis a drastic reduction of protein biosynthesis occurs that precedes the loss of cell viability and the irreversible commitment to cell death [45, 46]. In fact, prior to and during the pro-apoptotic signal several factors with translational activity are modified. These modifications mainly include a specific caspase activity, prevented by the cell-permeable caspase-inhibitor z.VAD.FMK, and changes in the translation factors phosphorylation rates. In particular, eIF4B as well as eIF4GI, eIF4GII and eIF3j subunit are substrates of caspase-3 that mediates their cleavage and degradation following several pro-apoptotic stimuli [47]. In addition, eIF2, eIF4E and small 4E-BPs are highly phosphorylated during apoptosis with an inhibitory effect on protein synthesis [48]. The strong repression of translation initiation factors should lead to a complete inhibition of protein biosynthesis; however, a certain quote of cellular mRNA contain in their 5’ UTR an IRES region that allows a cap-independent translation. IRESs directed translation is relatively inefficient under physiological conditions which favour cap-dependent translation, whereas it functions when cap-dependent translation is compromised [49]. Thus it is not surprising that cellular genes containing IRESs in their mRNAs, usually code for proteins that are involved in different cellular processes, including apoptosis. For instance, cleaved fragments of eIF4GI are able during apoptosis to enhance IRES-translation of the apoptotic protease-activating factor1 (Apaf-1) [50]. Another interesting example of translational control during apoptosis is the eukaryotic initiation factor 5A (eIF5A) and its peculiar post-translational modifications. eIF5A activity is modulated by a series of modifications that trigger the formation of the unusual amino acid hypusine [N-(4-amino-2-hydroxybutyl) lysine]. Hypusine plays a key role in the regulation of eIF5A function, as only the hypusine-containing eIF5A form is active. Intracellular hypusine content measures also the activity of eIF5A, as hypusine is contained only in this factor. Reduction of eIF5A1 expression or inhibition of hypusine modification may cause induction or suppression of apoptosis, depending on the biological system [51].
Besides eIFs, also other translation factors are involved in pro-apoptotic signalling. For example, upon treatment with antitumoural agents, eEF2 was shown to be involved in the therapeutic mechanism of doxorubicin. Treatment of prostatic cancer cells (PC3) with doxorubicin suppresses protein synthesis by inhibition of the elongation phase and not the initiation phase. This effect is probably mediated by a kinase independent phosphorylation of eEF2. Furthermore, inhibition of elongation activity correlates with decreased expression of the anti-apoptotic cellular FLICE-like inhibitor protein (cFLIPS), XIAP and survivin, all characterized by a short half-life and anti-apoptotic activity. These events result in a sensitization of the cells to the tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL) promoting, therefore, the doxorubicin apoptotic phenotype. TRAIL is a member of the TNF family capable to induce apoptosis in a wide variety of cancer cells upon binding to pro-apoptotic receptors, whereas it has no effect on the majority of normal human cells tested. Translation was found significantly inhibited in NIH3T3 cells also during taxol-induced apoptosis mediated by calpain [52]. Taxol treatment strongly decreased eIF4G, eIF4E and 4E-BP1 expression levels. However, a specific inhibitor of calpain, MDL28170, prevented reduction of eIF4G, but not of eIF4E or 4E-BP1 levels and did not block taxol-induced translation inhibition. Conversely, taxol treatment increased eEF2 phosphorylation in a calpain-independent manner thus supporting a role for eEF2 in taxol-induced translation inhibition [53].

3.1.1. Eukaryotic elongation factor 1A

Mentioning the relationship between apoptosis and translation, one cannot fail to mention the peculiar and fascinating role of the eukaryotic elongation factor 1A (eEF1A). In fact, eEF1A is a GTP binding protein that plays a central role in the elongation cycle of protein biosynthesis however, several studies suggest that eEF1A displays additional roles in different cellular processes far from its canonical role [54, 55]. eEF1A forms complexes with other cellular components like tubulin and actin. It has been observed that eEF1A cross-links actin filaments and it is implicated in microtubule binding, bundling or severing. Indeed, eEF1A mutants alter actin cytoskeleton organization but not translation, indicating a direct role of eEF1A on cytoskeletal organization in vivo [56]. In addition, eEF1A is known to be involved in several cellular process, including embryogenesis, senescence, oncogenic transformation, cell proliferation and organization of cytoskeleton [57]. In higher vertebrates, eEF1A is present in two isoforms (eEF1A1 and eEF1A2) with a different expression patterns and encoded by distinct genes [58]. The near-ubiquitous form, eEF1A1, is expressed in all tissues throughout development but is absent in adult muscle and heart expressing eEF1A2 instead [58, 59]. eEF1A2 is also found in some other cell types including large motor neurons, islet cells in the pancreas and enteroendocrine cells in the gut [60, 61]. Despite sharing 92% sequence identity, paralogous human eEF1A1 and eEF1A2 have different functional profiles. They exhibit similar translation activities but have different relative affinities for GTP and GDP [62] and, surprisingly, eEF1A2 appears to show little or no affinity for the components of the guanine-nucleotide exchange factor (GEF) complex eEF1B in yeast-two-hybrid experiments [63]. Moreover, as recent structural studies suggest,
the two eEF1A isoforms display different behavior as tyrosine phosphorylation substrate that could affect their interaction with different signalling molecules. In fact, while eEF1A1 is able to interact with adaptor proteins containing SH2 domains, eEF1A2 is instead able to bind both SH2 and SH3 protein containing domains [64] thus suggesting for eEF1A2 a greater involvement in phosphotyrosine-mediated signalling processes [65]. Interestingly, the two isoforms play opposite roles during apoptosis. eEF1A1 expression has a marked pro-apoptotic effect, whereas expression of eEF1A2 correlates with differentiation and works as an inhibitor of caspase-mediated apoptosis (see next paragraph). In particular, antisense eEF1A1 provides the cells with significant protection from cell death upon induction of apoptosis by serum deprivation, vice versa eEF1A1 over-expression leads to a faster rate of cell death [66]. Moreover, eEF1A1 protein levels undergo rapid increase upon treatment with lethal doses of H$_2$O$_2$; pre-treatment of rat heart myoblast cells H9c2(2-1) with transcriptional inhibitors fails to abolish the oxidant-induced increase in eEF1A1. Furthermore, eEF1A1 mRNA levels remain steady throughout H$_2$O$_2$ treatment, suggesting that the up-regulation of eEF1A1 is mediated post-transcriptionally. Transient depletion of eEF1A1 protects the cells against H$_2$O$_2$-mediated cytotoxicity in proportion to the degree of repression of eEF1A1 protein levels thus suggesting that up-regulation of eEF1A1 plays a role in expediting the execution of the apoptotic program in response to oxidative stress [67]. Interestingly, upon serum deprivation-induced apoptosis, eEF1A2 protein disappears and is replaced by eEF1A1 in dying myotubes. In addition, continuous expression of eEF1A2 protects differentiated myotubes from apoptosis by delaying their death thus suggesting a prosurvival function for eEF1A2 in skeletal muscle. In contrast, myotube death is accelerated by the introduction of the eEF1A1 homologues gene [68]. Investigations of eEF1A1 functional role related to apoptosis seems to be particulary promising as it may affect both protein synthesis and cytoskeletal organization, fundamental events during death signalling. A recent example of eEF1A1 involvement in apoptosis suggests that this protein mediates lipotoxic cell death through a mechanism independent from changes in the rates of protein synthesis. Since eEF1A1 plays an important role in remodelling microtubules and filamentous actin [56, 69, 70] and because the cytoskeleton undergoes dramatic changes during apoptosis and cell death, eEF1A1 may mediate cytoskeletal changes required to execute cell death programs in response to lipotoxic conditions [71].

3.2. Anti-apoptotic activity in cancer cells. Role of translation factors

Several factors of the translational machinery are involved at different levels in tumour progression with a strong support of the anti-apoptotic activity characteristic of cancer cells. In general, survival of most mammalian cells is dependent on extracellular signals that suppress programmed cell death. Recent studies have shown that survival factors prevent apoptosis through the activation of PI3 kinase (PI3k) pathway [72] and its downstream effector, the protein-serine/threonine kinase Akt [73]. PI3k/Akt signalling acts upstream of mitochondria preventing the release of cytochrome c and subsequent activation of cytosolic caspases [74]. However, the targets of PI3k/Akt signalling that promote cell survival remain to be fully elucidated. Interestingly, expression of the non-phosphorylatable eIF2B mutant
prevents cytochrome c release upon inhibition of PI3k whereas, inhibition of translation with cycloheximide induced cytochrome c release. Regulation of translation resulting from phosphorylation of eIF2B thus appears to affect the apoptotic cascade upstream of mitochondria, most likely interfering with PI3k/Akt signalling [75]. The mTOR/eIF4F axis is also an important contributor to tumour maintenance and progression program in terms of anti-apoptotic activity. Suppression of mTOR activity and that of the downstream translation regulators, including eIF4E, delays breast cancer progression, onset of associated pulmonary metastasis in vivo and breast cancer cell invasion and migration in vitro. eIF4E regulates the recruitment of mRNA to ribosomes, and thereby globally regulates cap-dependent protein synthesis. However, its over-expression contributes to malignancy by selectively enabling the translation of a limited pool of mRNAs that generally encode for proteins involved in cellular growth, angiogenesis, survival and malignancy. Translation of vascular endothelial growth factor (VEGF), matrix metallopeptidase 9 (MMP9) and cyclin D1 mRNAs, encoding for products associated with the metastatic phenotype, is indeed inhibited upon eIF4E suppression. Transgenic eIF4E-expressing mice show a marked increase in tumourigenesis by developing tumours of various histologies. Thus, eIF4E acts as an oncogene in vivo [76]. Moreover, over-expressed eIF4E prevents Myc-dependent apoptosis, at least in part, through a cyclin D1-dependent process [77] and in part by its ability to increase cellular levels of BclXl, a key apoptotic antagonist [78].

Because high level of protein synthesis is one of the characteristics of cancer cells, the elongation cycle has recently gained much more attention in this field as it seems to be directly involved in cell survival. eEF2, a critical enzyme of the elongation cycle, has been investigated as a target for new therapies and as a potential contributor to the success of conventional therapies. Interestingly, eEF2 is highly expressed in lung adenocarcinoma (LADC), but not in the non-tumour lung tissue. High eEF2 expression correlates with a significantly higher incidence of early tumour recurrence, and a significantly bad prognosis. Silencing of eEF2 expression increases mitochondrial elongation, cellular autophagy and cisplatin sensitivity. Moreover, eEF2 was found sumoylated and this sumoylation correlates with drug resistance. In particular, sumoylation of eEF2 is essential for protein stability and cell survival against cisplatin in LADC cells. Taken together, these results suggest eEF2 as an anti-apoptotic marker in LADC [79]. eEF2 protein is also over-expressed in 92.9% of gastric and 91.7% of colorectal cancers with no mutations in any of the exons of the eEF2 gene. Over-expressed eEF2 significantly enhances the cell growth through promotion of G2/M progression in cell cycle, activating Akt and cdc2 (G2/M regulator), and inactivating eEF2 kinase (negative regulator of eEF2). Conversely, knockdown of eEF2 inhibits cancer cell growth and induces G2/M arrest. These results provide a novel linkage between translational elongation and cell cycle mechanisms [80]. The implication of eEF2K (CaMKIII) in cancer was suggested by the observation that this kinase is up-regulated in various types of tumours such as malignant glioma and breast cancer. Inhibition of eEF2K results in a decreased viability of tumour cells. eEF2K was previously demonstrated to phosphorylate and in turn down-regulate eEF2 activity. However, eEF2K is not only a negative regulator of protein synthesis but also a positive regulator of autophagy, under environmental or
metabolic stresses. Similarly, aberrant activation of Akt promotes cell growth, survival and proliferation, and is associated with cancer development and progression. Akt represents an attractive target for therapeutic intervention against cancer. Akt inhibitors, such as the allosteric small molecule MK-2206, induce either apoptosis or autophagy. Interestingly, recent studies demonstrated that silencing of eEF2K, upon Akt inhibition, can blunt autophagy and augment apoptosis, thereby modulating the sensitivity of cancer cells to Akt inhibitors. Thus, targeting eEF2K reinforces the anti-tumour efficacy of Akt inhibitors, such as MK-2206, by promoting the switch from autophagy to apoptosis [81].

As widely demonstrated, eEF1A is also clearly connected with cancer progression and survival. In humans, eEF1A2 shows oncogenic properties when over-expressed; moreover, it is implicated in ovarian, breast, pancreatic, liver and lung cancer thus becoming one of the most intriguing putative onco genes in the last decade [82]. Notably, eEF1A2 can either directly or indirectly activate the Akt signalling pathway. Previous studies assessed a direct interaction between eEF1A2 and phosphorylated Akt 1 and 2 (pAkt) in breast cancer. eEF1A2 regulates pAkt levels promoting cell survival, tumour progression and motility [83]. In mouse fibroblast cell line NIH3T3, eEF1A2 interacts with peroxiredoxin-I (Prdx-I), resulting in increased activation of Akt, reduced activation of caspases 3 and 8, and protection against apoptotic death [84]. Moreover, downregulation of eEF1A2 expression leads to decreased expression of pAkt1 and to less extent of pAkt2 and promotes apoptosis [85]. Thus, eEF1A2 interaction with pAkt represents an important mechanism for the regulation of Akt-dependent survival signalling pathways in cancer [85, 86].

4. Survival and chemotherapy failure

Many chemotherapeutic agents exert their cytotoxic effects through the induction of apoptosis however, despite the fact that many tumours initially respond to therapy, tumor cells can subsequently survive by gaining resistance to these treatments. Therefore, emergence of drug resistance during chemotherapy is a major cause of cancer relapse and consequent therapy failure. In the last years there is an increase evidence that also translation factors participate in the control of tumor chemoresistance with mechanisms not yet well understood. For example, the initiation factor 4E (eIF4E) that is overexpressed in many solid tumors, plays a role not only in cell growth and proliferation but also in the apoptotic response and in the acquisition of drug resistance [87]. In fact, eIF4E controls the translation on an increasing number of mRNAs encoding proteins with notable functions in all aspects of malignancy, including angiogenesis and invasiveness through the activation of the ras and phosphatidylinositol 3-kinase/AKT anti-apoptotic pathways [78, 88, 89]. These findings clearly suggest that eIF4E is a promising target for anticancer therapy.

Also translation elongation factors can be involved in the regulation of drug resistance. For instance, eEF2 is phosphorylated at Thr56 by eEF2K thus terminating peptide elongation by decreasing its affinity for the ribosome. eEF2K is up-regulated in several types of malignancies, including gliomas, and affects the sensitivity of cancer cells to treatment with
the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). This ligand is considered a promising candidate as an anticancer agent based on its ability to trigger rapid apoptosis and its specific cytotoxicity in malignant cells by binding to the death receptors DR4 (TRAIL-RI) and DR5 (TRAIL-RII). Inhibition of eEF2K by RNA interference (RNAi) or by a pharmacological inhibitor (NH125) recovers sensitization of tumour cells to TRAIL-induced apoptosis through down-regulation of the anti-apoptotic protein, Bcl-x. [90]. These results indicate a possible therapeutic strategy for enhancing the efficacy of TRAIL against malignant cells by targeting eEF2 kinase.

Taken together, these studies suggest a role for translation factors and translational control signalling pathways in drug resistance and chemotherapy failure. The elucidation of basic molecular mechanisms leading to chemoresistance is an essential step in the development of new anti-neoplastic therapies.

4.1. Interferon alpha chemotherapy resistance. A new mechanism mediated by eEF1A

Since the advent of genetic engineering technology, recombinant IFNα has been largely employed in solid tumours treatments. To date, interferon therapy is used in combination with chemotherapy and radiation as a treatment for many cancers, and several clinical trials are currently ongoing. However, IFNα displays a limited activity, and several cancers are resistant to its anti-tumour function having developed mechanisms not completely elucidated yet. In human epidermoid cancer cells, IFNα induces growth inhibition and apoptosis most likely through the activation of caspase cascade mediated by JNK-1 and/or p38 MAPK activation and the mitochondrial pathway [91]. Furthermore, a concomitant reduction of the hypusinated eIF5A1 expression levels and eIF5A1 activity is also observed (Figure 4) [92]. These anti-proliferative and pro-apoptotic activities are all antagonized by the epidermal growth factor (EGF); notably, IFNα was found to increase the functional expression of the epidermal growth factor receptor (EGFR), participating itself in the EGF-mediated survival pathway. Moreover, the increase of EGFR leads to an hyperactivation of the Ras-dependent MAPK (Ras->Raf-1->Mek1->Erk-1/2) signalling further stimulated by the addition of EGF and with a prominent role in the antiapoptotic effects exerted by EGF. In particular, Raf-1 activity is increased by either EGF or IFNα and is potentiated after EGF addition. Raf-1, also known as C-Raf, is a member of the Raf kinase family of serine/threonine-specific protein kinases, composed by three members: Raf-A, B-Raf and C-Raf. It functions downstream of the Ras subfamily of membrane associated GTPases to which it binds directly. Once activated, C-Raf can phosphorylate and activate the protein kinases MEK1 and MEK2 that in turn phosphorylate to activate ERK1 and ERK2. Interestingly, C-Raf is known to exert both kinase-dependent and kinase-independent tumour-promoting functions in several cancers [93]. Our studies correlate its increased activity in human epidermoid cancer cells, during the survival response upon IFNα treatment, with the over-expression and post-translational modifications of eEF1A.
In particular, in human epidermoid lung cancer cells H1355, we found that upon treatment of the cells with INFα, both eEF1A1 and eEF1A2 protein levels increased but with a different degree since eEF-1A2 was the most up-regulated isoform. These data suggested that eEF1A2 increase was largely responsible for the upregulation of total eEF1A. To investigate the potential role of the increase in eEF1A protein levels, the apoptotic response to IFNα treatment was evaluated in H1355 cells in which eEF1A was down modulated by siRNA. In cells expressing low levels of eEF1A, the apoptotic cell death induced by IFNα was potentiated thus suggesting that eEF1A participate in these cells in the regulation of apoptosis. The increase of eEF1A levels mediated by INFα was also associated to phosphorylation of eEF1A on serine and threonine residues. These post-translation modifications have shown to be directly involved in the EGF-mediated survival response (see above) since the C-Raf inhibitor (BAY 43–9006) induces a decrease of eEF1A phosphsphorylation. These data suggest the existence of an anti-apoptotic network between the translational factor 1A and the Ras-dependent signalling [94]. More specifically, we found that both eEF1A1 and eEF1A2 were singularly phosphorylated by B-Raf in vitro, whereas phosphorylation by C-Raf required the presence of both isoforms. Two new phosphorylation sites have been identified: T88 and S21. The former was specifically mediated by B-Raf on eEF1A1, whereas the latter was present on both eEF1A isoforms and mediated by both B- and C-Raf kinases. T88 phosphorylation was also identified on eEF1A1 expressed in proliferating COS 7 cells thus suggesting that this post-translational modification is isoform specific and probably due to structural differences between eEF1A1 and eEF1A2. PhosphoT88 might stabilize in vivo the elongation complex and improve then...
protein biosynthesis. In contrast, phosphorylation of S21, that belongs to the first GTP/GDP-binding consensus sequence (G14HVDSGKST in both eEF1A1 and eEF1A2), could potentially prevent the binding of eEF1A to guanine nucleotides thus switching eEF1A activity to different non-canonical functions. The finding that C-Raf required the presence of both eEF1A isoforms for its phosphorylation activity in vivo suggested that this switch might be regulated by the formation of a potential eEF1A heterodimer. Remarkably, eEF1A dimerization has been already described for *Tetrahymena* eEF1A. In this case, eEF1A bundles filamentous actin (F-actin) through dimer formation whereas eEF1A monomer do not [95]. A 3D model of the eEF1A1/eEF1A2 heterodimer was generated using as template the structure of yeast eEF1A (PDB ID: 1F60, chain A). As reported in Figure 5, the obtained docking model supports the possibility of a heterodimer between eEF1A1 and eEF1A2. In particular, this model shows that the M-domain of one isoform is in contact with the G-domain of the other and vice versa. The heterodimer formation somehow could induce a conformational change in one or in both eEF1A isoforms that allows the phosphorylation of S21. These speculations are confirmed by the finding that neither eEF1A1 nor eEF1A2, which were expressed in normal proliferating COS 7 cells, where the mitogenic cascade is particularly strong, showed any modifications of S21. However, phosphorylation on serine 21 might occur in tumour cells following the activation of a signal transduction pathway inducing tumourigenesis [96].

The discovered mechanism might gives a potential key resolution for the IFNα therapy resistance in lung cancer cells. Connection between translational control and mitogenic cascade open a new intriguing field of research in which studying the underlying mechanism of a potential Raf mediated regulation of eEF1A. The link between protein synthesis machinery and growth factor-elicited survival pathway represents an important molecular target to improve strategies based on apoptosis induction.

![Figure 5. Imitation of a 3D model of an eEF1A1•eEF1A2 heterodimer. The heterodimer representation was obtained from the molecular docking pdb file (r-1.pdb) using PyMol software (DeLano Scientific LLC, San Carlos, CA, USA). In both eEF1A isoforms, the position of S21 and T88 are highlighted.](image-url)
5. Conclusions

Events that cause alterations in protein synthesis and translational control have a particular role in the molecular mechanisms underlying cancer development and progression. Interestingly, several translation factors can be directly involved in signal transduction pathways, interact with oncogenes or probably act themselves as oncogenes. Alterations in translational control are also often associated with the molecular events participating in cell transformation, tumour development and progression, apoptosis induction or inhibition. All together, these evidences give translational control a central role in tumourigenesis and response to anti-neoplastic therapies. Thus, a deeper investigation of the specific changes in the translation apparatus for certain types of human cancers, in relation to their stage, grade, histopathology and exposure to standard anticancer therapies should be carried out. Understanding molecular alterations in translational control in each of these contexts can furnish possible indications to improve the use of therapeutic strategies in human cancer. In conclusion, we think that one possible way to improve tumour therapies is to better clarify specific cancer-associated changes in the translation machinery. This research could probably give the opportunity to develop selective anti-tumour translation inhibitors directed towards specific translational targets.

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Acknowledgement

This work was supported by funds from Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale 2008 (2008BKRFBH_003) and PON Ricerca e Competitivita’ 2007–2013 (PON01_02782). CS and PA were recipients of ‘Deutsch-Italienisches Hochschulzentrum’ (Progetto Vigoni 2008–2009). We are also grateful to Dr. Piero Ocone for valuable discussion and ideas.

6. References


[70] Pittman YR, Kandl K, Lewis M, Valente L, Kinzy TG. Coordination of eukaryotic translation elongation factor 1A (eEF1A) function in actin organization and translation
elongation by the guanine nucleotide exchange factor eEF1Balpha. J. Biol. Chem. 2009;284:4739-4747.


