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Review

# Conducting the initiation of protein synthesis: the role of eIF4G

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**Abstract**

The eukaryotic initiation factor eIF4G is a large modular protein which serves as a docking site for initiation factors and proteins involved in RNA translation. Together with eIF4E and eIF4A, eIF4G constitutes the eIF4F complex which is a key component in promoting ribosome binding to the mRNA. Thus, the central role of eIF4G in initiation makes it a valid target for events aimed at modulating translation. Such events occur during viral infection by picornaviruses and lentiviruses and result in the hijack of the translational machinery through cleavage of eIF4G. Proteolysis of eIF4G is also mediated by caspases during the onset of apoptosis causing inhibition of protein synthesis. We will review the role of eIF4G and protein partners as well as the cellular and viral events that modulate eIF4G activity in the initiation of translation.


## 1. The initiation of protein synthesis: a summary

Translation is a complex mechanism subject to important physiological regulation and plays a major role in the control of gene expression. Protein synthesis can be broadly divided into three steps: initiation, elongation and termination with the initiation phase being one of the major regulatory targets for translational control. Initiation is a multistep process that involves a complex interplay between eukaryotic initiation factors (eIFs) and ribonucleic acids such as mRNAs, rRNAs and tRNAs (see Fig. 1). Attachment of the 40 S ribosomal subunit onto the mRNA requires a complex series of events that begin with the formation of a ternary complex between eIF2, a molecule of GTP and a tRNA initiator (eIF2–GTP–Met– tRNAi). This ternary complex is then recruited by the 40 S ribosomal subunit which is associated with eIF1A and eIF3 to form the 43 S initiation complex. For the vast majority of eukaryotic mRNAs, binding of the 43 S occurs by attachment to the 5' capped end of the RNA transcript. This process requires ATP, a group of three initiation factors called the eIF4F complex (composed of eIF4E, eIF4A and eIF4G) and is enhanced by the presence of eIF4B. The role of eIF4F is to promote binding of the 43 S particle to the mRNA via a cap-eIF4E–eIF4G–eIF3 interaction to form the 48 S complex (see Fig. 1). For a number of viral and cellular mRNAs harboring specific sequences known as internal ribosome entry segment (IRES), ribosome binding occurs directly at an internal position through a direct eIF4G–RNA interaction.

Displacement of the mRNA-bound ribosomal complex requires both eIF1 and eIF1A to assist the linear migration of the preinitiation complex (40 S and associated proteins) during scanning process in order to identify the correct AUG start codon. Upon reaching the initiation site, eIF5 triggers GTP hydrolysis by eIF2 and this results in the dissociation of all 40 S-bound initiation factors to allow 60 S ribosomal subunit joining to form the elongation competent ribosome (80 S). This marks the end of initiation and the beginning of polypeptide elongation. A comprehensive description of eukaryotic translation initiation can be found in these recent reviews (Gingras et al., 1999; Pestova et al., 2001; Sachs and Varani, 2000). The reader is also referred to a review and a summary article focused on the role and function of eIF4G that have been published (Hentze, 1997; Morley et al., 1997). This review article is aimed to complement these two previously published articles on the progress made over the last 5 years on the role of eIF4G in translation initiation.

## 2. eIF4G: a general overview

### *2.1. The eIF4G family*

The initiation factor eIF4G has been isolated and cloned from many different species including the yeasts *S. cerevisiae* and *S. pombe* (Goyer et al., 1993), *Drosophila* (Zapata et

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al., 1994), wheat (Browning, 1996) and human (Gradi et al., 1998a; Yan et al., 1992). There are two isoforms of eIF4G in wheat (eIF4G and iso-eIF4G), *S. cerevisiae* (TIF4631 and TIF4632) and human (eIF4GI and eIF4GII; eIF4GI being the prototype member of the family) which exhibit moderate sequence conservation (46% identity in human, 53% in yeast) but share similar overall biochemical activities, although some differences were observed between the yeast and wheat isoforms (Gallie and Browning, 2001; Tarun et al., 1997). In addition to these functional homologues, the eIF4G family also comprises proteins that display sequence conservation only to the carboxy-terminus part of eIF4GI. For instance, the mammalian p97/NAT1/DAP-5 protein (30% homology with eIF4GI) was isolated independently by three different groups and is a ubiquitous and abundantly expressed protein in normal tissues and cell lines (Imataka et al., 1997; Levy-Strumpf et al., 1997; Yamanaka et al., 1997). The p97/NAT1/DAP-5 protein lacks the N-terminal extremity of eIF4GI containing the poly(A) and eIF4E binding sites but possesses interacting domains with both eIF3 and eIF4A (see Fig. 2). Overexpression of p97/NAT1/DAP-5 in cultured cells results in inhibition of both cap-dependent and IRES-mediated protein synthesis suggesting that it functions as a negative regulator of translation (Imataka et al., 1997; Yamanaka et al., 1997). However, a recent report has described that DAP-5 was produced by cap-independent translation and that the protein could promote internal ribosome entry on its cognate IRES (Henis-Korenblit et al., 2000).

Finally, two other proteins exhibit modest sequence homology with the central domain of eIF4GI and can bind eIF4A in vitro; these are the poly(A) binding protein interacting protein-1 (Paip-1) and DUG (for death-upregulated gene, Craig et al., 1998; Goke et al., 2002).

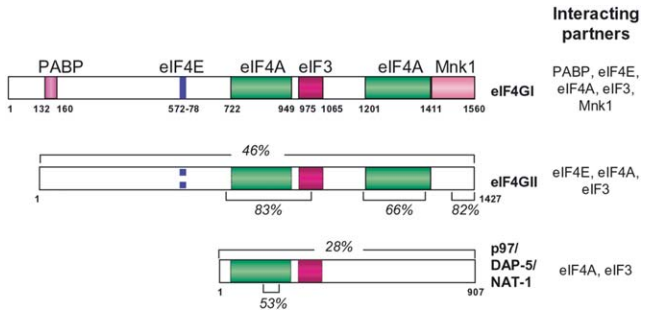


Fig. 2. eIF4G family in mammals and interacting partners. The eIF4GI, eIF4GII and p97/DAP-5/NAT-1 molecules are schematically represented. Domains of interaction between eIF4GI and eIF4A (Lomakin et al., 2000; Morino et al., 2000), eIF3 (Korneeva et al., 2000), Mnk-1 (Morino et al., 2000), PABP and eIF4E (Gingras et al., 1999b) are shown, and amino acids position of each individual domain is indicated. Putative corresponding binding domains on eIF4GII and p97/DAP-5/NAT-1 are represented with dashed lines. Homologies between eIF4GII and p97/DAP-5/NAT-1 with eIF4GI are expressed as percentage of amino acids identity. Numbering is with respect to the extended amino-terminal sequence (Piron et al., 1998; Imataka et al., 1998).

### *2.2. eIF4G synthesis and clones*

In the early years, eIF4GI was formerly called p220 due to its migration on SDS-PAGE as a cluster of four electrophoretic forms, the largest running around the 220 kDa Marker (Tahara et al., 1981). However, cloning of the cDNA from a human brain tumor library revealed a single reading frame encoding a protein of 154 kDa predicted molecular weight (Yan et al., 1992). Both chromosome mapping and Southern analysis indicated that the human gene coding for eIF4GI was found at only one copy (Yan and Rhoads, 1995). Studies with picornaviral proteases suggested that the origin of these isoforms was attributable to aberrant migration on SDS-PAGE of the amino-terminal part of the protein as a

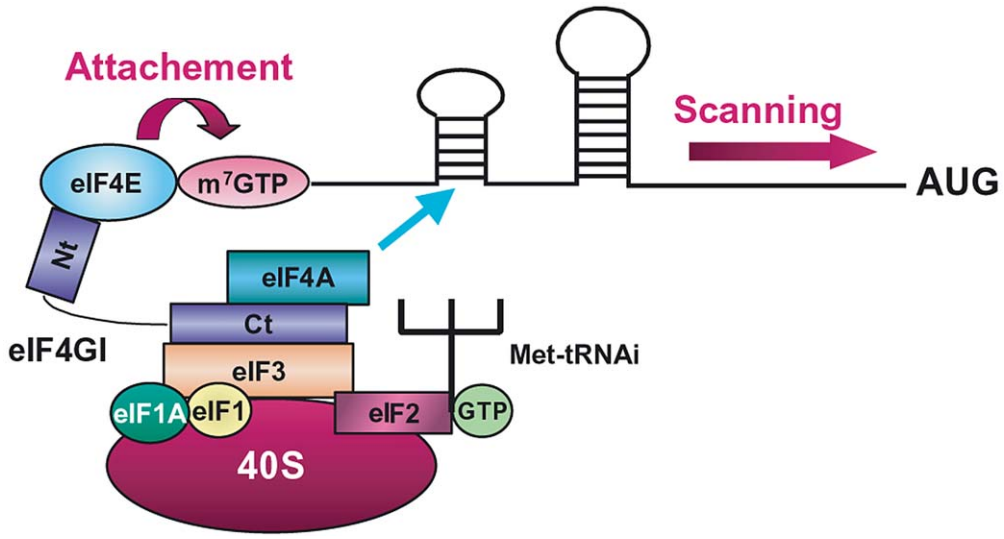


Fig. 1. General features of cap-dependent initiation of translation. The main initiation factors involved in cap-dependent translation are schematically represented on the cartoon. Upon recognition of the cap by eIF4E, the 40 S ribosomal subunit binds the 5' end of the mRNA via an eIF4E/eIF4GI/eIF3 interaction. Met-tRNAi is also recruited as part of a ternary complex with eIF2 and GTP. The preinitiation complex, in association with eIF1 and eIF1A, can then scan the 5' UTR to reach the AUG codon. During this process, eIF4A unwinds the secondary structures encountered between the cap and the AUG codon.

reflection of extensive post-translational modifications (Lamphear et al., 1995). However, it rather turned out that the original eIF4GI sequence was incorrect as first evidenced by the fact that homology between eIF4GII and eIF4GI stopped at a putative splice acceptor site suggesting that the published 5' boundaries of eIF4GI contained an intron (Gradi et al., 1998b). Thus, 5' RACE was performed and a new cDNA clone with a 156 amino acids N-terminal extension was obtained. This sequence is highly homologous to the corresponding region on eIF4GII, binds the poly(A) binding protein and functions in poly(A)-dependent translation (Imataka et al., 1998). More recently, yet another additional 340 nucleotides sequence extension to the 5' end has been added (Bradley et al., 2002; Byrd et al., 2002). In vitro translation of the RNA constructs derived from this cDNA sequence gave rise to a cluster of bands co-migrating with native eIF4GI, and these isoforms result from alternative translation initiation at distinct AUGs (Byrd et al., 2002). This was confirmed by mass spectrometric analysis, which allowed the characterization of several eIF4GI isoforms differing by their N-terminal extension (Bradley et al., 2002).

Synthesis of eIF4GI occurs through the use of an IRES element, and this has led to the attractive hypothesis that eIF4GI synthesis could continue under cellular stress conditions or during viral infections (Byrd et al., 2002; Gan et al., 1998; Gan and Rhoads, 1996; Johannes and Sarnow, 1998). However, a recent report has challenged this model showing that protein production driven by the 'IRES' of eIF4G (in the context of bicistronic RNAs) resulted in fact from translation of monocistronic constructs that were generated from a cryptic promoter (Han and Zhang, 2002).

### *2.3. Structure and function*

Human eIF4GI was first isolated as part of a large protein complex that could restore protein synthesis in lysates derived from poliovirus-infected HeLa cells (Tahara et al., 1981). This was later identified as eIF4F in which the initiation factor eIF4GI associates with eIF4E and eIF4A to form a heterotrimeric complex whose role is to promote attachment of the 43 S to the capped end of mRNAs. eIF4GI is a large modular polypeptidethat interacts with many different cellular and viral proteins such as (i) initiation factors eIF4E, eIF4A, eIF3 and the nuclear cap-binding protein CBP80 (Fortes et al., 2000; Imataka and Sonenberg, 1997; Korneeva et al., 2001, 2000; Lamphear et al., 1995; Mader et al., 1995; McKendrick et al., 2001) ; (ii) picornaviral proteases 2A and L (Foeger et al., 2002); (iii) viral proteins NS1 and NSP3 from influenza and rotavirus, respectively (Aragon et al., 2000; Piron et al., 1998); (iv) heat shock protein hsp27 (Cuesta et al., 2000a); (v) other proteins involved in RNA metabolism such as the poly(A) binding protein PABP (Imataka et al., 1998; Le et al., 1997; Tarun and Sachs, 1996) the decapping enzyme Dcp1 (Vilela et al., 2000), and the eIF4E kinase Mnk1 (Pyronnet et al., 1999).

eIF4GI possesses three domains that are roughly equivalent in size: the N-terminal part as defined by its cleavage by picornaviral proteases, the middle 'core' domain that is critical for assembly of the translation machinery and the carboxy-terminal fragment which appears to play a modular role in translation. The X-ray structure of the evolutionary conserved middle domain of eIF4GII has recently been solved and revealed the presence of five HEAT (named for Huntingtin, Elongation 3, A subunit of protein phosphatase 2A, Target of rapamycin) repeats (Marcotrigiano et al., 2001). HEAT repeats are found in proteins involved in large multiprotein complex assembly and are formed by the arrangement of five repeating pairs of antiparallel alpha helices (Andrade and Bork, 1995). Thus, these structural data confirmed the essential role of eIF4GI in co-ordinating all the components required to build the preinitiation complex onto the messenger RNA. Finally, the presence of three serum-stimulated phosphorylation sites located in the middle domain of the protein suggest that eIF4GI activity can be regulated by phosphorylation events (Raught et al., 2000).

## 3. eIF4G and interacting cellular partners

### *3.1. eIF4G within the eIF4F complex*

Twenty years ago, a complex of three proteins (size ranging from 24, 48 and 220 kDa) that could stimulate globin synthesis was purified from a ribosomal salt wash (Grifo et al., 1983). This complex is now recognized as the eIF4F holoenzyme which comprises eIF4E (24 kDa), eIF4A (48 kDa) and eIF4GI (220 kDa).

#### *3.1.1. eIF4E*

eIF4E is a small phosphoprotein of 24 kDa that exhibits high affinity for the $m^7$GTP cap structure present at the 5' end of virtually all eukaryotic mRNAs (Sonenberg et al., 1978). Strong binding of eIF4E to the cap occurs by interaction between two of the highly conserved tryptophan residues and the 7-methylguanosine cap (Marcotrigiano et al., 1997; Matsuo et al., 1997). Within eIF4F, eIF4E interacts with eIF4GI and mediates cap-dependent translation by promoting 48 S complex formation at the 5' end of the mRNA. The eIF4E binding site has been mapped by Lamphear and colleagues who have used picornaviral proteases to bisect eIF4GI (Lamphear et al., 1995). Upon cleavage, they observed that the N-terminal fragment of eIF4GI (from the cleavage site) could be retained on $m^7$GTP-sepharose chromatography indicating that eIF4E remained attached to it. This was confirmed by studying interactions between deletion mutants of eIF4GI with eIF4E in far western blot and co-immunoprecipitation assays (Mader et al., 1995). This led to the characterization of a 49 amino acids region of eIF4GI which is present both in mammals and in yeast. Within this sequence, a shorter motif (**Y**DREF**LL**) presents a high degree of conservation between the human, yeast, plant and rabbit forms of eIF4G (in bold, amino acids conserved between all the four species mentioned above). Interestingly, this motif is also found in the amino acid sequence of the

translational repressor 4E-BP, and disruption of this region by point mutation abrogates interaction with eIF4E. Therefore, it implies that binding of eIF4GI and 4E-BP to eIF4E is mutually exclusive and mediated via a common motif (Mader et al., 1995).

The initiation factor eIF4E can be isolated alone or as part of the eIF4F complex; interestingly, the eIF4F complex has 10-fold higher affinity for the $m^7$GTP structure than eIF4E (Ray et al., 1983). In fact, cross-linking of eIF4E alone was found to be very inefficient especially at low concentration of the protein (Haghighat et al., 1996). In vitro studies using purified recombinant initiation factors revealed that the interaction between eIF4GI and eIF4E dramatically enhances binding of eIF4E to the cap structure (Haghighat et al., 1996). This could be explained by the fact that eIF4GI can bind to the mRNA directly through RNA recognition motifs (RRMs) located in the middle domain of the protein (Goyer et al., 1993) and these additional eIF4GI–mRNA interactions may stabilize the eIF4E–eIF4GI complex to the cap structure. However, the increased association of eIF4E with the cap, as well as with cap analogues, can be reproduced by the addition of fragments of eIF4G that bind to eIF4E but not to the mRNA (von Der Haar et al., 2000). Moreover, a 98 amino acids region overlapping the eIF4E binding site of eIF4G from *S. cerevisiae* was shown to undergo structural conformational change upon binding of eIF4E which corresponds to an unfolded-to-folded transition suggesting a chaperoning activity of eIF4E for eIF4G (Hershey et al., 1999). Therefore, this implies that the binding of eIF4G to the dorsal site on eIF4E has the ability to strengthen interaction between eIF4E and the cap structure and suggests that the eIF4E–eIF4G complex, rather than eIF4E alone, is required for optimal activity.

### *3.1.2. eIF4A*

eIF4A is a 46 kDa polypeptide with RNA-dependent ATPase activity which also exhibits RNA annealing and unwinding activity in vitro (Pause et al., 1993; Pause and Sonenberg, 1992; Rozen et al., 1990). eIF4A is the prototype member of a large family of RNA helicases containing the specific DEAD box motif (Linder et al., 1989; Rogers et al., 2002). A current model for the action of eIF4A in cap-dependent initiation is that it binds to the 5' cap structure as part of the eIF4F complex and, together with eIF4B, unwinds the 5' proximal secondary structures to create a single stranded RNA segment accessible for ribosome binding. Then, the preinitiation complex moves in a 5'–3' direction to the AUG start site and during this process, eIF4A cycles in and out of the eIF4F complex to melt secondary structures in an ATP-dependent manner (Pause et al., 1994). By using picornaviral proteases to map functional domains of eIF4GI, Lamphear and colleagues have assigned the eIF4A binding site on the carboxy-terminal part of eIF4GI (Lamphear et al., 1995). Surprisingly, this domain of interaction is lacking in the yeast homologues, although eIF4A is essential for translation in the yeast system (Goyer et al., 1993). Also puzzling is the fact that the middle domain of eIF4GI can promote the association of eIF4A to the EMCV IRES, given that eIF4B is present (Pestova et al., 1996a). Therefore, it did not come as a surprise when a second independent binding site for eIF4A was mapped to the middle domain of eIF4GI (Imataka and Sonenberg, 1997). The presence of two binding sites raises the question of whether one or two molecules of eIF4A can simultaneously be bound on eIF4GI. This was directly addressed by Korneeva and colleagues who have used a combination of purified eIF4A and recombinant expressed eIF4GI fragments to show that eIF4A can interact with each of the individual binding site in a 1:1 ratio, whereas fragments of eIF4GI containing the two binding sites exhibited a 1:2 stoichiometry (Korneeva et al., 2001). Kinetic binding by surface plasmon resonance indicated that eIF4A associates and dissociates faster with the central than with the carboxy-terminal binding site. These results suggest that two molecules of eIF4A can bind in a co-operative manner to each individual binding site on eIF4GI (Korneeva et al., 2001).

However, a second model has been proposed on the basis of data obtained by immunoprecipitation and pull-down experiments in cells (Li et al., 2001). In these experiments, recovery of eIF4F complexes from HEK 293 cells on $m^7$GTP-sepharose beads indicated that both isoforms of eIF4A (eIF4AI and eIF4AII) could be incorporated into eIF4F with comparable efficiency. However, immunoprecipitation with antibodies specific to either eIF4AI or eIF4AII revealed that eIF4GI could be associated with either eIF4AI or eIF4AII but never with both. These data and pull-down experiments with tagged proteins showed that the mammalian eIF4F complex exhibits a ratio of one molecule of eIF4A to one molecule of eIF4GI (Li et al., 2001). This is in agreement with a previous model from Sonenberg and colleagues suggesting that one molecule of eIF4A is sandwiched between the two binding sites on eIF4GI (Morino et al., 2000).

Functional assays have shown that the carboxy-terminal last third of eIF4GI containing the second eIF4A binding site was not essential for translation, although its presence stimulated IRES-driven translation and was suggested to play a modulatory role (De Gregorio et al., 1999; Imataka and Sonenberg, 1997; Morino et al., 2000). Interestingly, although a direct eIF4G–eIF4A interaction exists in *S. cerevisiae*, purified eIF4F complexes do not contain eIF4A (Dominguez et al., 2001; Neff and Sachs, 1999). Thus, loss of eIF4A during the purification procedure may reflect a weaker eIF4A–eIF4G interaction in yeast than in mammals and this may be explained by the presence of a single binding site on yeast eIF4G.

Finally, the exact position of the carboxy-terminus eIF4A binding site has been mapped between aa 1201–1411 by Morino et al. (2000) while the upstream binding site is defined as amino acids 672–970 by Morino et al. (2000), 672–876 by Korneeva et al. (2000, 2001) and 722–949 by Lomakin et al. (2000) (see Fig. 2).

*3.1.3. eIF3*

eIF3 is the largest of the mammalian translation initiation factors and consists of a complex composed of 11 subunits of molecular weight ranging from 35 to 170 kDa (for a review on eIF3 see Hannig, 1995). Electron microscopy studies revealed that eIF3 has a triangular shape configuration and is bound to the protuberance of the 40 S subunit (Behlke et al., 1986). Three main biochemical functions are fulfilled by eIF3: (i) stabilization of the eIF2.GTP.Met-tRNAi binding to the 40 S ribosomal subunit; (ii) mRNA binding to the ribosomes; (iii) prevention of the formation of the 80 S ribosome by impeding the joining of the 40 S subunit to the 60 S. The binding site for eIF3 was initially mapped to the middle domain of eIF4GI (Lamphear et al., 1995; Ohlmann et al., 1996; Rau et al., 1996). Recently, titration, competition experiments and surface plasmon resonance refined the position of the eIF3 binding site between amino acids 975–1065 on the central domain of eIF4GI, a region that does not overlap the upstream eIF4A binding site nor the EMCV-IRES binding motif (see Fig. 2, Korneeva et al., 2000). Interestingly, this also established that the binding of eIF4A and eIF3 to the central region was mutually co-operative with a fourfold increase of eIF3 binding to eIF4GI in the presence of eIF4A. Reciprocally, eIF4A binding to the middle domain of eIF4GI was enhanced by more than twofold in the presence of eIF3 (Korneeva et al., 2000). Once more, this suggests that eIF4GI is not a static docking molecule, but is rather a dynamic scaffold which is likely to adopt many different conformational states in order to build the ribonucleoprotein complex necessary for translation initiation.

*3.2. The poly(A) binding protein*

*3.2.1. Physical interaction*

The poly(A) binding protein (acronym Pab1p in yeast and PABP in higher eukaryotes; for the remainder of the paper PABP will be used to avoid confusion) binds the poly(A) tail and is the prototype member of a family of proteins containing conserved RRMs. PABP is essential in yeast and is involved in several processes such as translation, mRNA stability and mRNA 3' end processing (Gallie, 1998). More than 10 years ago, both genetic and biochemical evidences have suggested that PABP could be involved in the initiation step of translation. Genetic screens showed that two mutant suppressors that could overcome lethality induced by PABP mutants were affected in 60 S subunit metabolism (Sachs and Davis, 1990, 1989). Biochemical data indicated that addition of a cap and a poly(A) tail on a reporter mRNA resulted in translational enhancement in a synergistic manner (Gallie, 1991). However, direct evidence for a cross-talk between the 5' end and the 3' end was first described in *S. cerevisiae* and involved a PABP–eIF4G interaction in an RNA dependent manner (Tarun and Sachs, 1996). The PABP interacting domain was mapped to a 114 amino acid region at the N-terminal part of the yeast eIF4G protein expressed from the Tif4632p gene. This sequence is conserved on the Tif4631p homologue and the latter was found to also bind the PABP/poly(A) complex in vitro (Tarun et al., 1997). Mutations in the first and second RRM of PABP impaired association to eIF4G and abolished poly(A) tail-dependent translation in yeast extract (Kessler and Sachs, 1998). On the basis of these results, a model of RNA circularization through a PABP–eIF4G interaction was proposed (see Fig. 3, Tarun and Sachs, 1996). However, formal proof for a 5'–3' circularization came from visualization by atomic force microscopy of eIF4E/eIF4G/PABP complexes assembled with recombinant proteins on a capped and polyadenylated RNA (Wells et al., 1998). Direct interaction between PABP and eIF4G has also been reported in plants (Le et al., 1997) and in mammals (Imataka et al., 1998). The main interaction site in mammals is located within the extended N-terminal sequence of eIF4GI (see Fig. 2) and is conserved in eIF4GII, but does not exhibit any sequence homology with the yeast PABP interacting domain except for a rich content of basic amino acids (Imataka et al., 1998; Tarun et al., 1997).

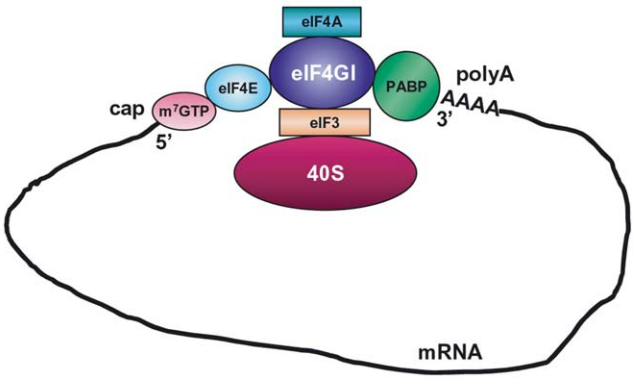


Fig. 3. 5'–3' cross-talk on the mRNA. Schematic model of mRNA circularization during translation. eIF4GI binds the 5' end of the mRNA via a cap/eIF4E interaction, and the 3' end via a PABP/polyA interaction.

*3.2.2. Physiological relevance of the eIF4G–PABP interaction*

Functional assays showed that simultaneous interactions between the 5' cap and the 3' polyadenylated end of the mRNA act together to enhance in vitro and in vivo translations (Iizuka et al., 1994; Preiss and Hentze, 1998; Tarun and Sachs, 1995; Tarun et al., 1997). This stimulation requires PABP and correlates with increased 40 S ribosomes recruitment to the 5' end of the mRNA (Tarun and Sachs, 1995). In yeast, this 5'–3' interaction is not necessary for cell viability as mutations within the PABP binding site of eIF4G only became lethal when the eIF4E binding domain was also disrupted (Tarun et al., 1997). This suggests that interaction of eIF4G to the 5' end of the mRNA can be promoted by either eIF4E or PABP. Consistent with this hypothesis, the poly(A) tail alone can deliver 40 S ribosomal subunits on an mRNA devoid of a cap structure in a yeast cell-free translation system (Preiss and Hentze, 1998; Tarun et al., 1997).

In vitro, cap and poly(A) tail stimulation of translation has different quantitative effects depending on the model used

(e.g. yeast, *Drosophila* or mammals). Recently, cap/poly(A) synergistic stimulation has been reproduced from reticulocyte lysate partially depleted in ribosomes and associated factors (Michel et al., 2000) and HeLa cell extracts (Bergamini et al., 2000). In these systems, translation of polyadenylated IRES-containing constructs was found to be stimulated from 3- (for EMCV) to 6–10-fold for Poliovirus (Bergamini et al., 2000; Michel et al., 2001). Specificity of this stimulation was demonstrated by the fact that the translation driven by the HCV IRES was not influenced by the presence of a poly(A) tail (Michel et al., 2001). In the case of IRES elements of picornaviral origin, it has been proposed that circularization of the mRNA does not occur via a cap-eIF4E–eIF4GI– PABP interaction, but rather through an IRES–eIF4GI–PABP complex (Michel et al., 2001).

These experiments have contributed to the general agreement that 5'–3' end cross-talk is an important determinant of eukaryotic RNA translation. However, one is left to wonder how circularization functionally contributes to enhance translation initiation and several models have been suggested.

Non-covalent linking of 5'–3' termini could be a way to ensure that the mRNA is intact and has not been post-transcriptionally modified through exo- or endoribonuclease removal of the 5' and 3' regions. Thus, this would represent a proof-reading mechanism to restrict translation only to intact and correctly processed mRNAs in order to prevent aberrant protein production. RNA circularization may also be required to facilitate initiation factors and ribosomes recycling through interaction of the 5' and 3' ends of the messenger. This is supported by the experiments with GCN4 in yeast showing that a significant proportion of 40 S subunits remained associated with the mRNA following translation termination of a small open reading frame (for a recent review see Hinnebusch and Natarajan, 2002). If a similar phenomenon could occur on a larger cistron, it can be hypothesized that 5'–3' interactions could guide 40 S subunits to the 5' end to participate in a new round of translation (see Fig. 3). Recently, a major component of the termination machinery (GSPT/eRF3) has been shown to interact with PABP suggesting that termination and initiation may be controlled by an eIF4F–PABP–eRF3 complex (Hoshino et al., 1999; Uchida et al., 2002).

Recent in vitro data suggest that free exogenous poly(A) can stimulate non-polyadenylated capped RNA translation to the same extent as the presence of a poly(A) tail on these RNAs suggesting that circularization per se is not required. This *trans* stimulation exhibits the same properties as previously described: PABP–eIF4GI interaction, synergistic activity of the cap and exogenous poly(A) tail and increased association of eIF4F–PABP complex to the cap (Borman et al., 2002). In plants, association of PABP with eIF4F was shown to increase the affinity of eIF4F for the cap structure by about 40-fold (Wei et al., 1998) with the PABP–eIF4F complex showing a tighter affinity for poly(A) than free PABP (Le et al., 1997). Thus, poly(A) tail stimulation could simply reflect enhanced binding of the PABP–eIF4F complex to the 5' or 3' end of the mRNA.

### *3.3. Other cellular interacting partners*

eIF4GI also contains binding sites for polypeptides that are not directly involved in translation initiation, but which can modulate the rate of protein synthesis. Among these, the MAP kinase-integrating protein kinase Mnk1, which is the kinase responsible for eIF4E phosphorylation (Fukunaga and Hunter, 1997; Pyronnet et al., 1999). Mnk1 interacts with the carboxy-terminal part of eIF4GI to form a eIF4E– eIF4GI–Mnk-1 trimeric complex which appears to be a prerequisite for eIF4E phosphorylation as a mutant form of eIF4E that cannot associate with eIF4GI did not undergo phosphorylation (Pyronnet et al., 1999).

In *S. cerevisiae*, the enzyme Dcp1 is a key protein in the decapping process during mRNA degradation (for a review on mRNA degradation see Tucker and Parker, 2000) and has recently been reported to interact with eIF4G (Vilela et al., 2000). This association occurs within the N-terminal domain of eIF4G but is very weak and by no means can compete with the eIF4E or PABP binding sites. In order to interact with eIF4G and to access the 5' capped end, Dcp1 must rely on dissociation of the eIF4E–eIF4G complex. Thus, by modulating interactions between components of the translational and degradation machinery, eIF4G may act as a sensor molecule to control the switch between translation and mRNA decay.

Last but not the least, a synthetic lethal interaction between mutations in eIF4G and the nuclear cap binding protein (CBP80) has been described in *S. cerevisiae*. In vitro and in vivo experiments characterized a direct physical interaction between CBP80 and a region of eIF4G partially overlapping the eIF4E and the eIF4A binding sites. The CBP80–eIF4G interaction was antagonized by eIF4E suggesting that the nuclear cap binding complex (CBC) was displaced from newly exported mRNAs by competitive interaction with initiation factors in the cytoplasm prior to the first round of translation (Fortes et al., 2000). Association between eIF4G and CBC has now been described in mammalian cells and occurs in the nucleus where a fair amount of eIF4G is localized (McKendrick et al., 2001). Finally, CBP80 bound mRNAs are able to participate in a ‘pioneer’ round of translation suggesting that 40 S ribosomal subunits can be recruited on cellular capped mRNAs even in the absence of eIF4E provided that eIF4G is present (Ishigaki et al., 2001). Consistent with this, addition of CBP80 has the ability to stimulate translation by 2.5-fold in a yeast translation extract derived from strains harboring a mutated form of eIF4G impaired for eIF4E and PABP binding (Fortes et al., 2000). These data suggest that eIF4G can bind newly synthesized mRNA (via the CBC) to participate in the nuclear export process and cytoplasmic exchange of CBC for eIF4F.

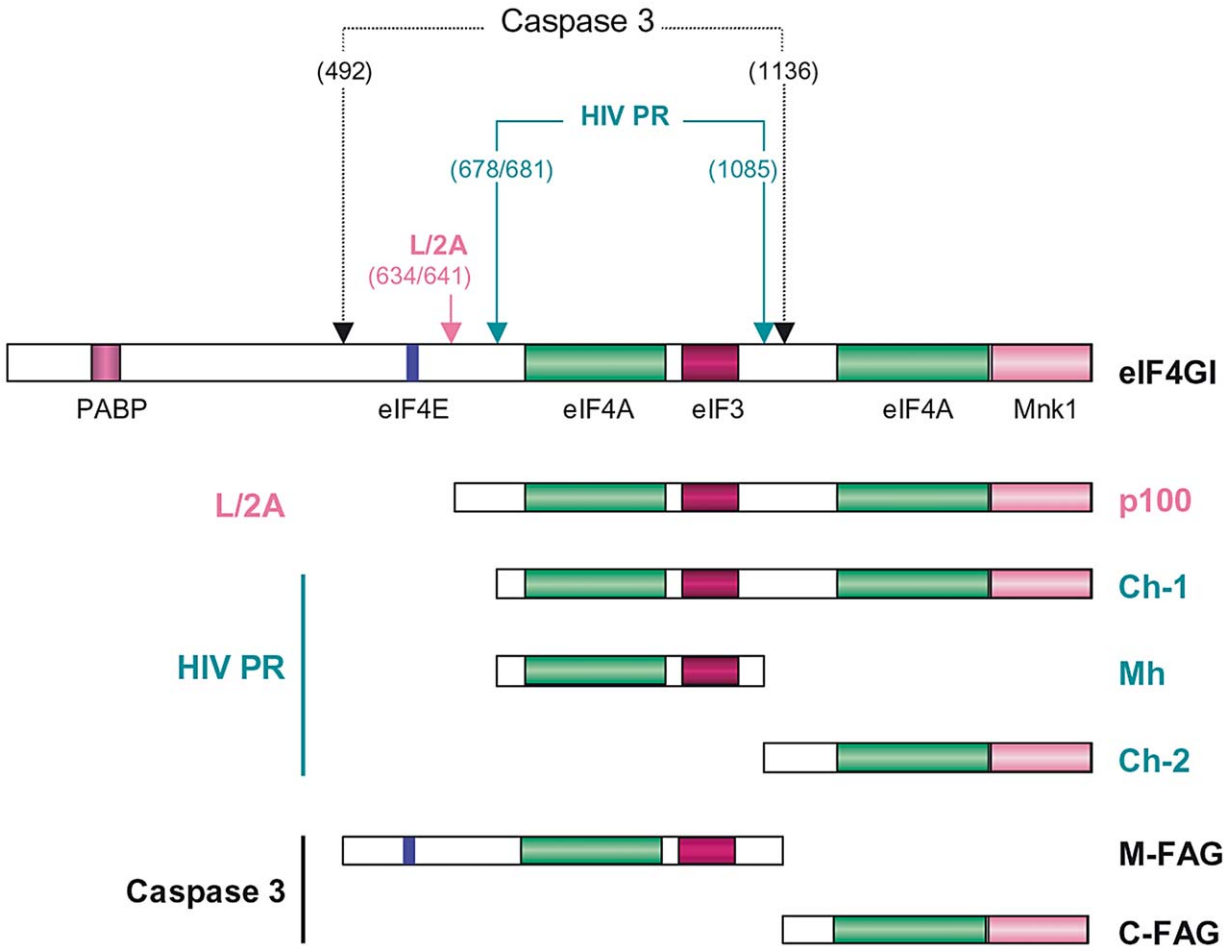


Fig. 4. Map of the cleavage sites on eIF4GI. The eIF4GI mammalian protein and its domains of interaction are schematically represented. The positions of the cleavage sites by picornaviral (L/2A), retroviral (HIV PR) and caspase 3 proteases are indicated. The corresponding C-terminal cleavage fragments generated by proteolysis are depicted below.

## 4. Modifications of eIF4G

### *4.1. Cleavage of eIF4G by picornaviral proteases*

The picornavirus family comprises a large number of human and animal pathogens and is made up of six genera, Enterovirus, Rhinovirus, Hepatovirus, Cardiovirus, Aphthovirus and Parechovirus. The virus genome is an uncapped single stranded RNA molecule of approximately 7.5 kb in length which codes for a large polyprotein that is matured by virally encoded proteases. Infection of cells with the poliovirus is accompanied by a rapid shut-off of host cell protein synthesis, whereas poliovirus RNA translation is not affected (Leibowitz and Penman, 1971). This abrupt decrease of host cell translation is not due to cellular mRNA degradation (Fernandez-Munoz and Darnell, 1976), but rather results from alteration of one of several initiation factors (Helentjaris and Ehrenfeld, 1978). It was initially reported that a purified initiation factor eIF4B could restore translational activity in poliovirus-infected HeLa cell extracts, but it later turned out that the 'restoring activity' was due to eIF4GI that had co-purified with eIF4B (Sonenberg et al., 1978; Trachsel et al., 1980). The poliovirus encoded 2A protease was found to mediate proteolysis of eIF4GI and this event was correlated with the profound decrease of cap-dependent protein synthesis (Etchison et al., 1982; Krausslich et al., 1987). Cleavage of eIF4GI also occurs with 2A protease from rhino- and coxsackievirus and the leader protein (L) of the foot-and-mouth-disease-virus (FMDV, Devaney et al., 1988; Lloyd et al., 1988). Sequencing and individual cloning of the 2A protease from rhino-, polio- and coxsackievirus, as well as the Lb form of the L protease from FMDV (Kirchweger et al., 1994; Liebig et al., 1993) have allowed the mapping of the position of cleavage sites on eIF4GI. It was found that the primary cleavage site was identical for the 2A proteases from polio-, rhino- and coxsackieviruses, whereas L protease cleavage occurs seven amino acids upstream (see Fig. 4, Kirchweger et al., 1994; Lamphear et al., 1993). The fact that 2A and L which are enzymes with distinct biochemical activities can both cleave eIF4GI within the same region suggests the presence of an exposed protease-sensitive site.

However, the order of events that leads to eIF4GI cleavage has been the subject of intense debates. It has been suggested that poliovirus protease 2A, rather than cleaving eIF4GI directly, could activate a quiescent cellular protease which

would, in turn, proteolyse the initiation factor. Evidence supporting this model mostly came from the observations that eIF4GI degradation products and 2Apro could not be co-purified from poliovirus-infected extracts (Bovee et al., 1998a; Lloyd et al., 1986). It was also suggested that eIF3 and a cellular protein of 55 kDa molecular weight of unknown origin was required as co-factors for 2A protease-mediated processing of eIF4GI (Wyckoff et al., 1990, 1992).

Availability of bacterially expressed recombinant 2Apro (from rhino- and coxsackievirus) and L from FMDV showed that these enzymes could act directly on the eIF4F substrate with no further requirement for additional proteins (Kirchweger et al., 1994; Liebig et al., 1993). Direct processing of eIF4GI was also supported by the use of a temperature-sensitive mutant of the HRV2 2A proteinase (Liebig et al., 2002). However, recombinant eIF4GI as well as synthetic eIF4GI peptides are poor substrates for the 2A protease unless they are associated with eIF4E (Bovee et al., 1998b; Haghighat et al., 1996). Similar results were obtained with the leader protease from FMDV showing that the eIF4GI–eIF4E complex rather than eIF4GI alone was the preferred target for the enzyme. This has led to the model whereby binding of eIF4E may change the conformation of eIF4GI, rendering it more susceptible to protease cleavage (Ohlmann et al., 1997). Interestingly, in *S. cerevisiae* it has recently been shown that eIF4G adopts an unfolded-to-folded transition upon binding of eIF4E which may expose the 2A and L cleavage sites (Hershey et al., 1999).

Recently, the 3C protease from FMDV was shown to process eIF4GI to generate fragments different from those generated by L protease, but a role for the cleavage fragments in translation remains to be determined (Belsham et al., 2000).

### *4.2. Cleavage of eIF4G and the inhibition of protein synthesis*

Although it is clear that picornavirus infection leads to the shut-off of host protein synthesis (for review see Gale et al., 2000), the cause of this is still subject to debate. It is widely accepted that the drastic inhibition of protein synthesis in cells infected with entero-, rhino- and aphthoviruses is largely due to the cleavage of eIF4GI mediated by virally encoded proteases (see Fig. 5). However, a large body of evidence suggests that destruction of eIF4GI cannot solely account for the host translational shut-off. First of all, eIF4GI proteolysis only occurs upon infection of cells by entero-, rhino- and apthoviruses but not by cardioviruses, although there is a shut-off of host protein synthesis. In the case of the cardiovirus EMCV, dephosphorylation of 4E-BP largely accounts for the inhibition of cap-dependent protein synthesis (Gingras et al., 1996). Also puzzling is the fact that during the course of poliovirus infection, treatment of cells with various inhibitors of viral replication (e.g. monensin, nigericin or guanidine HCl) results in only a partial inhibition of cellular translation whereas the pool of eIF4GI is substantially degraded (Bonneau and Sonenberg, 1987; Irurzun et al., 1995; Perez and Carrasco, 1992). Conversely, persistent infection of a human erythroleukemic cell line by poliovirus led to substantial degradation of the eIF4GI protein with no concomitant loss of host protein synthesis (Lloyd and Bovee, 1993) and injection of 2A protease into *Xenopus* oocytes results in only a modest inhibition of endogenous protein synthesis despite complete degradation of endogenous eIF4GI (Keiper and Rhoads, 1997). Taken together, all these data suggest that the cleavage of eIF4GI is not solely responsible for the host shut-off in infected cells.

Thus, identification and cloning of eIF4GII have prompted Sonenberg and colleagues to investigate the fate of eIF4GII in cells infected by polio- and rhinoviruses. This study showed that proteolysis of eIF4GII occurred with delayed kinetics compared to the cleavage of eIF4GI and the time of complete disappearance of eIF4GII correlated well with the complete inhibition of host cell protein synthesis. Consequently, the authors have proposed that the destruction of eIF4GII was also responsible for the collapse of host cell translation (Gradi et al., 1998b; Svitkin et al., 1999).

Recently, virally encoded 2Apro (from polio- and coxsackievirus) and 3C (from coxsackievirus) proteases have also been shown to cleave PABP following infection, and this event was proposed to contribute to the host translational shut-off (Joachims et al., 1999; Kerekatte et al., 1999).

In summary, the poliovirus induced host translational shut-off of translation can be explained by the concerted proteolysis of, at least, three components of the translation initiation machinery: eIF4GI, eIF4GII and PABP. Individual contribution of these proteins in this mechanism remains to be determined.

### *4.3. Modifications of eIF4G by other viruses*

Other viruses have also developed strategies to detour eIF4GI from its utilization by the cellular machinery to facilitate production of their viral proteins (see Fig. 6). For instance, rotavirus RNAs which are capped but non-polyadenylated code for a non-structural protein (NSP3) that binds tightly to the 3' end of the viral RNA and can also interact with the amino-terminal part of eIF4GI upstream to the eIF4E binding site. Formation of an NSP3–eIF4GI complex evicts PABP from the eIF4F complex and inhibits host cell translation, whereas that of viral RNAs remained unaffected by pseudo-circularization of the transcript via an NSP3–eIF4GI interaction (Piron et al., 1998).

Influenza virus also encodes a non-structural protein NS1 which is an RNA binding protein that interacts both with the 5' untranslated region of viral transcripts and the amino-terminal part of eIF4GI. This interaction specifically favors translation of influenza virus transcripts by allowing selective recruitment and positioning of eIF4F on the 5' end of viral mRNAs (Aragon et al., 2000).

Expression of adenovirus (Ad) late viral gene products provokes the inhibition of cellular protein synthesis which correlates with dephosphorylation of eIF4E (Huang and Schneider, 1991; Zhang et al., 1994). Recently, the 100 kDa

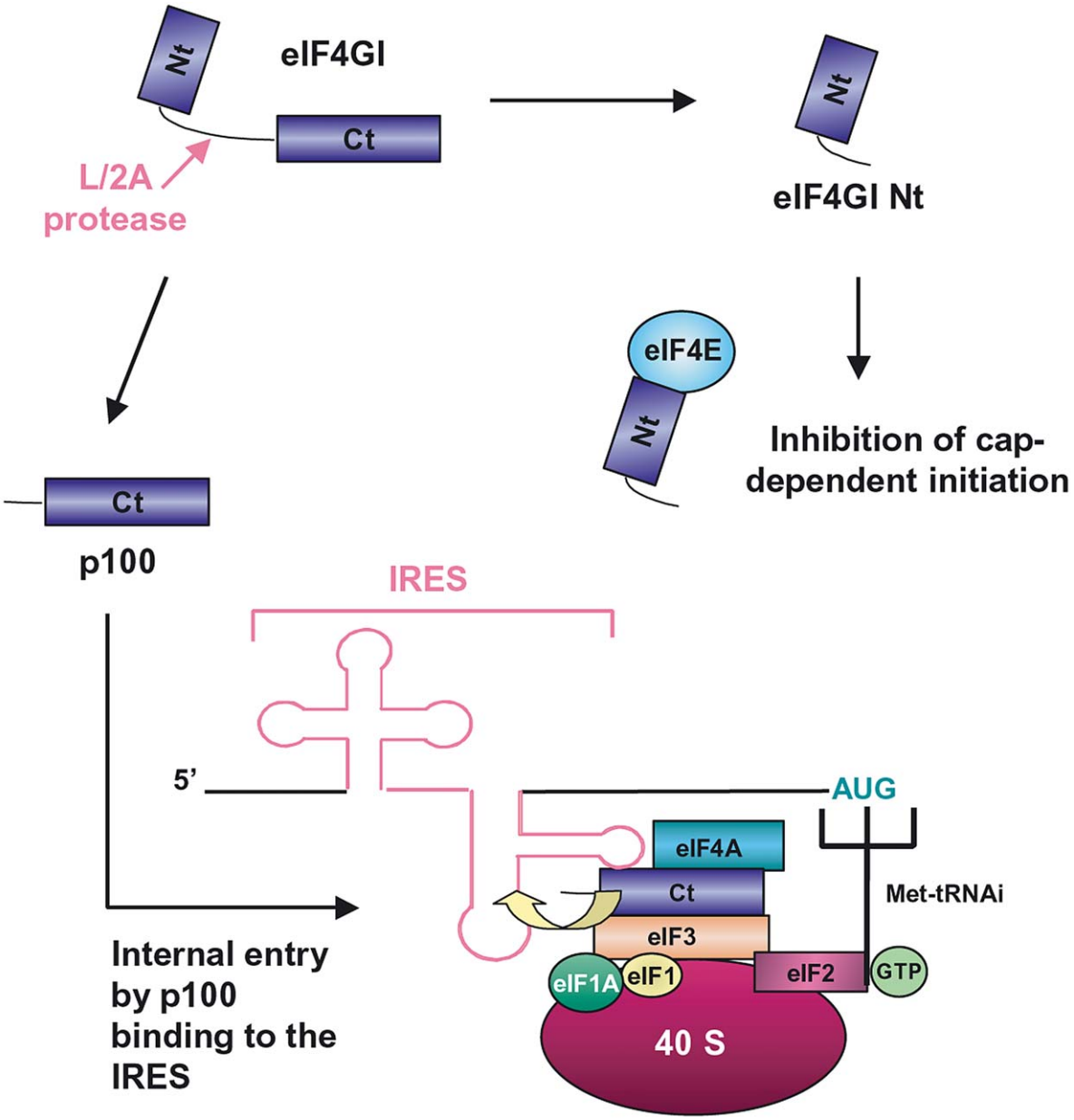


Fig. 5. Consequences of eIF4GI cleavage by picornaviral proteases. Proteolysis of eIF4GI by L/2A picornaviral proteases and the resulting cleavage fragments are represented on the cartoon. Upon this cleavage, the N-terminal fragment of eIF4GI which contains the binding site for eIF4E can no longer be utilized by capped mRNAs, leading to an inhibition of cap-dependent translation. However, the C-terminal part of eIF4GI (p100), is able to bind directly to the picornaviral IRESes and is sufficient to promote internal initiation.

adenoviral late protein (100k) was shown to be directly involved in this mechanism by evicting the Mnk1 kinase from eIF4F. This occurs by competition between 100k and Mnk1 for the binding site on the carboxy-terminus extremity of eIF4GI and results in progressive loss of Mnk1 and impairment of eIF4E phosphorylation (Cuesta et al., 2000b).

Finally, degradation of eIF4GI has been observed in CD4+ cells infected with the human immunodeficiency virus type 1 (HIV-1). Infection was accompanied by a decrease in total protein synthesis, and western blot analysis showed that this event correlated with the proteolysis of eIF4GI although a significant amount of the latter remained unaffected during infection (Ventoso et al., 2001). Expression of the HIV-1 protease by the vaccinia vT7 system in COS-7 cells resulted in proteolysis of eIF4GI suggesting that the retroviral enzyme could be responsible for this effect. This was confirmed by in vitro data showing destruction of eIF4GI by addition of recombinant HIV-1 protease to a cell free system (Ohlmann et al., 2002; Ventoso et al., 2001). In the rabbit reticulocyte translation system, cleavage with the retroviral enzyme occurs at multiple sites and generates a series of amino-terminal fragments, some of which retain the ability to bind to $m^7$GTP sepharose (Ohlmann et al., 2002). On the carboxy-terminal part of the molecule, three cleavage sites have been mapped at amino acid positions 678 and 681 generating a 100 kDa fragment (named Ch-1) and further downstream at

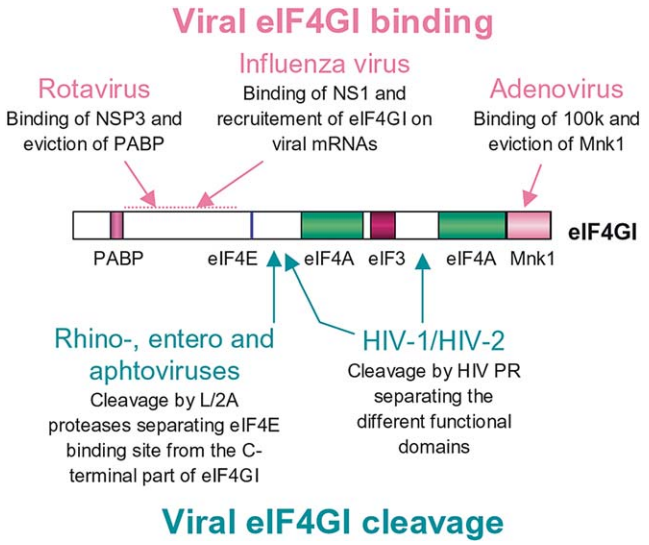


Fig. 6. Modifications of eIF4GI during viral infections. The eIF4GI mammalian protein and its domains of interaction are schematically represented. Sites of interaction between virally encoded proteins and eIF4GI are represented on the upper part of the cartoon. Positions of the cleavage sites by picornaviral and retroviral proteases are indicated on the lower part of the figure.

position 1085 bisecting Ch-1 into a 45 kDa and 55 kDa fragments (named Mh and Ch-2, respectively; see Fig. 4). Interestingly, in the reticulocyte lysate, the HIV-1 protease efficiently processes eIF4GI, but not eIF4GII, despite the conservation of one of the cleavage sites (aa 1085) on the eIF4GII molecule (Ohlmann et al., 2002; Schlick and Skern, 2002). In the RRL, cleavage of endogenous eIF4GI results in impairment of cap-dependent translation due to the loss of the eIF4E binding site (Ohlmann et al., 2002; Ventoso et al., 2001). However, the effect of this proteolysis on IRES-mediated translation is subject to debate. Ventoso and colleagues have reported that EMCV-IRES-driven translation was not affected by the treatment of the lysate with the retroviral enzyme. Moreover, translation of a transcript containing the authentic 5' UTR driving the synthesis of HIV-1 Gag was strongly stimulated (fourfold) by the addition of its cognate protease (Ventoso et al., 2001). Our results are in contrast with these data as we have repeatedly observed moderate but significant decrease of IRES-driven translation (including HIV-1 translation) upon cleavage with HIV-1 protease and the reason for this discrepancy is unknown.

From our results and the results of Ventoso and colleagues, two models can be put forward. The first model predicts that HIV-1 uses a similar strategy to that utilized by poliovirus in which cleavage of eIF4GI inhibits host cell translation and stimulates viral protein production (Ventoso et al., 2001). However, in lentivirus, the protease is released in its mature form at the end of the viral cycle at a stage when most of the structural proteins and enzymes have been produced (Freed, 1998; Wlodawer et al., 1989). Thus, our model predicts that proteolysis of eIF4GI by the HIV-1 protease impairs both cellular and viral translations. As a consequence, this event would induce polysome dissociation to allow packaging of genomic RNA and viral assembly.

### *4.4. Cleavage of eIF4G during apoptosis*

Apoptosis, or programmed cell death, is characterized by a series of morphological changes including cell shrinkage, membrane blebbing, chromatin condensation, and DNA fragmentation that ultimately result in loss of cell viability. Two independent research groups have reported that eIF4GI undergoes proteolysis during apoptosis. Using B and T cell lines exposed to serum deprivation, or cycloheximide treatment, Clemens and colleagues have observed the selective degradation of eIF4GI which occurs early upon the induction of apoptosis (Clemens et al., 1998). Similar results were obtained by Marissen and Lloyd in HeLa cells treated with the antitumor agents cisplatin and etoposide (Marissen and Lloyd, 1998). Both groups have also used a wide spectrum of different apoptosis inducers (UV light, proteasome inhibitors and death receptor ligands) and all of them caused almost complete loss of eIF4GI. Prevention of eIF4GI cleavage by the use of general caspase inhibitors such as Z-VAD.FMK and Z-DEVD.FMK established that these enzymes were the effectors of proteolysis and further investigation identified caspase 3 as both necessary and sufficient for targeted degradation of eIF4GI in vitro and in vivo (Bushell et al., 1999; Clemens et al., 1998; Marissen and Lloyd, 1998). The proteolytic processing of eIF4GI yields a 76 kDa fragment (referred to as M-FAG for middle fragment of apoptotic cleavage of eIF4GI) that retains binding sites for eIF4E, eIF4A and eIF3 (see Fig. 4, Bushell et al., 2000). The cleavage of eIF4GII has also been described in apoptotic cells. This is mediated by caspase 3 activation and occurs at multiple sites to yield many different fragments that are probably not active in the translation process (Marissen et al., 2000).

During apoptosis, proteolysis of eIF4GI occurs rapidly and is accompanied by the inhibition of cellular translation. However, the apparently complete processing of eIF4GI correlates with not more than 65% inhibition of translation to the control rate suggesting that the residual translational activity may be due to utilization of one, or more, of the apoptotic cleavage fragments (Morley et al., 1998). This is in agreement with the fact that under severe apoptotic conditions, some cellular IRES-driven mRNAs such as c-myc, Apaf-1, XIAP and DAP-5 continue to be translated, and this is thought to be mediated by the apoptotic cleavage fragments of the DAP-5 and eIF4GI proteins (Henis-Korenblit et al., 2002; Nevins et al., 2003; Stoneley et al., 2000).

## 5. The role of eIF4G in translation

### *5.1. Cap-independent translation*

Early work established that extracts from poliovirus-infected cells promoted the translation of poliovirus mRNA (Buckley and Ehrenfeld, 1987); this activity appeared to be

mediated by the presence of the 2A protease gene but could not exclusively be explained by reduced competition for the translation machinery (Hambidge and Sarnow, 1992). Similarly, human rhinovirus RNA translation was stimulated by the addition of its cognate protease in a HeLa cell-free system (Liebig et al., 1993) and FMDV-L protease could enhance translation driven by IRESes from both rhino- and enteroviruses (Ziegler et al., 1995b). Thus, it was proposed that the proteolytic cleavage of a set of cellular proteins, including eIF4GI, could be responsible for the enhancement of IRES-driven translation (Ziegler et al., 1995a, b). Using a preparation of in vitro translated L protease from FMDV, we have shown that addition of the enzyme to the reticulocyte lysate could stimulate translation of uncapped forms of normally capped mRNAs (Ohlmann et al., 1995b). However, we failed to observe any stimulation of translation driven by the IRES element from the Theiler's murine encephalomyelitis virus (TMEV) suggesting that this effect was not universal in the picornavirus family.

At the same time, Lamphear and colleagues have used both recombinant L and 2A proteases in an attempt to map the binding sites for eIF4A, eIF4E and eIF3 on eIF4GI. At low concentration of proteases, the cleavage of eIF4GI occurs at a unique site and separates two functional domains: the amino-terminal fragment (1/3 of the molecule) which retains the interacting site for eIF4E and the carboxy-terminal fragment (aa 642–1560 referred to as p100) which contains the eIF4A and eIF3 binding domains (see Fig. 4). These results suggest that processing of eIF4GI uncouples the cap recognition domain from the ribosome binding and RNA helicase activity provided by eIF3 and eIF4A (Lamphear et al., 1995).

A role for the domains of eIF4GI in translation was established after manipulating levels of amino-terminal and carboxy-terminal fragments generated by cleavage of eIF4GI by the L protease from FMDV (Ohlmann et al., 1996). These experiments have shown that (i) requirement for eIF4F for internal initiation on IRES-driven mRNAs can be fulfilled by the carboxy-terminal eIF4GI fragment which retains the eIF3 and eIF4A binding sites (Fig. 5); (ii) upon cleavage, the amino-terminal part of eIF4GI remains associated with eIF4E and depletion of the two proteins from the lysate does not impair cap-independent translation suggesting that there is no requirement for either of these peptides; (iii) the carboxy-terminal fragment of eIF4GI can also stimulate translation of uncapped cellular RNAs which do not possess an IRES element. These results were confirmed by partial purification of the carboxy-terminal fragment of eIF4GI from a rabbit reticulocyte ribosome high salt wash that allowed direct analysis of its effects on translation in the absence of proteolysis (Ohlmann et al., 1997).

The individual role of canonical initiation factors in IRES-mediated translation has been directly assessed by using primer extension analysis in a reconstituted in vitro assay with purified components and recombinant proteins (Met-tRNA$_i$, 40 S subunits and initiation factors, Pestova et al., 1996b). This established that virtually all canonical initiation factors with the exception of eIF4E were required for internal ribosomal entry on the EMCV IRES. Interestingly, the minimal region on eIF4GI required for IRES binding has been mapped between amino acids 746 and 949 (Lomakin et al., 2000), a region that had previously been suggested to contain two motifs (RNP-1 and RNP-2; amino acids 757–762 and 855–862) typical of RRM (Goyer et al., 1993). However, introduction of point mutations or sequence insertions in the RNP-1 and RNP-2 motifs did not affect the RNA binding ability of eIF4GI on the EMCV IRES casting some doubts on the functional role of this RRM (Lomakin et al., 2000). It is noteworthy that the binding abilities of eIF4GI were magnified by the presence of eIF4A and recent data have now defined that 43 S ribosome recruitment needs formation of an eIF4GI–eIF4A complex onto the EMCV IRES to permit internal ribosome entry (Kolupaeva et al., 2003).

The critical role of p100 in uncapped and IRES-driven translation was further demonstrated by cloning and expression of recombinant variants of eIF4GI lacking the N-terminal domain (Borman et al., 1997; De Gregorio et al., 1998). In vitro, the central domain of eIF4GI (amino acids 642–1091) was sufficient to mediate stimulation of uncapped RNA translation suggesting that the C-terminal eIF4A binding site was dispensable for this effect. Further evidence for a critical role of the middle domain of eIF4GI came from experiments using a bicistronic construct in which the iron responsive element (IRE) was inserted in the intercistronic spacer (De Gregorio et al., 1999). Under these conditions, translation of the second cistron was very poor. However, when this construct was co-transfected with a plasmid coding for the central domain of eIF4GI expressed as a fusion protein with the RNA binding protein IRP-1 (iron responsive protein 1), translation of the downstream cistron was increased by about fivefold and increasing the number of IREs from 1 to 3 further stimulated translation by twofold. This established that the middle part of eIF4GI forms a ribosome recruitment core which is the minimal domain to enable internal ribosomal entry.

The minimal sequence required for cap-dependent translation was also investigated by using deletion mutants and toeprinting analysis and found to correspond to the middle domain of eIF4GI extended by some 60 amino acids to include the eIF4E binding site (Morino et al., 2000). These results suggest that the core domain of eIF4GI is absolutely required for capped, uncapped and IRES-driven translations. However, while this core domain of eIF4GI was sufficient to promote binding to the EMCV IRES, this binding was less efficient than that of p100 under the same conditions (Lomakin et al., 2000). Moreover, whereas ectopic expression of the C-terminal two third of eIF4GI could activate the EMCV IRES in cells, the central region alone failed to do so suggesting that the second binding site for eIF4A plays an important modulatory role in this process (Imataka and Sonenberg, 1997).

Finally, it should be noted that the IRESes of HCV (and related pestiviruses) together with the cricket paralysis virus IRES can initiate protein synthesis independently of eIF4GI. Ribosome binding occurs directly at the AUG codon and needs eIF2-GTP-Met-tRNA and eIF3 in the case of HCV (Pestova et al., 1998), whereas initiation on the cricket paralysis virus IRES does not require any of the known initiation factors and occurs directly from the A site of the ribosome in a tRNA-independent manner (Wilson et al., 2000).

### *5.2. Cap-dependent translation*

The use of recombinant picornaviral proteases in cell-free translation systems clearly showed that cleavage of eIF4GI inhibits translation of capped mRNAs due to the fact that the carboxy-terminal fragment of eIF4GI lacking the eIF4E binding site was unable to promote 48 S assembly (Ohlmann et al., 1995a, b; Rau et al., 1996; Ziegler et al., 1995a, b). However, it has recently been reported that recombinant p100 (see Fig. 4) can support capped mRNA translation in an eIF4GI depleted lysate (Ali et al., 2001). Moreover, addition of p100 had also the ability to reverse the effect caused by inhibitors of cap-dependent translation such as $m^7$GTP cap analogues or 4E-BP. It is noteworthy that the concentration of p100 required to support capped mRNA translation was fourfold higher than endogenous concentration of eIF4GI contained in the reticulocyte lysate suggesting that the relative efficiency of p100 is somewhat lower than that of eIF4G. Nevertheless it suggests that capped mRNA translation can occur in the absence of eIF4E and the mechanisms of ribosome recruitment in the absence of an IRES–eIF4G or cap-eIF4E–eIF4G interactions remain to be elucidated.

## 6. Conclusions and future prospects

Over the last years, a considerable amount of work has been carried out to decipher the role of eIF4G in translation initiation. It is now recognized that eIF4G has a key role in 43 S delivery to the mRNA molecule. This occurs at the 5' end of the capped mRNA by virtue of an eIF4E–eIF4G interaction and allows recruitment of the 43 S complex by a direct contact with eIF3. In the case of IRES-driven translation, binding of the 43 S complex can occur at internal sites and is mediated by direct eIF4G–RNA interaction. Thus, only for a few viral exceptions (namely HCV, pestivirus and cricket paralysis virus) eIF4G is critical to promote ribosome delivery to the mRNA. However, little is known about the role of eIF4G following this event and during migration of the 48 S complex to the AUG codon and this should deserve further investigation.

It would also be of considerable interest to determine, among all the eIF4G interacting proteins which ones can be simultaneously bound to eIF4G and how these ligands can induce co-operativity and structural conformational changes on the molecule. This is particularly important as eIF4G interacts and modulates the activity of a large number of initiation factors and other proteins which play a key role in the control of translation.

Finally, the growing number and the conservation across species of all the eIF4G isoforms and derivatives point out to an important function of these proteins. This should be explored further especially under conditions where eIF4G is modified or degraded.

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