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REVIEW

eIF4G: Translation's mystery factor begins to yield its secrets

SIMON J. MORLEY, PENELOPE S. CURTIS, and VIRGINIA M. PAIN

Department of Biochemistry, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, United Kingdom

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INTRODUCTION

Translational control is now recognized as a major contributor to the regulation of gene expression in eukaryotes. Within this process, most of the regulatory mechanisms characterized so far target the initiation stage, whereby the two ribosomal subunits bind sequentially to the 5′ end of a messenger RNA molecule, forming a complex at the AUG initiation site. Initiation is itself a complicated, multistep process, consisting of a sequence of binding events involving at least nine polypeptide initiation factors together with initiator tRNA, mRNA, and ribosomes (reviewed in Merrick, 1992; Rhoads, 1993; Browning, 1996; Merrick & Hershey, 1996; Morley, 1996; Pain, 1996). In terms of regulation, a particularly important step is the binding to the mRNA 5′ end of the 43S preinitiation complex, consisting of the 40S ribosomal subunit, the initiator tRNA (Met-tRNA$_i$), GTP, and a number of initiation factors, including eIF2 and eIF3.[1] This step is known to be an important target for physiological regulation of global rates of protein synthesis (Merrick, 1992; Rhoads, 1993; Morley, 1994, 1996; Merrick & Hershey, 1996; Pain, 1996; Sonenberg, 1996). However, its pivotal position as the entry point for an mRNA molecule into the translation process raises the more exciting possibility that its modulation plays a key role in changing the selective recruitment of mRNA species in response to signals that lead to re-programming of gene expression, such as those received during early development, mitogenic stimulation, or stress responses.

In mammalian cells, mRNA is modified posttranscriptionally, with a cap structure at the 5′ end and a poly (A) tail at the 3′ end, both of which, individually and in concert, play essential roles in the regulation of translation. Cap-dependent initiation of translation involves the assembly of initiation factors at the 5′ end of mRNA. These include: the cap-binding protein, eIF4E; eIF4A, an ATP-dependent RNA helicase essential for translation of all mRNAs (Altmann et al., 1990; Pause et al., 1994b); and the eIF4G polypeptide. Together these initiation factors form the eIF4F complex. There is a strong requirement for eIF4E and eIF4G in cap structure-dependent initiation, with the eIF4F complex believed to promote the unwinding of mRNA secondary structure to facilitate the binding of the 40S ribosomal subunit (reviewed in Merrick, 1992, 1994; Pause & Sonenberg, 1993; Rhoads, 1993; Morley, 1994; Pain, 1996; Hentze, 1997; Jackson & Wickens, 1997). Although there is a fair amount known about the structure and function of eIF4E and regulation of its activity, eIF4G, a protein of 154 kDa in mammalian cells, has a long history as the "mystery factor" in translation. Its functional importance was clear from an early stage, because its specific proteolytic cleavage in cells infected with picornaviruses is associated with a profound inhibition of host cell protein synthesis. More recently, mammalian eIF4G and its homologues in wheat and *Saccharomyces cerevisiae* have been cloned and sequenced, and in the last 2–3 years, there has been tremendous progress in elucidating the role of this protein. It is now clear that eIF4G is an adapter molecule par excellence; a highly conserved binding site for eIF4E has been defined, and regions of the molecule with binding activity toward eIF3, eIF4A, and RNA elements have been identified in different systems. Figure 1 depicts a current model of the "48S" [43S.mRNA] initiation complex, based on studies of the interaction of mammalian eIF4G with eIF4E, eIF4A, and eIF3. This figure illustrates how eIF4G can form a

Reprint requests to: Simon J. Morley, Department of Biochemistry, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, United Kingdom; e-mail: s.j.morley@sussex.ac.uk.

Abbreviations: eIF, eukaryotic initiation factor; IRES, internal ribosome entry segment; EMCV, encephalomyocarditis virus; FMDV, foot-and-mouth-disease virus; Pab1p, poly(A) binding protein; RRM, RNA recognition motif.

[1] The nomenclature employed for initiation factors is as described in Merrick and Hershey (1996).

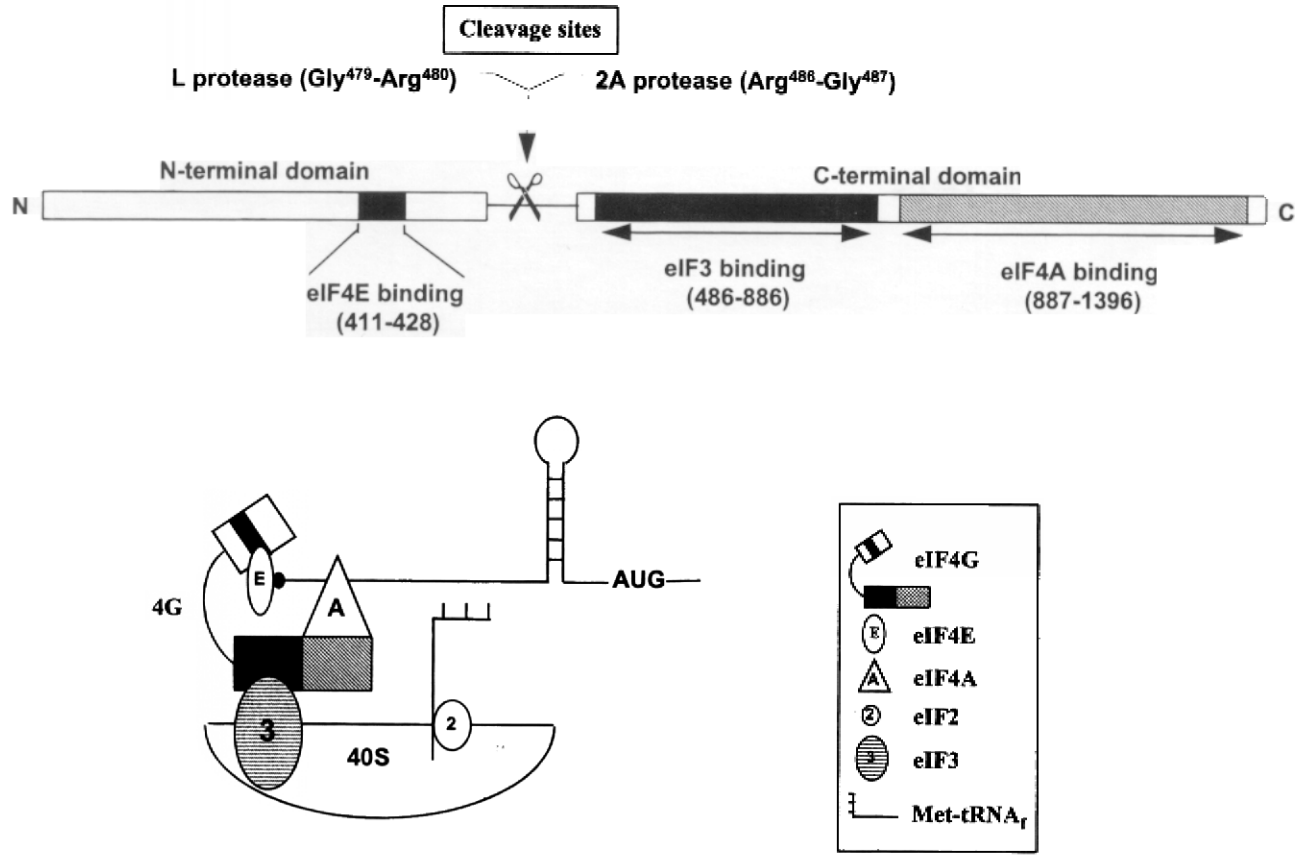


FIGURE 1. eIF4G forms a bridge in the formation of the 48S initiation complex. The upper panel is a diagrammatic representation of the functional domains of mammalian eIF4G, indicating the sites of direct cleavage by 2A and L proteases and the regions responsible for interaction with eIF4E, eIF3, and eIF4A (after Lamphear et al., 1993, 1995; Mader et al., 1995). The lower panel indicates how interaction of the N-terminus of eIF4G with eIF4E, and the C-terminus with eIF3 and eIF4A allow eIF4G to function as a bridge between the mRNA and ribosome in the formation of the 48S initiation complex (adapted from Lamphear at al., 1995).

bridge between the mRNA cap (via eIF4E) and the 40S ribosomal subunit (via eIF3, already part of the 43S preinitiation complex). However, it is becoming clear that eIF4G is a considerably more versatile molecule than depicted in this model. For example, it also appears to play an essential role in a distinct, cap-independent mechanism employed in the translation of picornavirus RNAs and a few cellular mRNAs. This function can be fulfilled by a proteolytic degradation product lacking the eIF4E binding site and may involve direct binding to mRNA. In addition, a very exciting recent development has been the identification of an element in the yeast protein that binds the poly(A) binding protein, Pab1p, opening up the interesting possibility that eIF4G forms part of a physical link that could account for the functional interaction between the 5′ and 3′ ends of mRNA that is rapidly emerging as a major force in translational regulation (Gallie, 1996; Jacobson, 1996; Richter, 1996; Wickens et al., 1996; Jackson & Wickens, 1997; Sachs & Buratowski, 1997; Sachs et al., 1997).

In this review, we aim to draw together information derived from sequence analysis and biochemical binding studies to assess the function and regulatory significance of eIF4G in the control of translation. The reader is referred to a number of reviews on the initiation of translation in general (Merrick, 1992; Browning, 1996; Gallie, 1996; Merrick & Hershey, 1996; Pain, 1996) and on the mRNA binding step in particular (Rhoads, 1993; Merrick, 1994; Morley, 1994, 1996; Rhoads et al., 1994; Sonenberg, 1994, 1996; Jackson & Wickens, 1997; Sachs et al., 1997). An excellent summary article on the structure and function of eIF4G has also appeared (Hentze, 1997).

STRUCTURE AND FUNCTION STUDIES ON eIF4G

Overview

Mammalian eIF4G

eIF4G was first identified as part of a complex with the ability to restore protein synthesis in picornavirus-infected HeLa cell lysates (Tahara et al., 1981; Lloyd et al., 1987; Ehrenfeld, 1996) and has been cloned sub-

sequently. Sequencing of a cDNA encoding human eIF4G revealed a 154-kDa polypeptide (Yan et al., 1992; Figs. 2, 3). Until recently, little was known about the structure and function of eIF4G, but the use of virally encoded proteases that mediate the inhibition of host protein synthesis in picornavirus-infected cells has proven invaluable in addressing this relationship. It has been known for many years that infection of cells with picornaviruses of the enterovirus group or with foot-and-mouth-disease virus leads to cleavage of eIF4G, with concomitant inhibition of cap-dependent initiation (reviewed in Belsham & Sonenberg, 1996). This can be reproduced in translation systems in vitro, where cleavage of eIF4G by viral proteases, such as 2A of poliovirus, coxsackie virus, and human rhinovirus (Etchison et al., 1982; Liebig et al., 1993) or the leader (L) protease of FMDV (Devaney et al., 1988; Ohlmann et al., 1995, 1996, 1997; Ziegler et al., 1995; Borman et al., 1997), disrupts translation of capped mRNAs, but permits that of picornavirus RNAs. Picornavirus RNAs are naturally uncapped and possess highly structured elements (called internal ribosome entry segments) within their 5′ untranslated regions (5′ UTRs) that direct 40S ribosomal subunits to bind internally rather than at the extreme 5′ end of the mRNA (reviewed in Hellen & Wimmer, 1995; Jackson & Kaminski, 1995; Belsham & Sonenberg, 1996; Ehrenfeld, 1996). 2A and L protease specifically cleave eIF4G at distinct sites in a region postulated to have a high probability of β-turns. 2A protease cleaves between amino acids Arg-486 and Gly-487 (Lamphear et al., 1993) and L protease between Gly-479 and Arg-480 (Kirchweger et al., 1994; Fig. 1). Following such cleavage, the cap-binding function of the eIF4F complex is separated from the eIF4A helicase function (Fig. 1) and, as a consequence, the cleaved factor is unable to promote initiation on capped mRNA. This mechanism, discussed in detail below, is consistent with earlier work showing that the failure of cap-dependent translation of cellular mRNAs that occurred concomitantly with cleavage of eIF4G in poliovirus-infected cells (Etchison et al., 1982) was linked to impaired ability of eIF4E to be crosslinked to the mRNA cap structure (Lee & Sonenberg, 1982), and to the appearance of complexes of eIF4E with one of the cleavage products of eIF4G (Lee et al., 1985; Etchison & Smith, 1990; Buckley & Ehrenfeld, 1992). On the other hand, direct studies with in vitro systems have demonstrated that cleaved eIF4G is able to support translation of sequences downstream of a picornavirus IRES elements (Thomas et al., 1992; Liebig et al., 1993; Ohlmann et al., 1995; Ziegler et al., 1995) and can even enhance translation driven by enterovirus or rhinovirus IRES elements (Liebig et al., 1993; Ziegler et al., 1995). In addition, cleavage of eIF4G enhances translation of uncapped transcripts lacking an IRES (Ohlmann et al., 1995). Studies with the reticulocyte lysate have shown that the C-terminal fragment of eIF4G is both necessary and sufficient to reproduce the effect of eIF4G cleavage on translation of uncapped and IRES-containing mRNAs (Ohlmann et al., 1996, 1997; Borman et al., 1997). Furthermore, transient transfection of the C-terminal domain of eIF4G into COS7 cells has been shown to enhance the translation of a luciferase reporter gene downstream of the encephalomyocarditis IRES sequence (Yamanaka et al., 1997). Thus, mammalian eIF4G fulfils at least two distinct functions in the initiation of translation. The intact protein is required to mediate mRNA recruitment via the mRNA cap, whereas the C-terminal two-thirds of the protein is sufficient to support IRES-driven translation. This may explain why, with the exception of eIF4E, there appears to be little difference between the requirement for canonical initiation factors, including the eIF4F complex, in supporting cap-structure-dependent translation on the one hand and IRES-driven initiation on the other (Anthony & Merrick, 1991; Scheper et al., 1992; Pause et al., 1994b; Ohlmann et al., 1995, 1996; Pestova et al., 1996a, 1996b; Borman et al., 1997). A further clue as to the role of eIF4G in the latter process has been provided recently by Pestova et al. (1996a, 1996b), reconstituting IRES-mediated initiation in vitro using purified components. These workers demonstrated that the essential role of the eIF4F complex in the formation of an initiation complex on EMCV mRNA could be fulfilled by truncated recombinant eIF4G variants lacking the eIF4E binding domain, and, moreover, that such variants could bind directly to the IRES element of this mRNA in the absence of ribosomes (Pestova et al., 1996a, 1996b).

eIF4G and eIF4F in lower eukaryotes and plants

Over the years, many groups have isolated from yeast (Goyer et al., 1989; Lanker et al., 1992), plants (Browning, 1996), and *Drosophila* (Zapata et al., 1994) eIF4E:eIF4G complexes that interact with the mRNA cap structure. In *S. cerevisiae*, two genes (*TIF4631* and *TIF4632*) were identified as encoding the largest component of the eIF4F complex (Figs. 2, 3), the latter also included eIF4E and a 20-kDa protein (Lanker et al., 1992; Altmann et al., 1997). Although both forms of eIF4G could interact with eIF4E, there appears to be some functional difference between *TIF4631* and *TIF4632*. Although *TIF4631*-disrupted strains exhibited a slow-growth phenotype, disruption of *TIF4632* failed to show any phenotype. A double disruption of these genes was lethal (Goyer et al., 1993). In plant cells, the situation appears to be more complicated, with two distinct cap-binding protein complexes being identified in wheat (Browning, 1996). One form, referred to as eIF4F, contains polypeptides of 26 kDa and 220 kDa, whereas the other, eIF*iso*4F, contains 28-kDa and 86-kDa proteins. Like the mammalian coun-

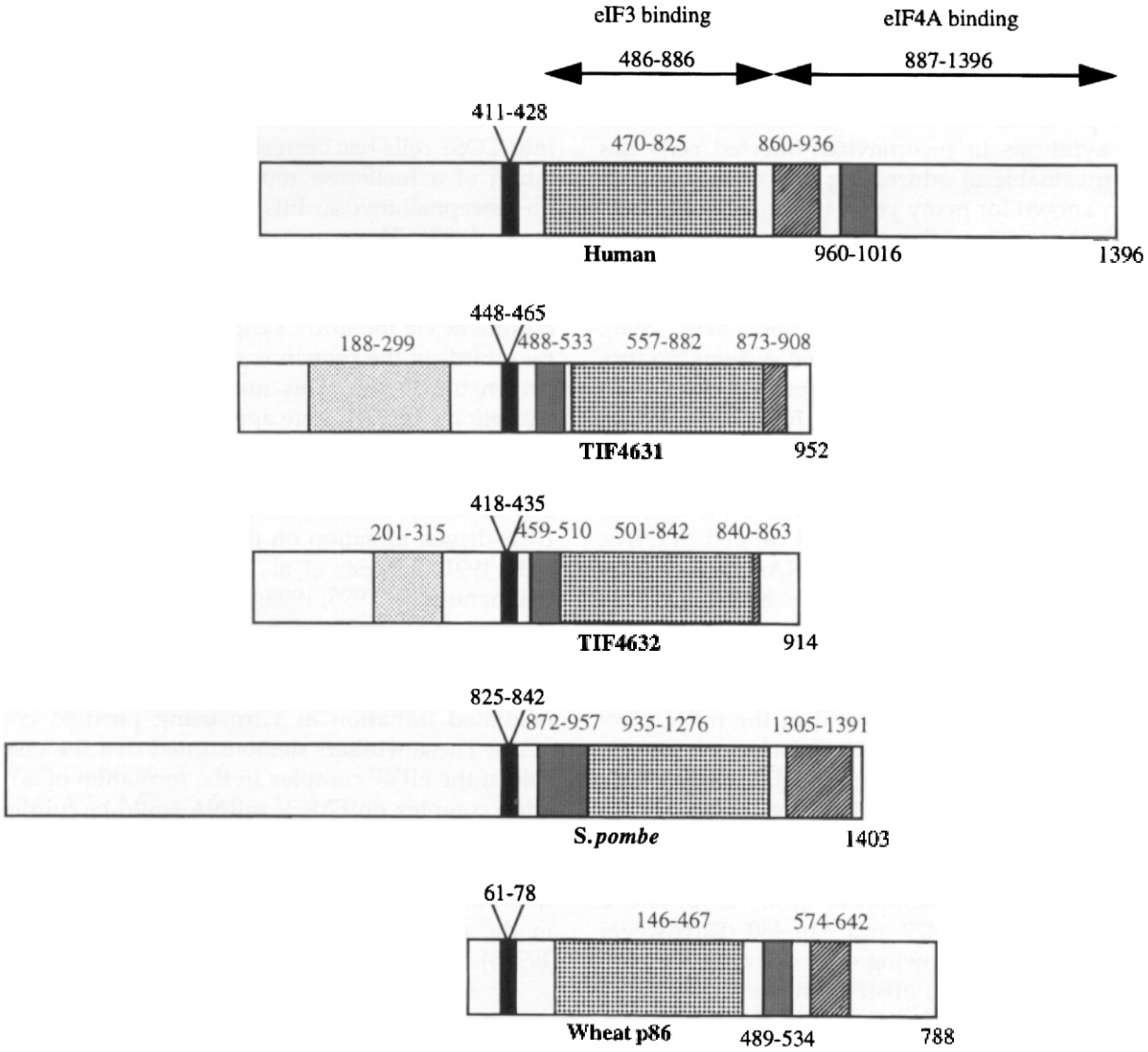


FIGURE 2. Schematic representation of eIF4G polypeptides from human, *S. cerevisiae*, *S. pombe*, and wheat germ, aligned through the eIF4E binding site. The eIF4E binding site on eIF4G is described in the text and in the following references: human (Lamphear et al., 1995; Mader et al., 1995); *S. cerevisiae*, TIF4631 and TIF4632 (Mader et al., 1995; Altmann et al., 1997); wheat (Metz & Browning, 1996). The putative eIF4G polypeptide sequence from *S. pombe* (cosmid c17C9, chromosome 1) was derived from the *S. pombe* sequencing project (The Sanger Centre) using a Blast search for similarity to both human eIF4G and TIF4631. The GAP program (gap weight 3.00, length weight 0.1) was used to identify, in the human, wheat, and *S. pombe* proteins, potential regions of similarity to the following features in the *S. cerevisiae* homologues: the RRM (Allen et al., 1992; Goyer et al., 1993); arginine/serine (R-S)-rich regions (Goyer et al., 1993); sites of interaction with Pab1p (Tarun & Sachs, 1996; A. Sachs, pers. comm.).

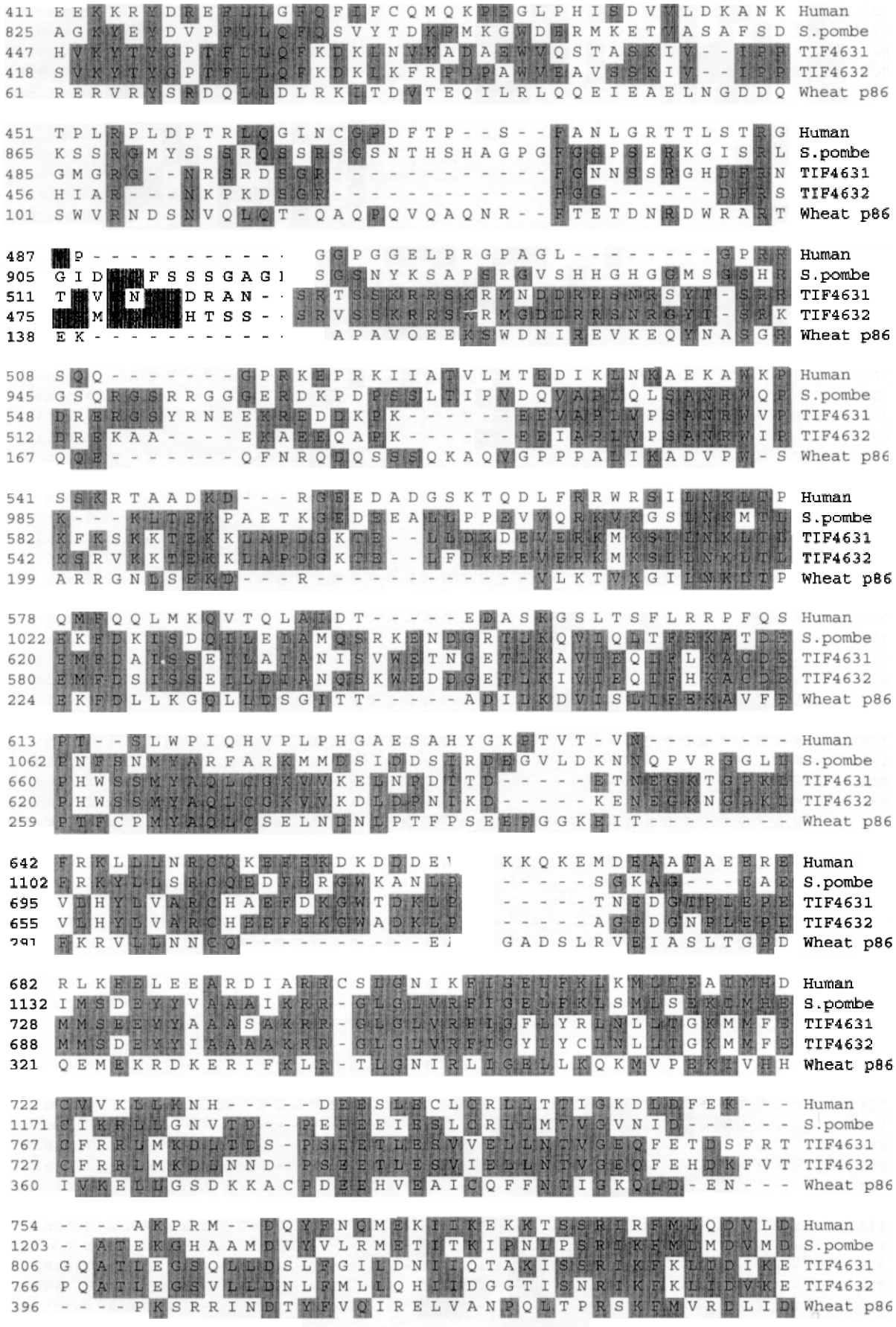
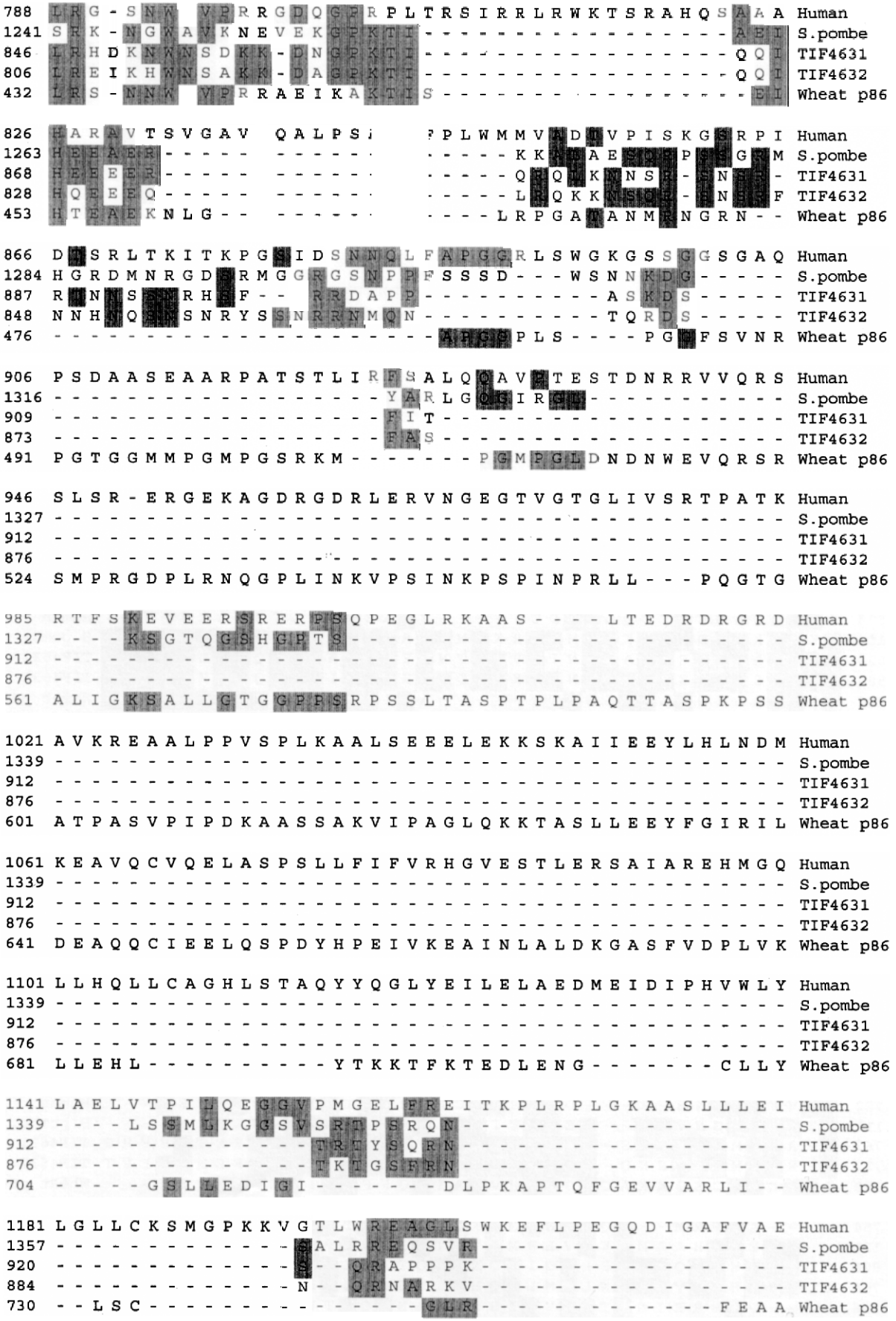


FIGURE 3. *(Figure continues on next page*



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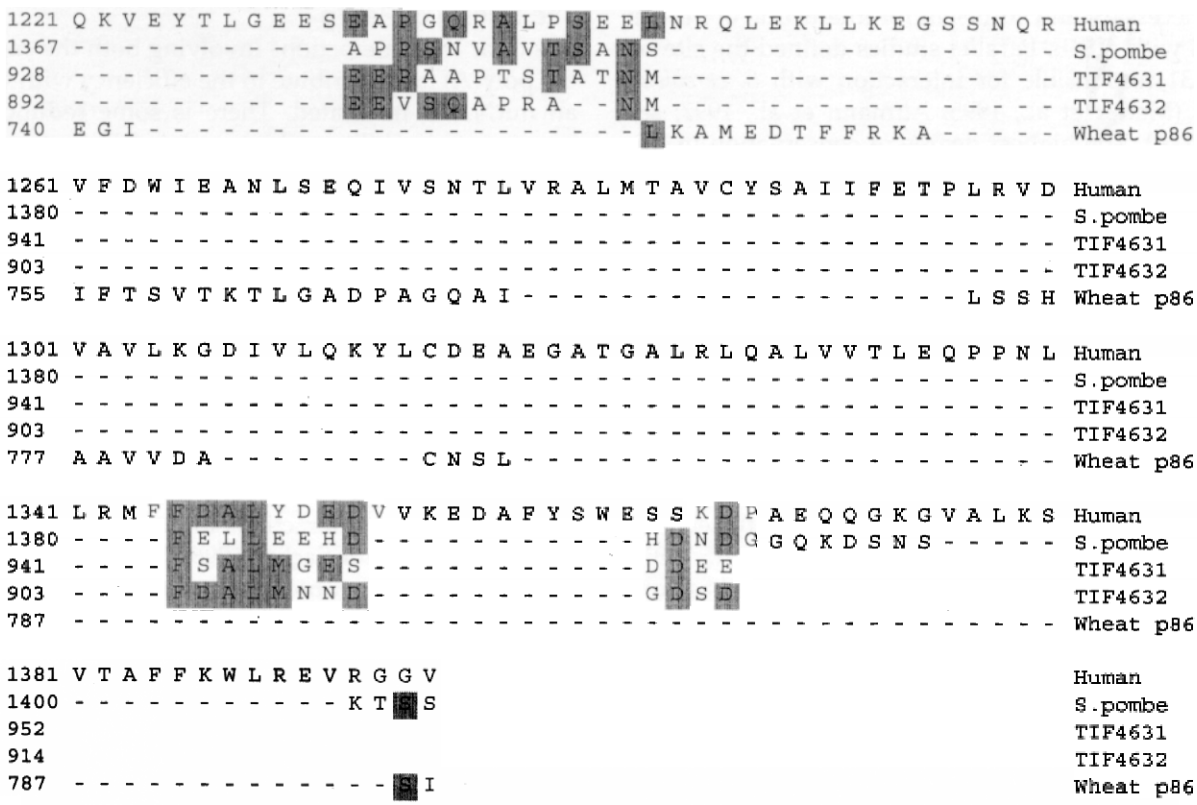

FIGURE 3. Sequence alignment of eIF4G from human, *S. cerevisiae*, *S. pombe*, and wheat. Accession numbers of the aligned sequences are: human eIF4G, D12686; *S. cerevisiae*, TIF4631, L16923; *S. cerevisiae*, TIF4632, L16924; wheat p86, M95747; *S. pombe*, cosmid c17C9 (The Sanger Centre). Sequence alignment of the amino acids N-terminal to the eIF4E binding site of these proteins suggested poor homology, apart from that of the Pab1p binding domain in TIF4631 and TIF4632 (see Fig. 2). Hence, multiple sequence alignment of various eIF4G homologues, downstream from the eIF4E binding site, was performed using the MegAlign software of Lasergene (DNASTAR), and the Clustal method (PAM250 residue weight table; Gap Penalty, 10; Gap Length Penalty, 10). Amino acids in shaded regions indicate sequence identity.

terpart, the isolated complexes may or may not contain eIF4A, depending upon the purification scheme employed (Webster et al., 1991; Browning, 1996). The eIF4E (p26) and eIF*iso*4E (p28) proteins share homology, and both have cap-binding activity (Browning, 1996; Gallie, 1996). Figures 2 and 3 show a comparison of mammalian eIF4G with the two *S. cerevisiae* homologues, TIF4631 and TIF4632, and with the p86 subunit of wheat eIF*iso*4F. The complete sequence of the p220 subunit of wheat eIF4F is not yet available, although a partial comparison with the p86 subunit can be found in Browning (1996). In the fission yeast, *Schizosaccharomyces pombe*, the eIF4G component has yet to be purified and characterized biochemically (Ptushkina et al., 1997). However, analysis of the partially completed *S. pombe* genomic DNA database reveals a putative gene encoding a polypeptide with homology to the mammalian and *S. cerevisiae* eIF4G sequences (Figs. 2, 3). The areas exhibiting the strongest conservation are a motif in the N-terminal domain, identified as the eIF4E binding site, and a more extensive region found near the center of the mammalian protein, corresponding to the amino-terminal half of the C-terminal cleavage fragment resulting from treatment with picornavirus proteases (Fig. 3). These domains are discussed in further detail below.

The N-terminal domain of eIF4G

The binding of eIF4E

Lamphear et al. (1995) have used viral protease cleavage of eIF4G to analyze the association of eIF4G with other initiation factors. Using a combination of m^7GTP-Sepharose affinity chromatography, sedimentation analysis, and immunoaffinity resins, they were able to show that eIF4E interacts with the N-terminal fragment of eIF4G (Fig. 2). In the reticulocyte lysate, cleavage of eIF4G with L protease resulted in the release of the N-terminal fragment of eIF4G from the ribosome and its co-purification with eIF4E on m^7GTP-Sepharose chromatography (Mader et al., 1995; Ohlmann et al., 1996; Rau et al., 1996). Mader et al. (1995) characterized the region of eIF4G responsible for interaction with eIF4E in human and yeast cells. Using a combination of interactive cloning, co-precipitation using tagged expressed proteins, and in vitro mutagenesis, they characterized a region of 18 amino acids that is

both necessary and sufficient for interaction of human eIF4G with eIF4E. Parallel studies defined the site on TIF4631 responsible for interaction with *S. cerevisiae* eIF4E (Mader et al., 1995; Altmann et al., 1997; see Figs. 2, 4). The highest degree of conservation in this region between human eIF4G, TIF4631, TIF4632, and a putative eIF4E binding domain in *S. pombe* corresponds to an 18-amino acid motif (6 of 12 conserved; Fig. 4). Five of the six conserved amino acids are hydrophobic residues with some degree of identity. Site-directed mutagenesis of the human protein to replace selected amino acids with alanine showed that replacement of either of the two phenylalanine residues with alanine had no effect on eIF4E binding. However, replacement of either the conserved tyrosine or the two conserved leucines with alanine yielded a mutant protein that retained less than 5% of binding efficiency to eIF4E compared to the wild type (Mader et al., 1995).

A motif for interaction of eIF4E with eIF4G has been identified in plants. The generation of bacterially expressed proteins, mutational analysis, and the yeast two-hybrid system have been used to examine the functional domains of the wheat p86. Metz and Browning (1996) showed that the loss of the first 90 amino acids of p86 rendered it unable to interact with p28 and abrogated its ability to promote translation. Further delineation demonstrated that the site of interaction of p28 with p86 resides within amino acids 52–90 (Figs. 2, 4; Metz & Browning, 1996).

The binding of Pab1p

It is clear that interactions involving both the cap and the poly(A) tail contribute to the efficiency with which an mRNA is translated. There is some redundancy, because some ability to be translated is conferred by possession of either the cap structure or a poly(A) tail. However, studies in several systems have demonstrated that translational efficiency can be stimulated synergistically when the cap structure and the poly(A) tail are both present (Gallie, 1991, 1996; Gallie & Traugh, 1994; Iizuka et al., 1994; Tarun & Sachs, 1995). This has led to some attractive models that propose interaction between the 5′ and 3′ ends of mRNA molecules during the initiation of translation (Gallie, 1996; Jacobson, 1996; Richter, 1996; Sachs & Buratowski, 1997; Sachs et al., 1997). Recent studies now indicate a central role for eIF4G in such interactions in both yeast and plant systems. Tarun and Sachs (1996) have demonstrated direct interactions of both forms of *S. cerevisiae* eIF4G (TIF4631 and TIF4632) with the yeast poly(A) binding protein, Pab1p. Pab1p was shown to co-purify and co-immunoprecipitate with TIF4631 and TIF4632 in an association requiring the presence of poly(A) or RNA (Tarun & Sachs, 1996). The poly(A)-dependent site of interaction of Pab1p with TIF4632 was mapped to a 114-amino acid region on the N-terminal side of the eIF4E binding site (Tarun & Sachs, 1996; see Fig. 2), and residues 188–299 have been identified as a similar domain in TIF4631 (A. Sachs, pers. comm.; see Fig. 2).

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Mammalian: 411EEKKRYDREFLLGFQFIF428
TIF4631:   447HVKYTYGPTFLLQFKDKL465
TIF4632:   418SVKYTYGPTFLLQFKDKL436
S.pombe:   825AGKYEYDVPFLLQFQSVY842
Wheat p86:  61HERVRYSRDQLLDLRKIT78
eIF4E-BP1:  49GTRIIYDRKFLLDRRNSP66
eIF4E-BP2:  49GTRIIYDRKFLLDRRNSP66
p20:         3  KYTIDELFQL      12
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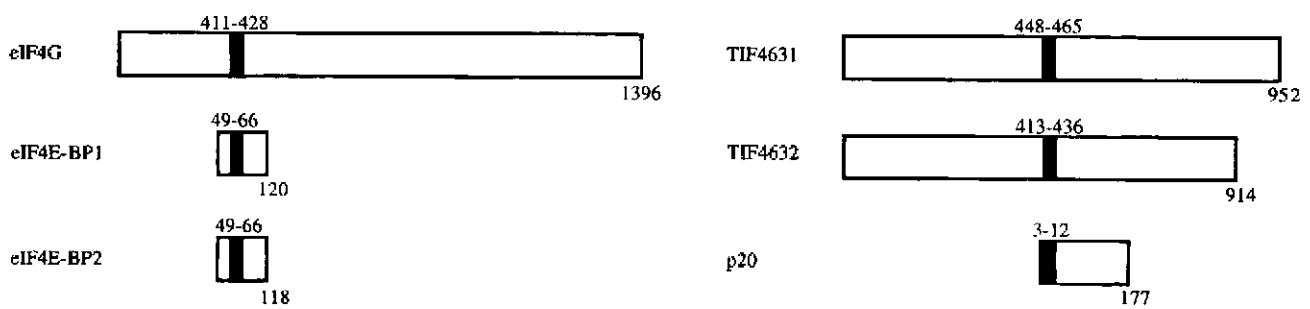

FIGURE 4. Sequence alignment of the eIF4E binding site of eIF4G. The minimal sequence required for interaction of eIF4E with eIF4G was identified as described in the text and in Lamphear et al. (1995), Mader et al. (1995), Metz and Browning (1996), Altmann et al. (1997). These sequences were aligned with that of mammalian (4E-BP1, 4E-BP2; Haghighat et al., 1995; Mader et al., 1995) and *S. cerevisiae* (p20; Altmann et al., 1997) eIF4E-binding proteins. Numbers refer to the amino acids at the boundaries of these domains and amino acids in bold face indicate identity between sequences.

Tarun and Sachs (pers. comm.) have now been able to demonstrate in a yeast in vitro translation system that disruption of the Pab1p binding site in TIF4632 removes the translational advantage for mRNAs of bearing a poly(A) tail, but does not affect cap structure-stimulated translation. Interestingly, however, although this mutant form of TIF4632 was, predictably, unable to bind to recombinant Pab1p in a purified system (Tarun & Sachs, 1996), it was recovered in anti-Pab1p immunoprecipitates derived from crude yeast extracts (A. Sachs, pers. comm.), suggesting that Pab1p and eIF4G may also associate indirectly by virtue of mutual interactions with other components, such as the initiation factor eIF4B.

The lack of any clear regions of sequence similarity near the N-terminus of any of the eIF4G homologues, except TIF4631 and TIF4632, makes it difficult at present to predict potential sites of interaction between eIF4G and poly(A) binding protein in non-yeast systems. However, in the wheat system, both eIF4F and eIF4B have been implicated in mediating the interaction between the cap structure and poly(A) tail (Gallie, 1996), and these factors are both able to reverse the inhibition of translation that occurs when exogenous poly(A) is added to a wheat lysate (Gallie & Tanguay, 1994). Using far-western analysis and biophysical techniques, in vitro studies with wheat lysates have now also shown direct protein:protein interaction between p86, poly(A) binding protein, and eIF4B (Le et al., 1997). In contrast with the *S. cerevisiae* study (Tarun & Sachs, 1996), these interactions could be observed in the presence or absence of poly(A). However, RNA mobility shift assays conducted in the presence of poly(A) showed that a functional consequence of the interaction of poly(A) binding protein with p86 was a stabilization of poly(A) binding protein:poly(A) interaction. Using deletion analysis, the interaction site on p86 was mapped to the N-terminal region (Fig. 2), close to that identified as responsible for interaction with eIF*iso*4E and eIF4A (Metz & Browning, 1996). However, at this time, the mapping is not yet so precise as to allow detailed comparison with the Pab1p binding site on TIF4632.

The C-terminal region

Lamphear et al. (1995) showed that, in addition to the primary cleavage at Gly 479–Arg 480, which separates the N-terminal region of mammalian eIF4G from the C-terminal region in vivo (Fig. 1), prolonged incubation of the isolated eIF3:eIF4F complex with the FMDV L protease in vitro resulted in secondary cleavage of eIF4G at several additional sites. Using antisera recognizing specific regions of the eIF4G molecule, they were able to map the resultant fragments and to delineate which parts of the C-terminal region interact with eIF3 and eIF4A (Lamphear et al., 1995; see Fig. 2). These studies suggested that the primary C-terminal cleavage fragment of mammalian eIF4G is best considered as consisting of two distinct domains, a "central domain" (amino acids 480–886), which was found to interact with eIF3, and a "C-terminal domain" (amino acids 887–1396), which was found to interact with eIF4A. This nomenclature will be used for the remainder of this article. More recently, the central domain was demonstrated by Pestova et al. (1996b) to bind directly to the IRES of EMCV RNA and, in conjunction with either eIF4B or the carboxy-terminal third of eIF4G, to potentiate UV crosslinking of eIF4A to this RNA. These findings are consistent with an earlier report that eIF4B can be crosslinked to the FMDV IRES element (Meyer et al., 1995).

Detailed inspection of the amino acid sequences of the C-terminal region of different eIF4G molecules (Fig. 3) shows that the central region of the human protein, particularly that between amino acids 496 and 814, includes several areas exhibiting strong similarity with sequences in the yeast and plant homologues, as discussed previously by Allen et al. (1992). Within this region, Goyer et al. (1993) highlighted in the *S. cerevisiae* proteins a hexapeptide (A/IVIEQI) and an octapeptide (RGLGLVRF) similar to the RNP2 and RNP1 motifs characteristically located in the 80-amino acid RNP domain found in many RNA binding proteins (reviewed in Burd & Dreyfuss, 1994; Nagai et al., 1995). The existence of potential RNA recognition motifs in the central domain of eIF4G is clearly of critical interest given observations of direct RNA binding activity of *S. cerevisiae* TIF4631 and TIF4632 (Goyer et al., 1993) and of plant p86 (Sha et al., 1995), as well as the interaction of the central portion of eIF4G with the EMCV IRES (Pestova et al., 1996b). However, the equivalent sequences in plant p86 and human eIF4G exhibit somewhat weaker conservation with the RNP2 consensus sequence (see Figs. 2, 3). Moreover, even in TIF4631 and TIF4632, the intervening sequence between the RNP2 and RNP1 motifs (90 amino acids) is much longer than the maximum of around 30 amino acids seen in RNP domains of other proteins (Nagai et al., 1995). Goyer et al. (1993) have also identified two regions relatively rich in arginine and serine residues in TIF4631 (box A: amino acids 488–553; box B: amino acids 873–908) and TIF4632 (box A: amino acids 459–510; box B: amino acids 840–863; see Figs. 2, 3), suggesting that they may be involved in RNA binding activity. Sequence analysis of the human and plant eIF4G homologues revealed regions bearing similarity to these arginine–serine-rich regions, but these were located differently relative to the conserved region in the central domain (see Fig. 2).

Human eIF4G includes a long C-terminal domain, which was proposed by Lamphear et al. (1995) to include the binding site for eIF4A. Wheat p86 also has a C-terminal domain, which shares some regions of similarity to the mammalian protein (Fig. 2). However,

unlike the case with its mammalian counterpart, truncation of the N-terminal domain of wheat p86 decreased the ability of the protein to interact with eIF4A, without affecting the ability to promote ATP hydrolysis (Metz & Browning, 1996). These data indicate that, for wheat p86, at least a part of the eIF4A binding domain resides in close proximity to the p28 binding site in the N-terminal domain of the protein, and that ATP hydrolysis does not require the interaction of p86 with eIF4A. Consistent with the ATP hydrolysis data, the C-terminal region of wheat p86 includes a potential ATP binding site (GX_3GK; amino acids 569–574; Browning, 1996). A similar motif can be seen in human and rabbit eIF4G (GX_4GK; Fig. 3, human protein amino acids 891–897), but we know of no information concerning the ability of the mammalian protein to promote ATP hydrolysis. The C-terminal domain appears to be absent from the eIF4G homologues of both budding and fission yeast (Fig. 2), although it seems clear that eIF4A is essential for translation of virtually all mRNAs in eukaryotic systems (Altmann et al., 1990; Pause et al., 1994b).

REGULATION OF eIF4G AND eIF4F ACTIVITY

Overview

As one of the initiation factors involved in binding mRNA and other initiation factors to the ribosome (Fig. 1), eIF4G would seem to be an ideal target for regulation of translation initiation. This may be mediated in part by: (a) competitive interactions that modulate the ability of eIF4E and eIF4G in the cell to form eIF4F, the physiological cap-binding protein complex (reviewed in Pain, 1996; Hentze, 1997); (b) phosphorylation of eIF4G; and (c) regulation of the cellular concentration of eIF4G by modulation of its synthesis and/or its stability. A specific, and extreme, example of this type of regulation is the proteolysis of the factor in response to infection by some types of picornavirus, as discussed above.

Regulation of eIF4F complex formation by modulation of the availability of eIF4E

Cellular concentrations of eIF4E have been estimated to be low relative to those of other components of the translational machinery (Hiremath et al., 1985; Duncan et al., 1987) and this factor is widely regarded as potentially rate-limiting in amount (reviewed in Morley, 1994, 1996; Sonenberg, 1996; Pain, 1996). However, for the reticulocyte lysate, this conclusion has been challenged recently (Rau et al., 1996). Genetic manipulation strategies that alter the intracellular levels of eIF4E have shown that artificial overexpression of eIF4E can exert profound effects on cell growth, resulting in aberrant morphology, increased focus formation, anchorage-independent growth, and the ability to produce tumors when injected into nude mice (De Benedetti & Rhoads, 1990; Lazaris-Karatzas et al., 1990). Overexpression of eIF4E may increase the level of eIF4F complex present in the cell and therefore, by reducing the competition for eIF4F, enhance the efficiency of translation of certain critical mRNAs (Kozak, 1991; Sonenberg, 1994, 1996). In contrast, overexpression of eIF4E in yeast had little effect on growth rates and did not influence the translation of mRNAs with secondary structure in their 5′ untranslated regions (Lang et al., 1994). Down-regulation of the levels of eIF4E has the opposite effect on cell growth; overexpression of antisense RNA to eIF4E caused a slowing of the rate of cell growth, a decrease in translation rates, disaggregation of polysomes, and, surprisingly, a decrease in the cellular levels of eIF4G (De Benedetti et al., 1991), suggesting some direct link between the cellular levels of these initiation factors (see below).

It is now clear that effective concentrations of eIF4E can also be modulated physiologically on an acute basis. Pause et al. (1994b) and Lin et al. (1994) screened a placental cDNA expression library to identify proteins that interact with eIF4E. In addition to eIF4G, the screen identified a number of other proteins, two of which have been characterized in detail (4E-BP1, 4E-BP2; Lin et al., 1994; Pause et al., 1994a). 4E-BP1 was found to be 93% identical to PHAS-I, which had been identified previously as a protein phosphorylated rapidly in adipose tissue in response to insulin (reviewed in Denton & Tavaré, 1995). Sequence comparisons between 4E-BP1 and eIF4G revealed no significant overall identities, suggesting that these proteins are not related evolutionarily (Mader et al., 1995). Interaction of 4E-BP1 with eIF4E inhibits cap structure-dependent translation both in vitro and when the protein is expressed in cells (Rousseau et al., 1996). In vitro studies showed that 4E-BP1 prevented the binding of mRNA to the ribosome. It did not interfere with the cap recognition by eIF4E, but competed with eIF4G for binding to eIF4E and prevented the assembly of eIF4E into the eIF4F complex (Haghighat et al., 1995). Conversely, eIF4E already in a complex with eIF4G could not interact with 4E-BP1 (Haghighat et al., 1995; Rau et al., 1996). A sequence similarity has been identified between a region of 4E-BP1 (amino acids 49–66) and 4E-BP2 and the eIF4E binding site on eIF4G (Mader et al., 1995; Haghighat et al., 1996; Rousseau et al., 1996; see Fig. 4). Five of the six amino acids in this region conserved between human and yeast eIF4G are present in the same arrangement in 4E-BP2, with two additional charged residues present at similar positions in eIF4G and the 4E-BPs. Mutants of 4E-BP1 that were deficient in their binding to eIF4E failed to inhibit the interaction between eIF4G and eIF4E or translation itself (Haghighat et al., 1995). It is believed that, in vivo, the hormone-induced hyperphosphorylation of 4E-BP1

causes it to dissociate from eIF4E, thereby allowing eIF4E to interact with N-terminal domain of eIF4G via the conserved motif described above, leading to enhanced levels of eIF4F complex formation (reviewed in Flynn & Proud, 1996; Morley, 1996; Pain, 1996; Sonenberg, 1996). Physiological regulation of eIF4E-4E-BP1 association has been demonstrated in a wide variety of cell types (Graves et al., 1995; Lin et al., 1995; Morley & Pain, 1995b; Azpiazu et al., 1996; Diggle et al., 1996; Kimball et al., 1996). In some of these cases, the relevance of these effects to the control of eIF4F complex formation has been corroborated by observations of parallel, but reciprocal, changes in the degree of association of cellular eIF4E with eIF4G and 4E-BP1 (Morley & Pain, 1995b; Kimball et al., 1996, 1997; Svanberg et al., 1997). With the demonstration of completely distinct expression profiles for the 4E-BPs between mammalian tissues (Lin & Lawrence, 1996), it is likely that the relative importance of 4E-BP1 and 4E-BP2 in the regulation of eIF4F complex formation will vary markedly. In the yeast *S. cerevisiae*, the p20 protein found associated with eIF4E has been demonstrated to have sequence homology with eIF4G of both yeast and mammalian cells, and with mammalian 4E-BP1 and BP2 (Fig. 4; Altmann et al., 1997). As with the mammalian system, both eIF4G and p20 interact with eIF4E and compete for binding to eIF4E. p20 expression inhibited cap-structure-dependent translation, but did not affect cap-structure-independent translation. As one would predict, disruption of the CAF20 gene encoding p20 stimulated the growth rate of cells, whereas overexpression led to a slow growth phenotype (Altmann et al., 1997). Earlier work had identified p20 as a phosphoprotein (Zanchin & McCarthy, 1995).

Phosphorylation of eIF4G

Little is known about the role of protein phosphorylation in modulating the activity of eIF4G. In some studies, enhanced phosphorylation has been correlated with increased rates of translation (reviewed in Morley, 1994, 1996). Treatment of reticulocytes with phorbol ester or 3T3-L1 cells with insulin was found to enhance the phosphorylation of eIF4G on several sites, via what appears to be both protein kinase C-dependent and independent pathways (Morley & Traugh, 1989, 1990, 1993; Morley et al., 1991). Phosphorylation of eIF4G was also increased in epithelial cells in response to treatment with okadaic acid and epidermal growth factor (Donaldson et al., 1991; Bu et al., 1993) in human cells following influenza infection (Feigenblum & Schneider, 1993), in T lymphocytes following phorbol ester or concanavalin A activation (Morley & Pain, 1995b), and in *Xenopus* oocytes during meiotic maturation (Morley & Pain, 1995a). It is interesting to note that in many of these cases, phosphorylation of eIF4G in vivo was also associated with its enhanced recovery in association with eIF4E, reflecting increased levels of eIF4F complex formation. Increased levels of eIF4F complex formation have also been reported during differentiation of HL60 cells, but it is not known whether there are changes in phosphorylation (A.E. Willis, pers. comm.). Two dimensional iso-electric focussing of wheat p86 has indicated that this protein is modified posttranslationally in a manner consistent with phosphorylation (Gallie et al., 1997). However, further work is required to confirm the nature of this modification. eIF4G is a substrate for protein kinases in vitro, including protein kinase A (PKA), protease-activated kinase II, and protein kinase C (PKC) (Tuazon et al., 1989), but currently, the physiological sites of phosphorylation of eIF4G are not known and few data are available as yet on the possible regulatory significance of in vivo phosphorylation. However, in vitro assays showed that phosphorylation of eIF4F complex with a multipotential S6 kinase that favored eIF4G over eIF4E increased the interaction of eIF4G with the mRNA cap structure and stimulated the binding of mRNA to 43S pre-initiation complexes (Morley et al., 1991).

Regulation of cellular eIF4G concentrations

It is frequently the case that proteins with important roles in growth regulation are present in cells at low molar concentrations, have short half-lives, or exhibit regulated levels of expression. In the field of translational control, there has been much debate on which initiation factors are rate-limiting for translation (reviewed in Hershey, 1994). In the case of the eIF4F complex, this question was raised initially by measurements indicating very low molar concentrations of eIF4E in mammalian cells relative to other initiation factors and ribosomes (Hiremath et al., 1985; Duncan et al., 1987; Goss et al., 1990), although more recent data suggest eIF4E concentrations were underestimated severely in these studies (Rau et al., 1996).

Information on cellular concentrations of eIF4G is relatively scarce, probably because there are no direct methods for isolating the homogeneous protein, and recombinant protein preparations suitable for use as assay standards are only now becoming available. However, using comparative genomic hybridization, recent studies have identified amplification of the gene encoding eIF4G in independent squamous cell lung carcinomas (Brass et al., 1997). These data suggest that, as described for eIF4E, the overexpression of eIF4G may have a central role in cell transformation and the loss of growth control. The possibility that eIF4G concentrations may have a regulatory role is further reinforced by the observation that the factor is present in *S. cerevisiae* at an extremely low concentration (only 0.1 mol per mol ribosomes, compared to 1 mol/mol ribosomes for eIF4E; Altmann et al., 1997), suggesting that it may be rate-limiting for translation initiation. This would

be consistent with the finding that most of the eIF4G in the reticulocyte lysate is ribosome-associated (Rau et al., 1996). As discussed in the following paragraphs, evidence is beginning to accumulate from a number of sources for rapid changes in the cellular concentration of eIF4G in response to physiological events additional to the well-known case of picornavirus infection. Clearly, an important question is whether such regulation is exerted on the synthesis or the degradation of the factor. Although definitive measurements of synthesis and degradation rates have yet to be made, a wide range of recent observations on the properties and behavior of the eIF4G protein indicate a strong potential for regulation of both these processes. The work leading to this conclusion has not been reviewed to date, and it seems timely to do so here.

Rhoads' laboratory has presented a number of interesting observations relevant to the question of eIF4G turnover. First, they demonstrated that the 5′ noncoding region of eIF4G mRNA contains an element that directs cap-independent internal initiation (Gan & Rhoads, 1996). This would permit an autoregulatory mechanism allowing continued synthesis of eIF4G in cells infected by picornaviruses, but internal initiation was also discussed as a potential mechanism for upregulating eIF4G expression in cells undergoing, or recovering from, other stresses that impair cap structure-dependent initiation (Gan & Rhoads, 1996). Second, they found that expression of antisense mRNA against eIF4E in HeLa cells resulted not only in diminished eIF4E levels, but in the virtual elimination of immunoreactive eIF4G from the cells (De Benedetti et al., 1991). The kinetics of decay of eIF4G and of overall translation rate paralleled closely those for eIF4E, consistent with the possibility that eIF4G may be destabilized when unable to form a complex with eIF4E, a phenomenon sometimes observed for proteins that normally exist in higher-order structures (see references in Rechsteiner & Rogers, 1996). In the context of protein stability, Yan et al. (1992) pointed out that the N-terminal half of mammalian eIF4G includes five high-scoring PEST regions, possession of which shows high correlation both with a short physiological half-life and with regulated stability (Rogers et al., 1986; Rechsteiner & Rogers, 1996). Using the PESTFIND program developed by Rechsteiner and Rogers (1996), we have extended the search for PEST regions to the other eIF4G homologues, and find that all except wheat p86 contain multiple PEST regions with very high scores (Table 1). In human eIF4G, these are largely, but not exclusively, located toward the N-terminal end of the protein, in regions of secondary structure predicted to be rich in loops. One might predict on this basis that, when eIF4G is cleaved proteolytically in picornavirus-infected cells, the C-terminal fragment would be more stable metabolically than either the N-terminal fragment or, indeed, the intact protein prior to infection. It would thus be of interest to determine whether the C-terminal fragment actually accumulates in infected cells, facilitating IRES-driven translation of viral RNAs. Indeed, when eIF4G cleavage in *Xenopus* oocytes was induced by the microinjection of coxsackie B4 protease, the C-terminal eIF4G fragment exhibited greater stability than the N-terminal fragment (Keiper & Rhoads, 1997). Both the *S. cerevisiae* eIF4G homologues contain PEST regions throughout the protein, with TIF4631 particularly rich in high-scoring motifs (Table 1). PEST regions can also be identified in the *S. pombe* eIF4G sequence.

The degradation of many PEST-sequence-bearing proteins has now been attributed to the ubiquitin-proteasome pathway (Rechsteiner & Rogers, 1996), but in some cases it is possible that PEST sequences regulate proteolysis by calpains (reviewed in Barnes & Gomes, 1995). In particular, a number of calmodulin-binding proteins possess PEST sequences, and it has been suggested that the binding of calmodulin may modulate calpain-mediated degradation of these proteins (Barnes & Gomes, 1995). This strikes an interesting chord with several observations concerning mammalian eIF4G. First, it seems likely that the factor is a calmodulin-binding protein, because it can be purified partially from crude extracts on calmodulin-Sepharose columns (Wyckoff et al., 1992; Morley & Pain, 1995a). Second, calpains have been invoked in the physiological degradation of eIF4G in uninfected mammalian cells and extracts derived from them. Wyckoff et al. (1990, 1992) found that addition of calcium accelerated the degradation of eIF4G in HeLa cell extracts and identified calpains I and II as responsible at least partially for the proteolytic activity. More recently, rapid degradation of eIF4G has been observed in brain following ischemia (DeGracia et al., 1996). This could be prevented by concurrent treatment with MDL-28,170, a specific inhibitor of calpain I. Moreover, degradation of the factor in incubated brain extracts was blocked by addition of either MDL-28,170 or calpastatin (R.W. Neumar, D.J. DeGracia, L.L. Konkoly, B.C. White, & G.S. Krause, pers. comm.).

The small amount of evidence available so far on mechanisms of degradation of eIF4G in uninfected cells suggests that the patterns of cleavage differ from those seen in cells infected by picornaviruses. The calcium-dependent protease activities studied by Wyckoff et al. (1990, 1992) exhibited purification properties distinct from the proteolytic activity toward eIF4G found in poliovirus-infected cells. More recent evidence has come from studies in Lloyd's laboratory of the effects of persistent poliovirus infections on human erythroleukemia cell lines. As part of a study of the effects of inducing differentiation in these cells, Benton et al. (1996) found that hemin treatment induced cleavage of eIF4G even in control cells that were not subjected to virus infection. However, this resulted in the ap-

TABLE 1. Predicted PEST sequences of human and *S. pombe* eIF4G.[a]

Position	PEST score	Comment	Sequence
Human eIF4G			
57–108	12.49	Good	SPSESQPSSPSPTPSPSPVL/../PGDTMTTIQMSVEESTPISR
164–229	13.15	Good	EPNGMVPSEDLEPEVESSPE/../APSPPAVDLSPVSEPEEQAK
231–281	19.97	Good	EVTASVAPPTIPSATPATAP/../EEEDEEEGEVGEAGEGESEK
340–369	9.88	Good	EANPAVPEVENQPPAGSNPGPESEGSGVPP
371–380	15.54	Good	PEEADETWDS
670–679	5.85	Invalid	EMDEAATAEE
S. pombe			
233–251	6.36	Invalid	PTTSASNTNTSPANGAPTN
253–288	12.93	Good	PSTDINTTDPATQTTQVSASNSPALSGSSTPSNTSS
507–524	9.75	Good	AETPTAAPPQISEEEASQ
TIF4631			
172–191	27.94	Good	ETSDSTSTSTPTPTPSTNDS
193–203	6.29	Invalid	ASSEENISEAE
274–285	12.44	Good	QETPAEEGEQGE
348–371	14.53	Good	EETEVAETEQSNIEESATTPAIPT
373–389	9.04	Invalid	SDEAEAEVEAEAGDAGT
391–412	11.23	Invalid	IGLEAEIETTTDETDDGTNTVS
676–688	8.39	Good	ELNPDITDETNEG
774–803	7.35	Good	DLTDSPSEETLESVVELLNTVGEQFETDSF
928–952	9.55	Good	EEPAAPTSTATNMFSALMGESDDEE
TIF4632			
133–145	8.8	Good	QPLNTNPEPPSTP
312–331	6.72	Good	SVTFNEPENESSSQDVDELV
333–345	16.65	Invalid	DDDTTEISDTTGG
351–367	7.49	Invalid	SDDETINSVITTEENTV
369–390	6.8	Good	ETEPSTSDIEMPTVSQLLETLG

[a] PEST sequences were identified using the PC/Gene PESTFIND program (Rechsteiner & Rogers, 1996), and only those with scores of at least +5 and a minimal length of 10 amino acids are indicated. "Invalid" PEST regions (defined as containing no proline or no glutamic/aspartic acid or no serine/threonine) are included on the grounds that they are acceptable with scores greater than +5 (Rechsteiner & Rogers, 1996).

pearance of a set of cleavage products whose migration on SDS-PAGE gels differed markedly from those of products formed in response to poliovirus infection, suggesting cleavage at a site nearer the N-terminus of the protein.

An important question, still unresolved, is whether or not cellular proteolytic mechanisms are responsible for the cleavage of eIF4G in cells infected with picornaviruses. Early evidence suggested that, in infected cells, eIF4G was not cleaved directly by the viral 2A proteinase, but by a cellular enzyme activated indirectly, possibly via a protease cascade (Lloyd et al., 1986; Krausslich et al., 1987). More recently, this idea has been challenged strongly by the demonstration that the picornavirus proteases can cleave eIF4G directly in purified eIF4F preparations (Liebig et al., 1993; Kirchweger et al., 1994; Lamphear et al., 1995; Lamphear & Rhoads, 1996) or in a complex of recombinant eIF4G and eIF4E proteins (Haghighat et al., 1996). Interestingly, the eIF4G polypeptide appears to be a poor substrate for cleavage by picornavirus proteases unless it is in a complex with eIF4E (Haghighat et al., 1996; Ohlmann et al., 1997), suggesting that a conformational change of eIF4G upon its interaction with eIF4E is required to expose the cleavage site. However, the demonstration that 2A and L proteases *can* cleave the eIF4G in isolated or in vitro-assembled eIF4F complexes does not necessarily rule out a role for cellular proteases in the cleavage elicited during virus infection of intact cells. The poliovirus 2A protease, in particular, is reported to be inefficient in cleaving eIF4G, even in relatively crude systems that contain eIF4E (M. Bovee, B. Lamphear, R. Rhoads, & R. Lloyd, pers. comm.) and, moreover, cleavage of eIF4G is not impaired significantly in poliovirus-infected cells when viral RNA synthesis is blocked by guanidine, a treatment that inhibits the expression of viral proteins severely (R.E. Lloyd, pers. comm.). A number of candidate cellular protease activities have been purified partially from poliovirus-infected cells (Lloyd et al., 1986; Krausslich et al., 1987; Wyckoff et al., 1992) and, significantly, the most active eIF4G cleavage activity has been shown not to co-purify with the main peak of 2A protease activity (R.E. Lloyd, pers. comm.). More definitive conclusions await the complete purification and characterization of the cellular eIF4G cleavage activities from cells infected with polio and other picornaviruses.

PUZZLES AND HOT TOPICS

A new family of homologues to the C-terminal domains of eIF4G

Recently, a new family of proteins exhibiting similarity to the central and C-terminal domains of mammalian eIF4G has been described. These 97-kDa proteins, referred to variously as p97 (Imataka et al., 1997), DAP-5 (Levy-Strumpf et al., 1997), *Eif4g2* (Shaughnessy et al., 1997), and NAT1 (Yamanaka et al., 1997), are virtually identical. They show closest similarity to human eIF4G, whereas phylogenetic analysis (Fig. 5) indicates a more distant relationship to TIF4631, TIF4632, and wheat p86.

The identification of cDNAs or gene sequences encoding the 97-kDa protein resulted from independent work in four laboratories studying widely differing phenomena. The cDNA encoding "p97" was identified originally from a human embryo brain cDNA library by random cloning (Imataka et al., 1997), whereas the gene *Eif4g2*, encoding an identical protein, was found in a search for novel disease genes in the region surrounding retroviral integration sites found in myeloid leukemias in mice (Shaughnessy et al., 1997). Northern blot analysis indicated ubiquitous, high-level expression of *Eif4g2* mRNA in mammalian cells, with the gene localized to distal chromosome 7 (Shaughnessy et al., 1997). At the same time, Yamanaka et al. (1997) found that transgene expression of the apolipoprotein B mRNA editing enzyme (APOBEC-1) caused hepatic dysplasia and carcinoma in the mouse. Differential display revealed extensive editing of an ubiquitously expressed and conserved mRNA, leading to reduced levels of expression of the encoded protein, NAT1, which is 99% identical to p97. DAP5 was isolated as part of a search for gene products that had the ability to confer resistance to interferon-γ-induced apoptosis (Levy-Strumpf et al., 1997).

Sequence analysis revealed that the 97-kDa proteins exhibited around 30% identity with the C-terminal two-thirds of human eIF4G (Imataka et al., 1997; Levy-Strumpf et al., 1997; Shaughnessy et al., 1997; Yamanaka et al., 1997), beginning close to the position of primary cleavage of eIF4G by picornavirus proteases (see above). Further analysis by Levy-Strumpf et al. (1997) subdivided the 97-kDa protein into distinct N-terminal and C-terminal domains, with the the N-terminal domain showing a closer similarity to eIF4G (39% identity and 63% similarity to the central domain of mammalian eIF4G). Interestingly, expression of the full-length protein in vivo at high levels inhibited cell growth, but this activity and the ability to convey resistance to apoptosis appeared to reside in the C-terminal domain, which exhibited less sequence similarity to eIF4G (Levy-Strumpf et al., 1997). It therefore seems likely that the growth-modulatory effects of this protein can be attributed, at least in part, to unique biochemical and functional properties of the C-terminal domain, rather than to similarity of its N-terminal domain to eIF4G (Levy-Strumpf et al., 1997; Shaughnessy et al., 1997).

The 97-kDa proteins do not contain a motif equivalent to the eIF4E binding site on eIF4G (Imataka et al., 1997; Levy-Strumpf et al., 1997; Shaughnessy et al., 1997; Yamanaka et al., 1997) and do not interact with eIF4E (Imataka et al., 1997). Both NAT1 (Yamanaka et al., 1997) and p97 (Imataka et al., 1997) were found to inhibit both cap structure-dependent and independent translation in vitro and in intact cells. This is in contrast to the C-terminal two-thirds of mammalian eIF4G, which stimulates both translation of uncapped mRNA in vitro (Ohlmann et al., 1997) and EMC IRES-driven luciferase translation in transfected COS7 cells (Yamanaka et al., 1997). Both NAT1 and p97 were found to interact with eIF4A in vitro, and p97 was also shown to associate with eIF3 (Imataka et al., 1997). These findings led to suggestions that the 97-kDa proteins may compete with eIF4G for binding to one of these factors and, consequently, act as negative regulators of initiation, and thus of growth, in a manner analogous to the 4E-BPs (Imataka et al., 1997; Yamanaka et al., 1997). At present, however, the role of these proteins remains an

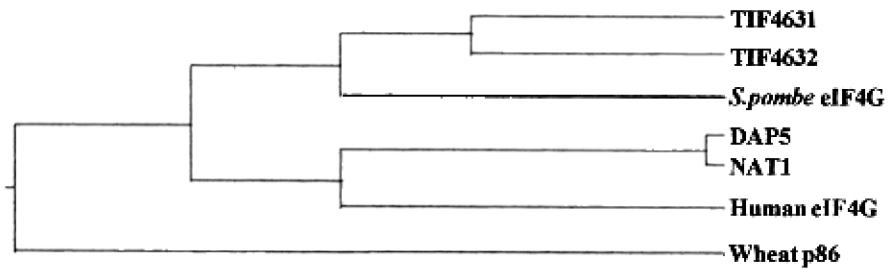

FIGURE 5. Phylogenetic analysis of eIF4G and related protein sequences. A phylogenetic tree of eIF4G from human, wheat, *S. cerevisiae*, and *S. pombe* and the NAT1, DAP-5, p97, and *Eif4g2* gene products was constructed using the Clustal method with the PAM250 residue weight table. For clarity, given that p97, DAP-5, and *Eif4g2* are identical, this branch is labeled DAP-5. Accession numbers are: human eIF4G, D12686; *S. cerevisiae*, TIF4631, L16923; *S. cerevisiae*, TIF4632, L16924; wheat p86, M95747; *S. pombe*, cosmid c17C9 (The Sanger Centre). DAP-5, X89713; p97, U73824; *Eif4g2*, U63323; NAT1, U76111.

open question, particularly because, at least in one case, the growth-regulatory properties appear to be conferred by the region of the molecule least similar to eIF4G (Levy-Strumpf et al., 1997).

The role of eIF4G in translation initiation complex formation

This article has focused on the ability of eIF4G to provide binding sites for a number of different molecules involved in protein synthesis, allowing it to function as a bridge between mRNA and the 40S ribosomal subunit (Fig. 1). It is particularly interesting that the factor can fulfil this function in different ways for two distinct mechanisms of translational initiation. In cap structure-dependent initiation, its eIF4E-binding domain allows it to build the foundations of the initiation complex at the 5′ end of the mRNA, whereas the interaction of the central domain with the eIF3 in the 43S preinitiation complex completes the link with the 40S ribosomal subunit. In contrast, the function of eIF4G in cap structure-independent translation can be fulfilled by the C-terminal two-thirds of the molecule independently, and here it now seems possible that the initial, localizing interaction with mRNA may involve direct binding via the central domain (Fig. 2; Pestova et al., 1996b).

There are still many unanswered questions concerning the details of the interactions involving eIF4G. The binding sites for eIF3 and eIF4A have so far only been assigned to global domains (Figs. 1, 2). In the case of eIF4A, it remains puzzling that the C-terminal domain in mammalian eIF4G thought to bind this factor appears to be lacking in the yeast homologues. Furthermore, even in the mammalian system, it is not clear how the central domain alone is able to promote the association of eIF4A to the EMCV IRES, in the absence of the C-terminus, if eIF4B is present (Pestova et al., 1996b). Although the central domains in the different eIF4G homologues are relatively well conserved and include motifs common in RNA-binding proteins (Fig. 2), the actual sites responsible for binding the EMCV IRES (Pestova et al., 1996b) and for the general RNA binding activity exhibited by yeast (Goyer et al., 1993) and plant (Sha et al., 1995) eIF4Gs have yet to be defined. The discovery of a direct interaction between eIF4G and Pab1p in yeast (Tarun & Sachs, 1996) and plant systems (Le et al., 1997) opens up exciting possibilities for explaining the functional link between the 5′ and 3′ ends of mRNA molecules in initiation (see Fig. 6). Pab1p is known to stimulate the binding of the 40S ribosome to mRNA (Tarun & Sachs, 1995) and to protect mRNA functionally from de-capping (Caponigro & Parker, 1995). These findings have led to proposals that eukaryotic mRNA may be circularized through the interaction of the 5′ and 3′ ends, thereby allowing stable mRNA structures or RNA–protein interactions at the 3′ end to regulate events at the 5′ end without interfering with ribosome scanning (Gallie, 1996; Jacobson, 1996; Tarun & Sachs, 1996; Hentze, 1997; Jackson & Wickens, 1997; Sachs & Buratowski, 1997; Sachs et al., 1997). It is important to establish whether this association also plays a role in other systems, particularly during early development, where changes in poly(A) tail length are known to play a key role in translational reprogramming (Richter, 1996; Wickens et al., 1996; Hake & Richter, 1997).

Another question barely addressed at the present time is the identification of the domains in initiation factors and other proteins required for interaction with eIF4G. Although there has been some work on the structural determinants of the cap-binding function of eIF4E (Teraoka et al., 1996; Friedland et al., 1997) and the crystal structure of eIF4E has been determined (Marcotrigiano et al., 1997), the domains or regions of the factor required for its association with eIF4G have yet to be identified clearly. Another critical interaction is that with eIF3, but it is still unclear which subunit(s) of eIF3 is involved in this interaction or how this may be regulated. The recent cloning of individual subunits of eIF3 (Asano et al., 1997; Methot et al., 1997; Naranda et al., 1997) eventually should contribute to progress on this question. Although considerably more is known about the structural basis of interactions of eIF4A and eIF4B with other ligands, again the sites involved in interaction of these factors with eIF4G are unknown. In the case of Pab1p, preliminary evidence indicates that the two N-terminal RNP domains are necessary for interaction with eIF4G (S. Kessler, J. Deardorff, & A. Sachs, unpubl. data). Finally, although increasing prominence is now given to the potential role of the cytoskeleton in mRNA localization (Curtis et al., 1995; St Johnston, 1995; Hesketh, 1996; Hovland et al., 1996), the role of interactions between initiation factors and cytoskeletal networks remains obscure. In this context, however, it is of interest that wheat p86 was found to have limited sequence similarity to a kinesin-like molecule and to exhibit microtubule binding activity (Bokros et al., 1995; Browning, 1996).

Although most available evidence indicates that the main, and possibly the only, function of eIF4G is to act as a multi-place adapter in the formation of the 40S subunit/mRNA initiation complex (Fig. 6), it is also becoming clear that this function is not unique to the factor. In particular, structure-function analysis of another initiation factor, eIF4B, suggests a parallel role (Naranda et al., 1994; Methot et al., 1996a, 1996b). By virtue of its possession of an N-terminal RRM, with specificity for ribosomal RNA, together with an additional, less specific, RNA-binding site, this molecule is also proposed to provide a link between mRNA and ribosomes (Methot et al., 1996a). Moreover, a well-conserved motif is now thought to confer the ability of eIF4B both to form homo-dimers and to associate with

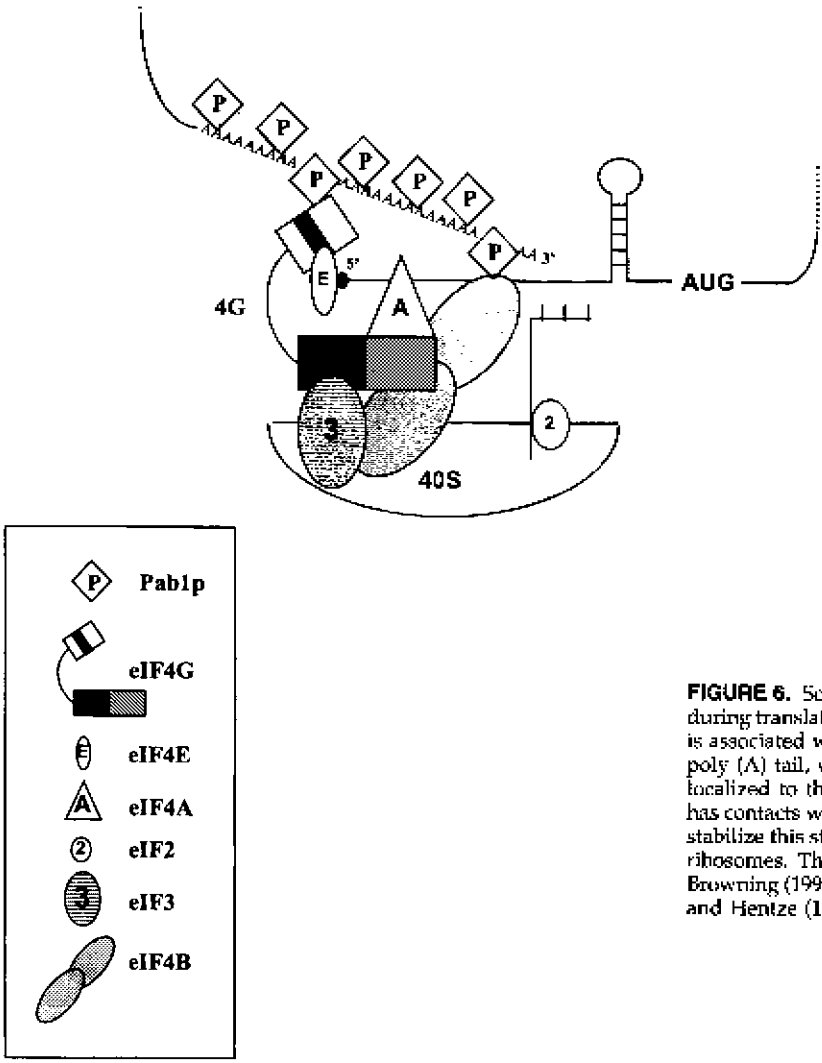

FIGURE 6. Schematic representation of the contacts made by eIF4G during translation initiation. The eIF4F complex (eIF4E:eIF4A:eIF4G) is associated with both the mRNA cap structure via eIF4E, and the poly (A) tail, via Pab1p and eIF4G. eIF4G can recruit eIF4A and is localized to the ribosome via its interaction with eIF3, which also has contacts with eIF4B. Protein/protein and protein/RNA contacts stabilize this structure, possibly to facilitate the efficient recycling of ribosomes. This scheme is based on recent models presented by Browning (1996), Gallie (1996), Jacobson (1996), Methot et al. (1996b), and Hentze (1997).

eIF3, providing a further mechanism for interaction with 43S preinitiation complexes (Methot et al., 1996b). As with eIF4G, there are also reports that eIF4B can interact with a picornavirus IRES (Meyer et al., 1995) and with poly(A) binding protein (Gallie, 1996). Unlike the case with eIF4G, physical association of eIF4B with eIF4A has proven difficult to demonstrate (Pause et al., 1994b), but functional interaction between these factors is well established both biochemically (Merrick, 1992, 1994) and genetically (Altmann et al., 1993; Coppolecchia et al., 1993). These observations are consistent with models depicting parallel bridges involving eIF4G and eIF4B within 40S subunit/mRNA initiation complexes (Gallie, 1996; Methot et al., 1996b; see Fig. 6). What seems eminently likely, however, is that the relative importance of the links centered on these two factors shifts during the sequential stages of mRNA recruitment. eIF4F has long been known as the factor complex that binds first to capped mRNAs in vitro (Merrick, 1992), and the consensus view is that interaction between eIF4E and eIF4G is a critical (and regulatable) stage in forging the *initial* contact beween the 40S subunit and a capped mRNA molecule. In the case of IRES-driven translation, this initial contact may be made via direct association with the IRES. Recent studies with eIF4B, on the other hand, suggest that it may promote RNA annealing, and thus play a later role in aligning the scanning 40S ribosomal subunit with the initiation codon (Altmann et al., 1995).

Consideration of this question has made us increasingly aware of how little we still know of the detailed sequence of events during the complicated process of initiation. It is still not clear whether the interactions involved in the initial binding of the 40S subunit to the 5′ end of mRNA occur in a strict order, and, if so, what that order is. There is at least partial evidence for each of three alternative pathways: binding of a preformed complex on the mRNA cap, followed by linking of the 43S preinitiation complex via eIF4G–eIF3 association (Merrick, 1994; Sonenberg, 1996); binding of eIF4E to

the mRNA cap, and, separately, eIF4G to the 43S preinitiation complex, followed by a link-up via eIF4E–eIF4G association (Joshi et al., 1994; Rhoads et al., 1994); binding of both eIF4G and eIF4E to the 43S preinitiation complex, followed by capture of the mRNA by association of eIF4E with the cap (M. Rau, S.J. Morley, E. Martin-Palma, & V.M. Pain, unpubl. data). If this is uncertain enough, even less is known about the detachment of these links as the 40S subunit scans to the initiation codon (Morley, 1996; Pain, 1996). Although some models predict the early release of eIF4E and eIF4G, others suggest the sequential binding of trimeric eIF4F complexes as a means of recruiting multiple eIF4A molecules to promote mRNA unwinding (Merrick, 1994). In contrast, evidence from Rhoads' laboratory (Hiremath et al., 1989; Joshi et al., 1994; Rhoads et al., 1994) and our own data indicate that, in in vitro translation systems, both eIF4E and eIF4G remain associated with 40S ribosomal subunits trapped at the initiation codon in the presence of nonhydrolyzable analogues of GTP. This would be in line with a model for initiation visualized for plants (Browning, 1996), whereby the eIF4E disengages from the cap as the 40S subunit is bound to the mRNA, but remains associated with the complex via p86. Finally, at what stage in the process is the association between Pab1p and eIF4G forged? In the yeast system, this seems to have a role distinct from interactions involving the cap, suggesting that it may be a later event. If it turns out to be universally important, a critical question will be whether it functions in recruiting mRNAs from the untranslated pool or whether it provides a means of ensuring continued translation of an mRNA molecule by "funnelling" 40S subunits released upon termination back to the initiation site (Gallie, 1996; Jacobson, 1996; Tanguay & Gallie, 1996).

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