Cell Death and Differentiation (2005) 12, 541–546

www.nature.com/cdd

News and Commentary

The eukaryotic initiation factor 4E-binding proteins and apoptosis

CG Proud*,[1]

[1] Division of Molecular Physiology, School of Life Sciences, University of Dundee, Dundee, UK
* Corresponding author: CG Proud. Tel: +44 1382 344919; Fax: +44 1382 345507; E-mail: c.g.proud@dundee.ac.uk

Cell Death and Differentiation (2005) **12,** 541–546.
doi:10.1038/sj.cdd.4401588
Published online 1 April 2005

Eukaryotic initiation factor 4E (eIF4E) is a key component of the translational machinery that is required for cap-dependent translation. It also exerts antiapoptotic effects and can transform cells. However, some mRNAs do not require eIF4E as they are translated by mechanisms involving internal ribosome entry. Overexpression of eIF4E is associated with cell transformation and can protect cells against apoptosis. eIF4E's function in cap-dependent translation is blocked by eIF4E-binding proteins, such as 4E-BP1. 4E-BP1 is inhibited by phosphorylation mediated through the mammalian target of rapamycin pathway, which can exert antiapoptotic effects. In response to cell stresses, for example DNA damage, 4E-BP1 undergoes dephosphorylation favouring its binding to eIF4E and thus inhibiting cap-dependent translation. Furthermore, in apoptotic cells, 4E-BP1 undergoes caspase-dependent cleavage, which removes an important regulatory motif and so causes its dephosphorylation and binding to eIF4E. These events impair cap-dependent translation and are likely to lead to a switch in protein synthesis to favour cap-independent mRNAs. Synthesis of several proteins that have pro- or antiapoptotic functions is thereby enhanced, altering the balance of apoptotic signaling.

eIF4E, eIF4F and translation initiation

Eukaryotic initiation factor eIF4E binds to the 5′-cap structure found on all nuclear-encoded mRNAs.[1] This structure includes a 7-methylated guanosine moiety linked by a 5′-5′ phosphodiester bond to the first nucleotide of the mRNA proper. This interaction is considered to play a key role in recruiting ribosomes to the 5′-end of mRNAs to facilitate their translation. The translation of most mRNAs is therefore dependent upon recruitment of eIF4E. However, the translation of certain mRNAs is independent of the 5′-cap and thus of eIF4E by virtue of the presence within them of internal ribosome entry sites (IRES). IRES-containing mRNAs are discussed in more detail below.

eIF4E also binds to partner proteins. In particular, in interacts with eIF4G, a multidomain scaffold protein that forms complexes containing a number of other proteins that are either components of the basal translation machinery or regulate this process. There are two eIF4G genes in mammals (eIF4GI, eIF4GII) and further complexity arises due to the possibility of alternate start site usage, which generates additional eIF4G isoforms (e.g. for eIF4GI).[2]

The partners for eIF4G include the ATP-dependent RNA helicase eIF4A. This protein also occurs as two main cytoplasmic isoforms (eIF4AI, eIF4AII). eIF4A is thought to facilitate ribosomal scanning from the 5′-cap by facilitating unwinding of secondary structure within the 5′-untranslated region (UTR) that would otherwise impede scanning. The heterotrimeric protein complex eIF4E/4G/4A is often termed eIF4F. eIF4A function is enhanced by eIF4B, which also binds eIF4G.[1] Binding to eIF4G also enhances the helicase activity of eIF4A, so that eIF4F exhibits higher helicase activity than free eIF4A.

eIF4G also binds the poly(A)-binding protein (PABP): the fact that eIF4E and PABP both bind eIF4G means that the two ends of the message can be brought into proximity, thus circularising it. This cap/tail interaction greatly increases the efficiency of translation.[3]

The component involved in recruiting the ribosome (actually, the 40S subunit) to the mRNA is eIF3, a multisubunit protein that also binds eIF4G. There are additional interactions of eIF4G with eIF5 (a GTPase-activator protein) and the MAP kinase-interacting kinases (Mnks), which phosphorylate eIF4E and modulate its affinity for capped mRNA.[4]

The interaction with eIF4G involves a region on the so-called 'dorsal' face of eIF4E. The region of eIF4G that interacts with eIF4E contains a motif containing key hydrophobic residues, which is also found in two other kinds of proteins. These are 4E-T (a protein that can shuttle eIF4E between cytoplasm and nucleus[5]) and the eIF4E-binding proteins (4E-BPs). There are three 4E-BPs in humans, 4E-BP1-3, of which 4E-BP1 is by far the best understood (see Figure 1). Its function is regulated by phosphorylation, which occurs at multiple sites and decreases its ability to bind eIF4E and thus block eIF4F complex formation.

Certain viruses (e.g. picornaviruses such as poliovirus[6]), whose mRNA is translated in a cap-independent IRES-driven manner, inactivate eIF4F and cap-dependent translation via cleavage of eIF4G such that the N-terminal fragment that binds PABP and eIF4E is separated from the C-terminal portion that binds eIF3, eIF4A and the Mnks. Since translation of the viral mRNA is cap-independent, it does not require intact eIF4G/eIF4F and can proceed, but cap-dependent host cell translation is inhibited. Host cell ribosomes and translation factors are thus not engaged in translating host cell mRNAs so are available to translate the IRES-driven viral mRNA. This secures efficient production of the virus-encoded proteins. As described below, some cellular mRNAs also contains IRESs.

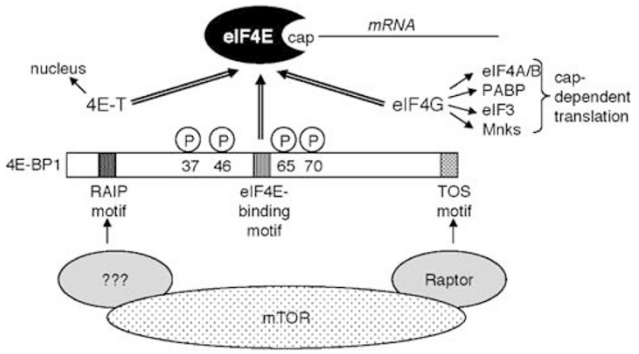

Figure 1 Motifs and function of 4E-BP1. 4E-BP1 binds to eIF4E through a motif towards in the centre of the protein. Selected phosphorylation sites in 4E-BP1 are shown, as are the RAIP and TOS motifs and the interaction of the latter with raptor, a protein binding partner for mTOR. ??? indicates a hypothetical and unknown component that may interact with the RAIP motif in 4E-BP1 and bind to mTOR. eIF4E binds the 5′-cap of the mRNA, and, when not associated with a 4E-BP, can bind either the nucleocytoplasmic shuttling protein 4E-T or to the scaffold protein eIF4G, which also binds the indicated proteins

The 4E-BPs block eIF4F complex formation

Since similar regions within 4E-BPs and eIF4Gs bind to eIF4E, their interactions with eIF4E are mutually exclusive. 4E-BPs thus block the binding of eIF4E to eIF4F and the formation of initiation factor complexes. The binding of 4E-BP1 to eIF4E is regulated by its phosphorylation, which occurs at multiple sites (Figure 1). Phosphorylation of 4E-BP1 is a rather complicated hierarchical process whereby the phosphorylation at some sites is required for, or at least favours, modification of others (see e.g. Gingras *et al.*,[7,8] Mothe-Satney *et al.*[9,10] and Wang *et al.*[11]). Thus, phosphorylation of the N-terminal threonines (Thr37/46 in human 4E-BP1) is apparently required for modification of other sites that impair the binding of 4E-BP1 to eIF4E (Ser65, Thr70: the precise roles of these sites in modulating eIF4E-binding is unclear and to some extent controversial, see for example Ferguson *et al.*[12]). Hypophosphorylated 4E-BP1 binds to eIF4E and blocks eIF4F complex formation, impairing cap-dependent mRNA translation.

Phosphorylation of some of the sites in 4E-BP1 and, more crucially, its binding to eIF4E, are influenced by signaling through the mammalian target of rapamycin (mTOR) as indicated by sensitivity to rapamycin. Rapamycin thus blocks the assembly of eIF4F complexes that is generally induced by agents such as insulin.[13] Insulin, and other anabolic or mitogenic stimuli, activate mTOR signaling, as do amino acids, especially leucine. Conversely, amino-acid starvation (reviewed in Proud,[14]) ischaemia or hypoxia,[15,16] and a range of other stressful conditions[17] lead to the dephosphorylation of 4E-BP1, which favours its binding to eIF4E, and thus to dissociation of eIF4F complexes.

4E-BP1 actually binds to a partner protein of mTOR, raptor, via its C-terminal TOR-signaling (TOS) motif (shown in Figure 1). mTOR contains a kinase domain, belongs to the family of PI 3-kinase related kinases, and displays protein kinase activity *in vitro*. The mTOR/raptor/4E-BP1 interaction is thought to facilitate 4E-BP1 phosphorylation: indeed, it does so markedly *in vitro*, although it remains unclear whether mTOR actually phosphorylates all the sites in 4E-BP1 *in vivo*.[18] There are several reasons to doubt this, including the facts that, *in vitro*, phosphorylation of 4E-BP1 by mTOR requires very high, nonphysiological, concentrations of Mn^{2+}; such phosphorylation is slow (even in the presence of raptor); mTOR only very weakly phosphorylates Thr70 and Ser65 *in vitro* and that phosphorylation of the sites that are most efficiently phosphorylated by mTOR *in vitro* (Thr37/46) is sensitive to rapamycin while their phosphorylation is largely insensitive to this agent in cells (see e.g. Gingras *et al.*,[7,8] Yang *et al.*,[19] and McMahon *et al.*[20]). Other protein kinases have been reported to phosphorylate 4E-BP1 *in vitro*: these include CK2 and ATM, which both phosphorylate the most C-terminal site,[21,22] ERK, which phosphorylates several sites, especially Ser65,[23,24] and a kinase that can associate with mTOR.[25] The physiological significance of these observations remains unclear.

eIF4E, apoptosis and cell transformation

Overexpression of eIF4E leads to dysregulation of cell growth and, in certain cases, to cell transformation, while decreasing eIF4E levels may reverse this.[26–31] This may involve effects of eIF4E on the translation of a variety of mRNAs, especially ones that, due to the existence of extensive secondary structure in their 5′-UTRs, have a high requirement for eIF4F (and in particular its eIF4A helicase activity; for recent reviews see Mamane *et al.*[32] and Clemens[33]). The possible links between eIF4E and cell transformation are underlined by the numerous observations that cancer cells often display high levels of eIF4E, especially tumors that are classed as aggressively metastatic.[34]

Overexpression of eIF4E can also inhibit apoptosis, for example, when it is induced by elevated levels of c-myc[35,36] (see also Tan *et al.*[37]) or by endoplasmic reticulum (ER) stress.[38] Indeed, in transgenic animals, eIF4E also counteracts c-myc induced cell death, while c-myc opposes eIF4E-induced cell senescence. Together eIF4E and c-myc promoted cell transformation (Ruggero *et al.*[39] in this study, since c-myc was expressed under the control of an immunoglobulin transcriptional element, lymphomagenesis). In a different transgenic mouse study, eIF4E proved to be a potent oncogene *in vivo*[40] with the resulting tumors showing a high proliferation/apoptosis ratio. The effects of eIF4E on cell survival and tumorigenesis were similar to those of expressing Akt (PKB; protein kinase B; a known antiapoptotic signaling component that acts downstream of phosphatidylinositide (PI) 3-kinase[40]). In particular, the expression of eIF4E conferred resistance to proapoptotic drugs such as the DNA-damaging agent doxorubicin (DXR), an agent used in cancer treatment.[40] While Akt overexpressing cells were sensitive to a combination of DXR and rapamycin, eIF4E overexpressing cells were not. One implication of the data is that deregulation of translation seems to play an important role in the effects of Akt in cell survival: this may reflect differential effects on the translation of pro- *versus* antiapoptotic mRNAs.

Conversely, one would expect that treatments or manipulations that impair eIF4E function would be proapoptotic and/or

reverse cell transformation. Enhanced expression of 4E-BP1 or 4E-BP2 does indeed reverse the ability of eIF4E to induce cell transformation.[41] Since phosphorylation of 4E-BP1 abrogates its ability to inhibit eIF4E, interfering with phosphorylation of 4E-BP1 would also be expected to block eIF4E function. As described above, the phosphorylation of specific sites in 4E-BP1 and its release from eIF4E are blocked by rapamycin: in this context it is important to note that the proliferation of PTEN-negative cells (in which Akt signaling is hyperactivated) is extremely sensitive to rapamycin.[42,43] The relative contributions to this of effects on cell proliferation (e.g. cell cycle) and cell survival remain to be clarified.

Rapamycin can induce apoptosis in certain tumor cell types, such as rhabdomyosarcoma cells.[44] These cells lack functional p53 and expression of p53 protects them against rapamycininduced cell death.[45] However, the ability of rapamycin to induce apoptosis is by no means universal (see e.g. Yao and Cooper,[46] McCarthy *et al.*[47] and Staruch *et al.*[48]), although numerous studies have shown it can synergise with other to promote apoptosis. It may be that some types of transformed cells are particularly sensitive to the proapoptotic effects of rapamycin (see, e.g. Houghton and Huang[44], Mohi *et al.*[49] and Thompson and Thompson[50]).

Increased expression of 4E-BPs represents an important way of restraining eIF4E function. Of especial interest in the present context are the findings reported by Houghton and Huang[44] that, for the rhabdomyosarcoma cell lines they studied, sensitivity to rapamycin-induced apoptosis correlated positively with levels of 4E-BP1 expression. Conversely, colon carcinoma cell lines that were relatively resistant to rapamycin showed rather low levels of expression of 4E-BP1,[51] and ectopic expression of 4E-BP1 in them greatly increased their sensitivity to this drug. Indeed, ectopic expression of 4E-BP1 is proapoptotic.[36,52,53] Expression of a variant 4EBP1 in which various phosphorylation sites were mutated (to prevent its dissociation from eIF4E) led to apoptosis, both in transformed and in normal fibroblasts. Expression of these constitutively active 4E-BP1 mutants also slowed cell cycle progression and inhibited myc-mediated cell transformation.[53] However, the most repressive mutants actually decreased the degree of apoptosis: this condition was found to favour cap-independent, IRES-driven, translation. It has been suggested that this may lead to increased translation of antiapoptotic IRES-containing mRNAs (see below). Interestingly, transformed cell lines showed a greater sensitivity to the proapoptotic effects of 4E-BP1 than nontransformed cells.[52] Thus, eIF4E and 4E-BP1 exert, respectively, proliferative and antiproliferative (or tumor-suppressive) effects.

An alternative approach to inhibiting eIF4E function – treating cells with cell-permeant peptides designed to bind eIF4E and thereby block its ability to interact with eIF4G – also led to very rapid cell death.[54] Although the speed of this effect is surprising, it is consistent with the antiapoptotic effects of ectopic expression of eIF4E.

These findings suggest that inhibition of mTOR signaling (especially the branch(es) of that pathway that control 4E-BP1) or of eIF4E function may be a promising avenue for cancer therapy. Considerable attention is now being focused on developing therapeutic strategies to target eIF4E or 4E-BP1.[44,55]

The data generally imply that inhibition of cap-dependent translation may favour apoptosis and thus that transformed cells may exhibit an increased dependency on cap-dependent translation for cell survival. This could arise, for example, if active eIF4E (factor available to form eIF4F complexes) favours translation of mRNAs encoding antiapoptotic proteins (perhaps because they have an particularly strong requirement for eIF4F and its helicase activity).[56] Such weakly competitive mRNAs include those coding for growth factors and cell cycle regulators (reviewed by Graff and Zimmer[57]). Alternatively, inhibition of eIF4E activity and thus of eIF4F complex formation may favour the translation of cap-independent, for example, IRES-driven, mRNAs for proapoptotic proteins such as APAF-1.[58] However, the data mentioned above, where the most repressive 4E-BP1 mutants actually showed decreased proapoptotic effects, suggest that the situation is more complex than this and may be rather delicately poised with the balance between survival and death being determined by both cap-dependent and -independent translation. As described below, some mRNAs for antiapoptotic proteins are also translated in an IRES-dependent manner.

Regulation of eIF4E during apoptosis

The above data indicate that enhanced 4E-BP1 activity can cause apoptosis. Is 4E-BP1 actually regulated during apoptosis? It is. Firstly, as described above, signaling through mTOR results in increased phosphorylation of 4E-BP1, leading to its inactivation. Treatment of cells with DNA-damaging agents such as etoposide causes inactivation of mTOR signaling and dephosphorylation of 4E-BP1, leading to increased binding of 4E-BP1 to eIF4E and concomitant loss of eIF4F complexes (Figure 2).[59] This will impair cap-dependent protein synthesis and likely favour cap-independent, IRES-driven, translation. Importantly, the impairment of mTOR signaling preceded the actual onset of apoptosis. Treatment of cells with staurosporine, another agent that induces

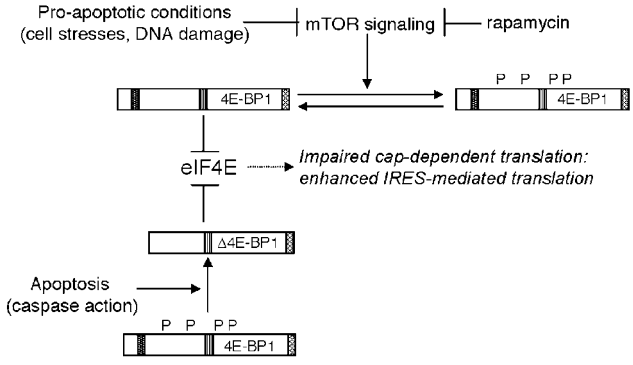

Figure 2 Regulation of 4E-BP1 and eIF4E in apoptosis. Prior to and during apoptosis, two effects leads to activation of 4E-BP1 and inhibition of eIF4E. Before the onset of apoptosis, a range of stressful conditions leads to impairment of mTOR signaling and dephosphorylation of 4E-BP1, causing it to bind to eIF4E and inhibit the function of the latter. In addition, in apoptotic cells 4E-BP1 undergoes caspase-dependent cleavage, resulting in inhibition of its phosphorylation and its increased association with eIF4E. The resulting major fragment of 4E-BP1 is indicated as 'Δ4E-BP1'

apoptosis, also led to inhibition of mTOR signaling.[60] Since mTOR signaling positively regulates cell growth and proliferation, and ribosome biogenesis, this shut-down of mTOR signaling may represent a response of cells to adverse conditions that may ultimately compromise cell survival. By affecting both cap-dependent and -independent translation, it may also alter the balance of protein expression and thereby modulate the events that trigger commitment to apoptosis, an issue that is dealt with in more detail below.

One way in which DNA damage could lead to inhibition of mTOR might involve p53. However, p53 was not required for the effects of DNA-damaging agents on mTOR signaling (Morley *et al.*[61] and Beugnet, Tee and Proud, unpublished data). Nevertheless, more recent studies have shown that p53 can cause inhibition of mTOR signaling, for example, the dephosphorylation of 4EBP1.[62] This may represent an additional mechanism by which p53 exerts its inhibition of cell proliferation. The mechanism(s) by which p53 impairs mTOR signaling remain to be established. Another possible alternative link between DNA damage and mTOR signaling is provided by the tyrosine kinase c-Abl, which is activated by DNA damage, and can phosphorylate and inactivate mTOR.[63]

Other cell stresses – hyperosmolarity, oxidative stress, ATP depletion[17,64,65] – also lead to impaired mTOR signaling and dephosphorylation of 4E-BP1, with consequent loss of eIF4F complexes, indicating, as suggested above, this may be a common cellular response to adverse situations.

4E-BP1 is subject to caspase-dependent cleavage in apoptotic cells

Apoptosis involves the activation of an array of proteinases termed caspases, which cleave many proteins as part of the apoptotic program. This results in the cleavage of a large number of cellular proteins, including a number of translation factors (see the article by Morley *et al.*, in this volume for further information). Of these, the cleavage of eIF4G (I and II) is the most relevant to the present discussion (Marissen *et al.*,[66] Bushell *et al.*[67] and Clemens *et al.*;[68] see Clemens *et al.*[69] for a review). Cleavage separates the part of eIF4G that binds eIF4E from that recruits eIF3 and the 40S ribosomal subunit to the mRNA. This is analogous to the cleavage of eIF4G during picornaviral infection: although the precise cleavage sites differ, the consequence is likely the same, that is, impairment of cap-dependent translation, but continued translation of IRES-containing mRNAs.

Importantly, 4E-BP1 is also cleaved in apoptotic cells and this event requires caspase activity.[70] The cleavage site has been identified. Cleavage removes the first 24 amino-acid residues of 4E-BP1, and the major fragment (Δ4E-BP1) therefore still contains all the known phosphorylation sites and the eIF4E-binding motif.[70] However, strikingly, phosphorylation of this fragment within cells is greatly reduced *versus* intact 4E-BP1, suggesting that the amino terminal region contains a feature required for normal phosphorylation of 4E-BP1. Further analysis revealed that this involves the four amino-acid motif Arg-Ala-Ile-Pro (RAIP), which is required for phosphorylation of Thr37/46/70 and Ser65[70,71] and appears to mediate a rapamycin-insensitive input from mTOR, which is dependent upon amino acids (especially leucine). A major consequence of this modification is that eIF4E becomes sequestered by Δ4E-BP1 (Figure 2), leading to decreased levels of eIF4F complexes and inhibition of cap-dependent translation (while IRES-driven translation can continue).

The eIF4F complex or its function can therefore be inactivated by two distinct mechanisms in response to proapoptotic stimuli or during apoptosis: (i) dephosphorylation of 4E-BP1, and consequent dissociation of eIF4E from eIF4G; and (ii) cleavage and functional dismemberment of its scaffold component, eIF4G. Thus, via several mechanisms, there appears to be a shift from cap-dependent to IRES-driven translation during apoptosis, and likely in response to a variety of cellular stresses.

Although rapamycin inhibits the formation of eIF4F complexes, it only has a surprisingly minor effect on ongoing protein synthesis,[16,72,73] but see also, for example Beretta *et al.*[74] This may reflect a low requirement of such complexes for continued translation of mRNAs that are already associated with ribosomes: it is thought that *de novo* initiation onto previously untranslated mRNAs may have a greater requirement for eIF4F, an idea for which there is some evidence.[75] There is thus an apparent paradox: rapamycin has only minor effects on overall protein synthesis but can induce or promote apoptosis. One likely explanation is that rapamycin, and thus impaired availability of eIF4E and eIF4F complexes, may have particularly pronounced effects on the translation of certain mRNAs, which exhibit a relatively high requirement for eIF4E/4F. This could be because they have highly structured 5′-UTRs. This issue has recently been discussed in some detail by Clemens.[76]

Regulation of specific mRNAs by 4E-BP1 and during apoptosis

As discussed above, regulation of 4E-BP1 impacts on the formation of translationally active eIF4F complexes and thus on cap-dependent and IRES-mediated translation. Several pro- or antiapoptotic proteins are encoded by IRES-containing mRNAs. What follows is not intended to be an exhaustive list but rather to highlight some of these mRNAs and proteins.

The mRNA for the X-linked inhibitor of apoptosis (XIAP) contains an IRES and the IRES-mediated translation of this mRNA is increased during cellular stress, including apoptosis induced by low-dose *ã*-radiation.[77,78] This mRNA/protein are the subject of a separate article in this volume and are accordingly not discussed further here. Similarly, translation of the mRNA for the cellular inhibitor of apoptosis (c-IAP1; also called HIAP2) is also mediated by an IRES and is stimulated by treatment of cells with proapoptotic agents or during ER stress.[79,80] The efficiency of the c-IAP1 IRES was apparently enhanced during ER stress, an effect which may involve a cleavage product of the protein p97/DAP5/NAT1 (which is similar in structure to the C-terminus of eIF4G; and also translated in an IRES-dependent manner[81,82]). Interestingly, the levels of XIAP are not increased during ER stress.

Bcl-2 also inhibits apoptotic signaling and one form of its mRNA also contains an IRES (this is the major form in many

cell types). Although this IRES exhibits rather weak activity in untreated cells, DNA damage or chemical stress substantially activates it.[83] The mechanism by which this occurs is currently unclear. It is initially a little puzzling that the synthesis of antiapoptotic proteins may continue following the apoptosis-associated inactivation of components required for cap-dependent (cleavage of eIF4G and its displacement from eIF4E by 4E-BP1). This likely reflects a complex interplay between pro- and antideath components, where the cell seeks to maintain antiapoptotic signaling until a proapoptotic threshold is exceeded and cells become committed to executing the apoptotic program.

The c-myc mRNA also contains an IRES and this apparently continues to function in cells that have suffered DNA damage.[84,85] Subkhankulova *et al*.[85] suggest that the continued synthesis of c-myc under such conditions may reflect a requirement for the c-myc protein in the process of repairing damaged DNA.

The mRNA for the apoptotic protease activating factor 1 (APAF-1) contains an IRES, which may allow continued production of this proapoptotic protein during stressful and apoptotic conditions.[58] This mechanism likely serves to maintain cellular levels of this important component of the apoptosome, which interacts with procaspase 9 (an initiator caspase) and thereby facilitates its cleavage and activation. The mRNA for protein kinase Cδ also contains an IRES that is active during apoptosis and may allow continued expression of this protein under such conditions, where it may exert proapoptotic effects.[86] Thus, the switch from cap-driven to IRES-directed translation which 'reprogram' translation during apoptosis does not cause a shift only to favour synthesis of antiapoptotic proteins, but also proapoptotic components too.

So far, this discussion has focused on the upregulation of translation of IRES-mediated translation of specific mRNAs: the other side of the coin is that, under the same circumstances, the translation of cap-dependent mRNAs will be inhibited. These include mRNAs for growth factors that may favour cell survival and a number of other proteins involved in the positive regulation of cell proliferation. Space precludes a detailed description here and the reader is referred to the recent review by Mamane *et al*.[32] for further information.

Implications for cancer therapy and other perspectives

As described above, the discovery that eIF4E plays a key role in cell survival and is apparently especially important in certain transformed cells suggests that targeting eIF4E either by inhibiting it directly or by activating 4E-BP1 may offer effective ploys for tumor therapy. In particular, inhibition of mTOR signaling is being explored as a possible route to achieving this. One possibility is the use of rapamycin analogs.[44,87] It may be especially appropriate to target specifically the inputs from mTOR to 4E-BP1, for example, the mechanism by which the RAIP motif works (such motifs are not found in other known mTOR targets) or the kinases that phosphorylate specific sites in 4E-BP1, assuming that all the sites are not direct targets for mTOR itself.

The roles of 4E-BP2 and 4E-BP3 are very poorly understood. 4E-BP1 and 4E-BP2 may be regulated differentially, for example, during cell differentiation.[88] A fraction of the cellular 4EBP3 is nuclear.[89] Recent data[90] have shown that eIF4GII, but not eIF4GI, is selectively recruited into eIF4F complexes during differentiation of multipotent human hematopoietic UT7-mpl cells. The mechanism by which this occurs and its impact on the process of mRNA translation are unknown. It is possible that eIF4GII may favour translation of specific (subsets of) mRNAs, although evidence for this is so far lacking. Indeed, the field of regulation of translation factors during differentiation programs may be a fruitful area for further work.

Acknowledgements

Work in the author's laboratory on eIF4E, 4E-BP1 and mTOR signaling has been supported by the Medical Research Council (UK), the Biotechnology and Biological Sciences Research Council, AstraZeneca and Biotica.

1. Gingras A-C *et al.* (1999) Annu. Rev. Biochem. 68: 913–963
2. Bradley CA *et al.* (2002) J. Biol. Chem. 277: 12559–12571
3. Sachs A (2000) In *Translational Control of Gene Expression* Sonenberg N, Hershey JWB, Mathews MB, eds (Cold Spring Harbor: Cold Spring Harbor Laboratory Press) pp. 447–465
4. Scheper GC and Proud CG (2002) Eur. J. Biochem. 269: 5350–5359
5. Dostie J *et al.* (2000) EMBO J. 19: 3142–3156
6. Belsham GJ and Sonenberg N (2000) In *Translational Control of Gene Expression* Sonenberg N, Hershey JWB, Mathews NB (eds) (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press) pp. 869–900
7. Gingras A-C *et al.* (2001) Genes Dev. 15: 2852–2864
8. Gingras A-C *et al.* (1999) Genes Dev. 13: 1422–1437
9. Mothe-Satney I *et al.* (2000) J. Biol. Chem. 275: 33836–33843
10. Mothe-Satney I *et al.* (2000) Mol. Cell. Biol. 20: 3558–3567
11. Wang X *et al.* (2003) Mol. Cell. Biol. 23: 1546–1557
12. Ferguson G *et al.* (2003) J. Biol. Chem. 278: 47459–47465
13. Harris TE and Lawrence JC (2003) Sci. STKE 2003; re15
14. Proud CG (2002) Eur. J. Biochem. 269: 5338–5349
15. Tinton SA and Buc-Calderon PM (1999) FEBS Lett. 446: 55–59
16. Martin ME *et al.* (2000) Biochem. J. 351 (Part 2): 327–334
17. Patel J *et al.* (2002) Eur. J. Biochem. 269: 3076–3085
18. Fingar DC and Blenis J (2004) Oncogene 23: 3151–3171
19. Yang D *et al.* (1999) FEBS Lett. 453: 387–390
20. McMahon LP *et al.* (2002) Mol. Cell. Biol. 22: 7428–7438
21. Fadden P *et al.* (1998) FEBS Lett. 435: 105–109
22. Yang D-Q and Kastan MB (2000) Nat. Cell Biol. 2: 893–898
23. Lin T-A *et al.* (1994) Science 266: 653–656
24. Haystead TAJ *et al.* (1994) J. Biol. Chem. 269: 23185–23191
25. Heesom KJ and Denton RM (1999) FEBS Lett. 457: 489–493
26. Lazaris-Karatzas A *et al.* (1990) Nature 345: 544–547
27. Lazaris-Karatzas A and Sonenberg N (1992) Mol. Cell. Biol. 12: 1234–1238
28. De Benedetti A and Rhoads RE (1990) Proc. Natl. Acad. Sci. USA 87: 8212–8216
29. De Benedetti A *et al.* (1991) Mol. Cell. Biol. 11: 5435–5445
30. Graff JR *et al.* (1995) Int. J. Cancer 60: 255–263
31. Zimmer SG *et al.* (2000) Anticancer Res. 20: 1343–1351
32. Mamane Y *et al.* (2004) Oncogene 23: 3172–3179
33. Clemens MJ (2004) Oncogene 23: 3180–3188
34. De Benedetti A and Graff JR (2004) Oncogene 23: 3189–3199
35. Li S *et al.* (2003) J. Biol. Chem. 278: 3015–3022
36. Polunovsky VA *et al.* (1996) Mol. Cell. Biol. 16: 6573–6581
37. Tan A *et al.* (2000) Oncogene 19: 1437–1447
38. Li S *et al.* (2004) J. Biol. Chem. 279: 21312–21317

39. Ruggero D *et al.* (2004) Nat. Med. 10: 484–486
40. Wendel HG *et al.* (2004) Nature 428: 332–337
41. Rousseau D *et al.* (1996) Oncogene 13: 2415–2420
42. Neshat MS *et al.* (2001) Natl. Acad. Sci. USA 98: 10314–10319
43. Podsypanina K *et al.* (2001) Proc. Natl. Acad. Sci. USA 98: 10320–10325
44. Houghton PJ and Huang S (2004) Curr. Top. Microbiol. Immunol. 279: 339–359
45. Huang S *et al.* (2003) Mol. Cell 11: 1491–1501
46. Yao R and Cooper GM (1996) Oncogene 13: 343–351
47. McCarthy SA *et al.* (1992) Transplantation 54: 543–547
48. Staruch MJ *et al.* (1991) Int. J. Immunopharmacol. 13: 677–685
49. Mohi MG *et al.* (2004) Proc. Natl. Acad. Sci. USA 101: 3130–3135
50. Thompson JE and Thompson CB (2004) J. Clin. Oncol. 22: 4217–4226
51. Dilling MB *et al.* (2002) J. Biol. Chem. 277: 13907–13917
52. Polunovsky VA *et al.* (2000) J. Biol. Chem. 275: 24776–24780
53. Li S *et al.* (2002) Mol. Cell. Biol. 22: 2852–2861
54. Herbert TP *et al.* (2000) Curr. Biol. 10: 793–796
55. Bjornsti MA and Houghton PJ (2004) Cancer Cell 5: 519–523
56. Koromilas AE *et al.* (1992) EMBO J. 11: 4153–4158
57. Graff JR and Zimmer SG (2003) Clin. Exp. Metastasis 20: 265–273
58. Coldwell MJ *et al.* (2000) Oncogene 19: 899–905
59. Tee AR and Proud CG (2000) Oncogene 19: 3021–3031
60. Tee AR and Proud CG (2001) Cell Death Differ. 8: 841–849
61. Morley SJ *et al.* (1998) FEBS Lett. 438: 41–48
62. Horton LE *et al.* (2002) Oncogene 21: 5325–5334
63. Kumar V *et al.* (2000) J. Biol. Chem. 275: 10779–10787
64. Dennis PB *et al.* (2001) Science 294: 1102–1105
65. Inoki K *et al.* (2003) Cell 115: 577–590
66. Marissen WE *et al.* (2000) Cell Death Differ. 7: 1234–1243
67. Bushell M *et al.* (2000) Eur. J. Biochem. 267: 1083–1091
68. Clemens MJ *et al.* (1998) Oncogene 17: 2921–2931
69. Clemens MJ *et al.* (2000) Cell Death Differ. 7: 603–615
70. Tee AR and Proud CG (2002) Mol. Cell. Biol. 22: 1674–1683
71. Beugnet A *et al.* (2003) J. Biol. Chem. 278: 40722
72. Anthony TG *et al.* (2001) J. Nutr. 131: 1171–1176
73. Mendez R *et al.* (1996) Mol. Cell. Biol. 16: 2857–2864
74. Beretta L *et al.* (1996) EMBO J. 15: 658–664
75. Novoa I and Carrasco L (1999) Mol. Cell. Biol. 19: 2445–2454
76. Clemens MJ (2001) J. Cell. Mol. Med. 5: 221–239
77. Holcik M *et al.* (1999) Nat. Cell Biol. 1: 190–192
78. Holcik M (2003) Ann. NY Acad. Sci. 1010: 249–258
79. Van Eden ME *et al.* (2004) RNA 10: 469–481
80. Warnakulasuriyarachchi D *et al.* (2004) J. Biol. Chem. 279: 17148–17157
81. Henis-Korenblit S *et al.* (2002) Proc. Natl. Acad. Sci. USA 99: 5400–5405
82. Henis-Korenblit S *et al.* (2000) Mol. Cell. Biol. 20: 496–506
83. Sherrill KW *et al.* (2004) J. Biol. Chem. 279: 29066–29074
84. Stoneley M *et al.* (2000) Mol. Cell. Biol. 20: 1162–1169
85. Subkhankulova T *et al.* (2001) Biochem. J. 359: 183–192
86. Morrish BC and Rumsby MG (2002) Mol. Cell. Biol. 22: 6089–6099
87. Sawyers CL (2003) Cancer Cell 4: 343–348
88. Grolleau A *et al.* (1999) J. Immunol. 162: 3491–3497
89. Kleijn M *et al.* (2002) FEBS Lett. 532: 319–323
90. Caron S *et al.* (2004) Mol. Cell. Biol. 24: 4920–4928