PLANT CAP BINDING COMPLEXES EUKARYOTIC INITIATION FACTORS eIF4F AND eIFiso4F: MOLECULAR SPECIFICITY OF SUBUNIT BINDING
Laura K. Mayberry, M. Leah Allen, Kelley R. Nitka, Lara Campbell, Patricia A. Murphy and Karen S. Browning
From Department of Chemistry and Biochemistry and the Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712
Running head: Subunit specificity of eIF4F and eIFiso4F
Address correspondence to: Karen Browning, Ph.D., 1 University Station A5300, University of Texas at Austin, Austin, TX 78712. Fax 512-471-8696; E-mail: kbrowning@mail.utexas.edu

Background: Plants have a unique form of cap-binding complex.
Results: Correct and mixed complexes show differential translation and mixed complex subunits have lower binding affinity than correct complex subunits.
Conclusion: The subunits of the cap-binding complexes show specificity for complex formation and the translational efficiency is determined by the large subunit.
Significance: The results suggest the potential for differential translation by the two plant cap binding complexes.

The initiation of translation in eukaryotes requires a suite of eukaryotic initiation factors (eIFs) that include the cap-binding complex, eIF4F. eIF4F is comprised of the subunits eIF4G and eIF4E, and often the helicase, eIF4A. The eIF4G subunit serves as an assembly point for other initiation factors, whereas eIF4E binds to the m7G cap of mRNA. Plants have an isozyme form of eIF4F (eIFiso4F) with comparable subunits, eIFiso4E and eIFiso4G. Plant eIF4A is very loosely associated with the plant cap-binding complexes. The specificity of interaction of the individual subunits of the two complexes was previously unknown. To address this issue, mixed complexes (eIF4E/eIFiso4G or eIFiso4E/eIF4G) were expressed and purified from E. coli for biochemical analysis. The activity of the mixed complexes in in vitro translation assays correlated with the large subunit of the respective correct complex. These results suggest that the eIF4G or eIFiso4G subunits influence translational efficiency more than the cap binding subunits. The translation assays also showed varying responses of the mRNA templates to eIF4F or eIFiso4F suggesting some level of mRNA discrimination is possible. The dissociation constants for the correct complexes have K_Ds in the sub-nanomolar range, whereas the mixed complexes were found to have K_Ds in the ~10 nM range. Displacement assays showed that the correct binding partner readily displaces the incorrect binding partner in a manner consistent with the difference in K_Ds. These results show molecular specificity for the formation of plant eIF4F and eIFiso4F complexes and suggest a role in mRNA discrimination during initiation of translation.

Initiation of protein synthesis requires the concerted effort of a large number of proteins and protein complexes, as well as multiple methods of control and control elements (1-5). Among the protein complexes required is eIF4F\(^1\), the cap-binding complex, which binds to the m\(^\text{7G}\) cap found at the 5' end of most eukaryotic cellular mRNAs. Mammalian eIF4F consists of three subunits, eIF4G, eIF4E and eIF4A. eIF4G is a large, multi-domain protein of ~180 kDa. eIF4G is largely responsible for ribosome attachment and enhancing efficiency of mRNA translation through multiple protein and/or RNA interactions (6). eIF4E is a small protein, ~24 kDa that directly binds the m\(^7\)G cap and facilitates initiation events. Mammalian eIF4F complexes contain eIF4A, the

\(^1\)eIF, eukaryotic initiation factor; m\(^7\)G, 7-methyl guanosine; ITC, isothermal calorimetry; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidene fluoride; SPR, surface plasmon resonance.
The prototype of the DEAD box helicase family (7,8); however, plant eIF4A is loosely associated and is easily removed during purification (9).

eIF4G is a multi-functional protein that is an important structural platform for the assembly or nucleation of several initiation factors (eIF4A, eIF3, eIF4B, eIF5, poly(A) binding protein) and interaction with the 40S ribosome during the initiation process (10,11). Furthermore, in mammals there is also interaction of eIF4G with MNK kinase that then phosphorylates associated eIF4E (12). Mammalian eIF4G consists of several domains for interaction with these factors. The eIF4E and PABP binding sites are located in the N-terminal region, whereas the C-terminal region contains three HEAT domains, MIF4G (eIF4A, eIF3), MA3 (eIF4A) and W2 (MNK) (1,13-18). However, yeast eIF4G has only retained the MIF4G domain and lost the MA3 and W2 HEAT domains. Plants have retained the MIF4G and MA3 HEAT domains, but have also lost the W2 HEAT domain (see Figure 1A (19).

Plants contain a second form of eIF4F not found in other eukaryotes, eIFiso4F (9,20). The amount of eIF4F present in wheat germ extracts is about 5-10 times less compared to eIFiso4F (21). eIFiso4F consists of two subunits, eIFiso4G and eIFiso4E and has activities in vitro similar to those of eIF4F (9,22). The eIFiso4G subunit contains an eIF4E binding site and the two HEAT domains found in plant eIF4G, but has lost most of the N-terminal region compared to eIF4G (see Figure 1A). Plant eIF4E and eIFiso4E are similar in molecular weight and are about 50% similar in amino acid sequence (23). Mutations in eIF4G or eIFiso4G, as well as eIF4E or eIFiso4E, result in resistance to various plant viruses; however, the precise roles of these translation factor subunits in viral replication have not been fully elucidated (reviewed in 24,25).

In the model plant Arabidopsis thaliana, there are two genes for eIFiso4G and a single gene for eIF4G. The functional deletion of the two eIFiso4G genes in Arabidopsis results in a phenotype that includes impaired growth/development, poor seed quality, lower fertility, altered stress responses and lower chlorophyll (19). This phenotype suggests eIFiso4G has a significant role in proper plant growth/development and response to environmental stress. Interestingly, the deletion of the single gene for eIFiso4E does not result in a strong phenotype, only virus resistance to certain potyviruses (26,27).

Having two forms of the cap binding complex in plants presents the question of whether specific roles in the initiation of translation exist for eIF4F and eIFiso4F. To begin to address this question, we determined whether or not there is molecular specificity in forming the eIF4F or eIFiso4F complexes. We find that in vitro, mixed complexes of eIF4G/eIFiso4E or eIFiso4G/eIF4E are able to form spontaneously and function in the initiation of translation in a manner similar to the eIF4F or eIFiso4F complex comprised of the respective large subunit. We find that very low levels of mixed complexes are found in vivo, consistent with the biochemical evidence that there is strong specificity in binding affinity of the correct binding partners of the complexes compared to the mixed complexes.

EXPERIMENTAL PROCEDURES

N-terminal and internal peptide analysis of wheat eIF4G and eIF4E. eIF4F was purified from wheat germ as previously described (28). The eIF4F complex was resolved into eIF4G and eIF4E subunits by SDS PAGE and transferred to PVDF or nitrocellulose. Trypsin digestion, N-terminal sequence analysis (Edmund degradation) and peptide sequence analysis of eIF4G or eIFiso4G were carried out by John Leszyk (University of Massachusetts Medical Center Proteomics Mass Spectrometry Center, Worcester, MA) or by William Lane (Harvard University Micro Sequencing Center, Cambridge, MA). The amino terminus (Edmund degradation) of eIF4E was determined at the Protein Microanalysis Laboratory of the University of Massachusetts Medical Center.
Laboratory (Institute for Cellular and Molecular Biology, University of Texas at Austin).

*Screening of the wheat cDNA library for eIF4G.* The λZAP cDNA library preparation and screening were as previously described (22,29). The expression library was screened using eIF4G affinity purified rabbit antibody to native wheat eIF4F (21).

*Screening of a wheat genomic library for eIF4G.* The preparation and screening of a λFIX II (Stratagene) wheat (var. Chinese Spring) genomic library was as previously described (30). The genomic library was screened with a [32P]-labeled probe made from the truncated cDNA template for eIF4G obtained from the λZAP cDNA (see above). The complete sequence of the eIF4G gene was obtained by primer walking on both DNA strands (Genbank JN091779).

*RACE analysis of introns in eIF4G gene.* *In silico* prediction of splice sites was carried out using the NetPlant Gene Server (31). DNA amplification primers were designed to confirm the presence or absence of the predicted introns. Total RNA was extracted from 3 day old seedlings (var. Chinese Spring) using a Plant RNeasy extraction kit (Qiagen). Reverse transcription (Retroscript, Ambion) and DNA amplification and sequencing were carried out with the appropriate primer sets to check the presence of the predicted introns.

*Preparation of mouse monoclonal and rabbit polyclonal antibodies.* Mouse monoclonal antibodies to native wheat eIF4F were prepared as previously described (32). Monoclonal antibodies were obtained to six distinct epitopes of wheat eIF4G and one monoclonal was obtained to eIF4E. Mouse ascites fluid (1/1000) was used for western blot analysis (21). Rabbit polyclonal antiserum was prepared to native eIF4F, native eIFiso4F, gel purified native eIFiso4E or eIFiso4G and to recombinant wheat eIFiso4E, eIFiso4G, eIF4G or eIF4E at the University of Texas MD Anderson Cancer Center, Dept. of Veterinary Science (Bastrop, TX). Western blots were carried out as previously described (21).

*Construction of expression clone for eIF4G.* The N-terminus of native wheat eIF4G was determined by Edmund degradation. The sequence obtained (see Fig. 1B) was identified on the deduced gene sequence. There are many potential translation start sites in this region of the sequence. It should be pointed out that any blocked N-termini from other start sites would have been missed by the N-terminal sequencing and thus other start sites may be used *in vivo.* However, for the purposes of biochemical analysis of eIF4G, the N-terminal sequence obtained was used as the start site for expression of recombinant protein.

The partial cDNA clone obtained from screening of the cDNA λZAP library was used as a template for DNA amplification using a forward primer containing an N-terminal NcoI site and a reverse primer containing a BamHI site. This DNA fragment was cloned into pET3d (Novagen) using the primer derived NcoI and BamHI restriction sites. A second fragment was obtained by digestion of the genomic clone (a region devoid of introns) with NcoI (naturally occurring, see Fig. 1B) and AgeI (naturally occurring, see Fig. 1B). This fragment was inserted into pET3d containing the cDNA between the NcoI (vector) and AgeI (insert) restriction sites. This construct expresses an N-terminal truncated version of eIF4G that is fully functional in translation assays (data not shown). A third fragment was amplified by reverse transcription and DNA amplification of poly A+ mRNA (var. Chinese Spring) with a forward primer containing a NcoI site corresponding to the N-terminus obtained from protein sequencing of eIF4G and a reverse primer complementary to the region 3’ to the naturally occurring NcoI site. This fragment was cloned into pET3d (containing fragments 1 and 2) at the NcoI restriction site located at the most 5’ end of the cDNA. Positive colonies were screened by PCR for the correct orientation. The complete reconstructed eIF4G cDNA for expression was sequenced (Genbank EF190330).
Cloning of wheat eIF4E. The cDNA previously obtained for eIF4E was not full length (29). The N-terminal protein sequence (AEDTETRPSAGAEEREEGEI) obtained by Edmund degradation was used to design an oligonucleotide primer to provide the missing sequence. The oligonucleotide was optimized for E. coli expression, but retained the correct wheat amino acid sequence (Genbank Z12616).

Construction of dicistronic eIF4F, eIF iso4F and mixed complexes. The cap-binding subunits, eIF4E or eIFiso4E, were amplified using a primer containing a BamHI restriction site, an E. coli ribosome binding site and 12-15 nucleotides of the respective cap-binding protein 5’ coding region in the forward direction. The reverse direction primer contained a BamHI restriction site, a termination codon and 12-15 nucleotides of the respective cap-binding protein 3’ coding region. The amplified DNA for eIF4E or eIFiso4E was cloned into the BamHI site that is 3’ to the termination codon of either the eIF4G (described above) or eIFiso4G (23) coding regions. The resulting plasmid constructs were screened for the correct orientation of the cap binding protein by DNA amplification and confirmed by DNA sequencing.

Expression of eIF4F, eIFiso4F and mixed complexes. The expression and purification of eIF4F and eIFiso4F was as previously described (33). The mixed complexes of eIF4G/eIFiso4E or eIFiso4G/eIF4E were purified similarly.

In vitro translation assay. The abilities of the cap binding complexes to support in vitro translation were determined as previously described (28,34). The fractionated assay is dependent upon the addition of eIF4F or eIFiso4F for activity. Briefly, 100 μl reactions included 5 pmol mRNA template (as indicated), recombinant eIF4F, eIFiso4F or mixed complex as indicated, 0.6 μg (10 pmol) of wheat recombinant eIF4B, 5 μg (7 pmol) native wheat eIF3, 10 μg (200 pmol) recombinant wheat eIF4A, 24 mM Hepes-KOH, pH 7.6, 2.9 mM MgAc2, 100 mM KAc, 30 mM KCl, 2.4 mM DTT, 0.1 mM spermine, 1 mM ATP, 0.2 mM GTP, 34 μM [14C]leucine, 50 μM 19 amino acids, 7.8 mM creatine phosphate, 3 μg creatine kinase, 0.75 A260 units of yeast tRNA, 1-2 A260 units of 1X washed wheat ribosomes, and 200 μg wheat germ 40-70% ammonium sulfate fraction. Incubation was for 30 min at 27 °C and the amount of [14C]leucine incorporated into protein was determined as previously described (28).

SPR Analysis. SPR (Biacore) experiments were carried out at the Center for Biomolecular Interaction Analysis (Dr. David Myszka, Director; University of Utah). Briefly, reactions were performed at 25 °C using a Biacore 3000 optical biosensor equipped with a CM4 sensor chip and equilibrated with running buffer (10 mM HEPES, pH 7.4, 150 mM NaCl). eIF4G or eIFiso4G were amine-coupled in individual flow cells to densities of 580 and 750 resonance units (RU), respectively. For each immobilization, the surface was activated for 5 min, the protein was coupled, and any remaining active sites on the surface were blocked with 1 M ethanolamine pH 8.9. eIFiso4E and eIF4E were tested for binding to the immobilized proteins in 20 mM HEPES, pH7.6, 100 mM KCl, 1.0 mM DTT, 0.1 mM EDTA, 100 μM m7GTP, 5% glycerol, 0.01% Tween-20, 0.1 mg/ml BSA. Starting at 50 nM, a three-fold dilution series of eIFiso4E or eIF4E were tested in triplicate. The response data were globally fit using Scrubber2 (Biologic Software Pty Ltd) to a 1:1 interaction model to extract binding constants.

Immunoprecipitation of eIF4E or eIFiso4E from wheat germ extracts. Magnetic protein A beads (40 μl, Genscript) were incubated with 100 μl rabbit sera to recombinant wheat eIF4G or eIFiso4G for 1 h at room temperature and washed according to the manufacturer’s instructions. The beads were then incubated for 3 h at 4 °C on a rotating platform with 1.0 ml of wheat germ extract supplemented with Complete protease inhibitor (40 μl of a 25X solution made from tablet, Roche). The beads were washed three times with IP buffer (50 mM Tris-Cl,
pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 0.1% Nonidet P-40, 1 mM PMSF) and eluted with 50 μl of 2X SDS sample buffer (35). Eluted proteins (20 μl) from the beads were separated on 10-20% gradient gels (Invitrogen) and transferred to nitrocellulose (Genscript). Monoclonal mouse antibody to elf4E (monoclonal 8F7) or rabbit antisera raised to native gel purified elfiso4E were used to probe the western blot using a One-Hour Western IP kit (Genscript) according to the manufacturer’s instructions.

Gel Shift Assay for displacement of cap binding protein from mixed complexes. Correct or mixed complexes (2.5 pmol) were presented cap binding proteins (0, 2.5, 7.5 or 20 pmol) in a 10 μl reaction containing 20 mM HEPES, pH 7.6, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol, 0.1 μg/μl BSA and 100 μM m⁷GTP. Reactions were incubated on ice for 10 min, then 10 μl native gel buffer (Invitrogen) was added and loaded on a 4-12% non-denaturing NOVEX gel (Invitrogen) and electrophoresed at 100 volts at 4 ºC for ~2.5 h. The gel was blotted to PVDF and probed with mouse monoclonal antibody to elf4E (8F7, 1/1000) and then stripped and probed with rabbit anti-native elfiso4E (1/1,000). The second antibody used was goat anti-mouse or goat anti-rabbit HRP (1/10,000, Kirkegaard-Perry) respectively.

RESULTS

Construction of cDNA expression clones. Construction of full length cDNA clones of wheat elf4E and elf4G were necessary to analyze their interactions and compare them with those of elfiso4E and elfiso4G that were previously constructed (22). A combination of protein sequence data, cDNA and genomic DNA sequencing and intron analysis were used as described in Experimental Procedures to construct the expression clones for elf4E and elf4G.

The genomic DNA sequence for elf4G indicates that there are numerous potential start sites in addition to the dual ATGs immediately preceding the N-terminal amino acid sequence obtained (see Figure 1B). It is not known at this time if any additional upstream start sites are used in the expression of wheat elf4G in vivo. If the amino-termini of alternative start sites were blocked, then Edmund degradation would not have identified them. However, since the goal was to obtain recombinant protein, the amino terminus identified by Edmund degradation was selected for construction of the recombinant protein.

The complete reconstructed cDNA was sequenced (GenBank EF190330) and eight differences (3 silent; 5 amino acid changes) were identified between the gene sequence and the expression construct as shown in Figure 1B. These represent mutations that either arose from the amplification process used to generate the N-terminal fragment or are allelic/cultivar differences in the source materials.

Dicistronic expression of wheat elf4F. To facilitate preparation of recombinant elf4F or elfiso4F for biochemical analysis, both subunits of the respective complex were placed into the same pET expression vector, co-expressed and purified as described (33,34). Recombinant wheat elf4F or elfiso4F prepared in this manner is highly pure (see Figure 2, lanes 2 and 5 respectively). The monoclonal antibodies obtained to native wheat elf4F react with the recombinant forms of elf4G and elf4E (see Fig. 3) indicating that all the epitopes represented in the native form are present in the recombinant form of elf4G.

Recombinant wheat elf4F or elfiso4F has similar activity to native elf4F or elfiso4F in in vitro translation (Fig. 4). These results suggest that the expressed dicistronic forms of elf4F and elfiso4F complexes are fully functional. Interestingly, elfiso4F displays sigmoidal behavior with some mRNA templates such as β-hemoglobin mRNA (shown in Fig. 4) or yeast polysomal mRNA (9). Other plant mRNA templates also display this behavior in vitro (data not shown) and this behavior may reflect a difference in the interaction of some mRNAs with elfiso4F compared to elf4F.
Do “mixed” complexes form? Preliminary experiments using the yeast two-hybrid system showed that wheat eIFiso4G could interact with either eIFiso4E or eIF4E and eIF4G could interact with either eIF4E or eIFiso4E suggesting that mixed complexes were possible (data not shown). To determine if stable mixed complexes could form in vitro and to test their ability to function in translation, dicistronic expression clones of eIF4G/eIFiso4E and eIFiso4G/eIF4E were prepared and the complexes expressed and purified similarly to eIF4F or eIFiso4F. As shown in Figure 2 (Lanes 3 and 4), stable mixed complexes were obtained and purified from E. coli. In vitro translation activity of mixed complexes. To determine if the mixed complexes were able to support initiation of translation in vitro, the mixed complexes were compared to recombinant eIF4F and eIFiso4F in the presence of five different mRNAs. The mRNAs selected include both capped cellular mRNAs (barley α–amylase, Arabidopsis thaliana HSP21, β-hemoglobin), capped viral RNA (AMV 4 RNA) and a non-capped viral RNA (STNV). As shown in Figure 5, the mixed complexes are able to fully support initiation of translation. Interestingly, the translational efficiency appears to correlate more closely with the large subunit rather than the cap-binding subunit. That is, the complex containing eIF4G/eIFiso4E has activity more similar to eIF4F, and the complex containing eIFiso4G/eIF4E is more similar to eIF4F. These results suggest that the large subunit provides some level of specificity of mRNA translation by eIF4F or eIFiso4F.

IP analysis. It was clear that the subunits are able to form functional mixed complexes in vitro. The question then was whether or not mixed complexes occur in planta since the predominant forms purified from wheat germ are the eIF4F and eIFiso4F complexes. To address this issue, wheat germ extracts were immunoprecipitated using rabbit antibodies to recombinant eIF4G or eIFiso4G. The resulting immunoprecipitated proteins were probed with antibodies to eIF4E or eIFiso4E to determine if mixed complexes are present in vivo. The data shown in Figure 6 suggests that very low levels of eIF4E are detected when eIFiso4G is immunoprecipitated. Conversely, no eIFiso4E was detected when eIF4G was immunoprecipitated. It is not clear whether this means that there is not any eIF4G/eIFiso4E mixed complex or if it reflects a detection limit as eIF4G is present in 5-10-fold lower amounts than eIFiso4G (21). These results show that very low levels of mixed complexes, at least of eIFiso4G/eIF4E are present in plants.

Binding affinity and displacement analysis. Although it is possible to form mixed complexes in vitro and in vivo, it is expected that there may be some level of specificity for complex formation. The binding affinity for association of the cap-binding subunits with the large subunits was measured to assess the level of specificity of complex formation. Initial experiments with isothermal calorimetry (ITC) indicated that there were significant differences in the binding affinity for correct and mixed complexes; however, the apparent sub-nanomolar binding affinities observed for the correct complexes are not accurately measured by ITC (Dr. Verna Frasca, personal communication). To obtain more accurate measurements of the binding constants, SPR (BiaCore) was carried out on the correct and mixed complexes. The SPR data clearly support a “preferred” binding partner (Figure 7 and Table 1) and show that the correct complexes have sub-nanomolar K_Ds. The K_D for the eIF4G/eIF4E complex is ~80-fold tighter than for the eIF4G/eIFiso4E mixed complex. Similarly, the eIFiso4G/eIFiso4E complex is ~148-fold tighter than the eIFiso4G/eIF4E mixed complex. The sub-nanomolar K_Ds for the correct complexes suggest that if a mixed complex is presented with the correct binding partner, that the correct binding partner should displace the incorrect binding partner preferentially forming the correct complex.

Displacement assays were performed using eIF4F, eIFiso4F and mixed
complexes. As shown in Figure 8, when the mixed complex of eIF4G/eIFiso4E was presented with increasing levels of eIF4E, the eIFiso4E was displaced by the eIF4E and eIF4E appears in the complex (Fig. 8A). Conversely, eIFiso4E disappears from the complex (Fig. 8C). Similarly, when eIFiso4G/eIF4E is presented with eIFiso4E, eIF4E is displaced from the complex (Fig. 8B) and eIFiso4E appears in the complex (Fig. 8D). However, increasing amounts of eIF4E could not displace eIFiso4E from the eIFiso4F complex (Fig. 8C, right side) and remains in the unbound state (Fig. 8A, right side), nor did eIFiso4E displace eIF4E from the eIF4F complex (Fig. 8B, right side) and remains unbound (Fig. 8D, right side). These results suggest preferential binding when the appropriate binding partner is present. Thus the low level of mixed complex of eIFiso4G/eIF4E in vivo demonstrated by immunoprecipitation likely reflects a very small excess of eIF4E over eIF4G that may be bound by any excess eIFiso4G not in complex with eIFiso4E. Interestingly, it was recently shown by an extensive proteomic analysis of mammalian cells that eIF4G and eIF4E were present in roughly equal molar amounts (36) which is consistent with the plant immunoprecipitation data indicating very low levels of excess subunits that form mixed complexes.

DISCUSSION

The discovery of a plant specific form of eIF4F, eIFiso4F, suggested that there may be specialized functions for these complexes in plant initiation of translation (9,20). Biochemical analysis of these complexes should be able to reveal important information about the specificity of binding of the subunits to form complexes and their interactions with mRNAs.

We constructed functional wheat discistrionic expression clones and have shown that the recombinant eIF4F and eIFiso4F complexes are functionally similar to native complexes in in vitro translation (Figure 4).

To assess the ability of the subunits of eIF4F and eIFiso4F to form mixed complexes, additional discistrionic plasmids were constructed that contained eIF4G/eIFiso4E and eIFiso4G/eIF4E and used to purify mixed complexes. As shown in Figure 5, these complexes are functional and support the initiation of translation in vitro. Interestingly, the large subunit appears to be dominant in determining the activity of the mixed complex. The mixed complex containing eIF4G/eIFiso4E behaved more similarly to eIF4F while the mixed complex containing eIFiso4G/eIF4E was more like eIFiso4F. These results suggest that the large subunit is most likely responsible for discrimination among groups of plant mRNAs.

The binding constants were determined using surface plasmon resonance (SPR) for the interaction of eIF4F, eIFiso4F and mixed complex subunits. The K_D for binding of eIF4G to eIF4E and eIFiso4G to eIFiso4E determined by SPR were in the sub-nanomolar range (0.18 and 0.08 nM respectively), whereas the mixed complexes were in the ~12-14 nM range. This represents a ~80 to 148-fold difference in binding affinity and is supported by the displacement data in Figure 8 that shows that the correct binding partner readily displaces an incorrect binding partner. Mammalian and yeast K_D values for eIF4G and eIF4E binding are reported in the 2.5-27 nM and 2-15 nM range respectively (see Supplemental Table A). The wheat mixed complexes (~12-14 nM) are more similar in K_D to those of the yeast and mammalian complexes. This may reflect that in contrast to mammalian and yeast systems, plants do not appear at this time to have the 4E binding proteins that participate in the regulation of the accessibility of eIF4E to bind eIF4G. Since such a system does not appear to function in plants, there is no apparent need for the subunits to readily dissociate as indicated by the strong binding constants and displacement data (Table I, Figure 8). Although there are reports of
plant proteins that interact with eIF4E in the yeast two-hybrid system (37,38), these interactions have not been studied biochemically and their biological role is unknown. One might speculate that there is a rich and varied set of protein interactions for the subunits of eIF4F or eIFiso4F that may regulate translation in plants; however, given the strength of the interactions of the subunits (sub-nanomolar) of the eIF4F or eIFiso4F complexes, there would most likely need to be a significant input of energy to dissociate these complexes in the form of ATP hydrolysis or other similar biochemical process. However, such a system has not yet been detected. It needs to be determined if plant eIF4F or eIFiso4F dissociation occurs and/or has a role in regulation.

The precise eIF4F or eIFiso4F subunit requirement for initiation of translation in plants remains to be determined. Preliminary evidence from generating multiple Arabidopsis T-DNA insertion mutants for the subunits of eIF4F or eIFiso4F suggests that a mixed complex of eIFiso4G and eIF4E is quite sufficient for normal growth and development; however, plants with only the mixed complex of eIF4G and eIFiso4E fail to thrive or proceed through normal development to flowering. This suggests that certain mRNAs may need eIFiso4G to be properly translated. We have recently shown that a double mutant lacking both eIFiso4G genes in Arabidopsis shows an impaired growth phenotype that includes less chlorophyll, reduced fertility and loss of long term seed viability (19). These results suggest that plant eIFiso4G is very important in plant growth and development, whereas eIF4G is not required or at least the lack of eIF4G does not present an obvious impairment. It remains to be determined if eIFiso4F provides optimal translation for a specific subset of mRNAs necessary for plant growth and development, or if, as recently shown for the two genes for yeast eIF4G, that it is the total amount of eIF4G (and/or eIFiso4G) present, not the particular gene product (39). It is also possible that plants have evolved different cap binding complexes for the pioneer round versus steady-state translation (40). There is still much to be learned about the roles for eIF4F and eIFiso4F in the initiation of plant translation and its regulation. The further elucidation of the roles of eIF4F and eIFiso4F subunits in plant initiation of translation may illuminate how other cap-binding complexes form and function in organisms that have multiple genes for eIF4G and eIF4E.

Acknowledgements--The authors thank Dr. John Leszyk (University of Massachusetts Medical Center Proteomics Mass Spectrometry Center, Worchester, MA) and Dr. William Lane (Harvard University Micro Sequencing Center, Cambridge, MA) for protein sequence analysis; Drs. Verna Frasca and Lung-Nan Lin (GE Lifesciences, Northampton, MA) for the ITC analysis and comments on the manuscript; and Dr. David Myszka (Director, Center for Biomolecular Interaction Analysis, University of Utah) for the BiaCore analysis and comments on the manuscript. This work was supported by the National Science Foundation to K.S.B. (MCB1052530 and MCB0745146).

REFERENCES

1. Marintchev, A. and Wagner, G. (2004) Q. Rev. Biophys. 37, 197-284
2. Pestova, T. V., Lorsch, J. R., and Hellen, C. U. T. (2007) The Mechanism of Translation Initiation in Eukaryotes. In Mathews, M. B., Sonenberg, N., and Hershey, J. W. B., editors. Translational Control in Biology and Medicine, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3. Mathews, M. B., Sonenberg, N., and Hershey, J. W. B. (2007) Origins and Principles of Translational Control. In Mathews, M. B., Sonenberg, N., and Hershey, J. W. B., editors. Translational Control in Biology and Medicine, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
4. Livingstone, M., Atas, E., Meller, A., and Sonenberg, N. (2010) Phys. Biol. 7, 021001.
5. Merrick, W. C. (2010) J. Biol. Chem. 285, 21197-21201
6. Park, E. H., Zhang, F., Warringer, J., Sunnerhagen, P., and Hinnebusch, A. G. (2011) BMC. Genomics 12, 68
7. Rogers, G. W., Jr., Komar, A. A., and Merrick, W. C. (2002) Prog Nucleic Acid Res Mol Biol 72, 307-331
8. Parsyan, A., Svitkin, Y., Shahbazian, D., Gkogkas, C., Lasko, P., Merrick, W. C., and Sonenberg, N. (2011) Nat. Rev. Mol. Cell Biol. 12, 235-245
9. Lax, S. R., Fritz, W., Browning, K. S., and Ravel, J. M. (1985) Proc. Natl. Acad. Sci. USA 82, 330-333
10. Hernandez, G. and Vazquez-Pianzola, P. (2005) Mech. Dev. 122, 865-876
11. Prévôt, D., Darlix, J. L., and Ohlmann, T. (2003) Biol. Cell 95, 141-156
12. Buxade, M., Parra-Palau, J. L., and Proud, C. G. (2008) Front Biosci. 13, 5359-5373
13. Korneeva, N. L., Lamphear, B. J., Hennigan, F. L. C., and Rhoads, R. E. (2000) J. Biol. Chem. 275, 41369-41376
14. Marintchev, A., Lomakin, I. B., Sonenberg, N., Pestova, T. V., Hellen, C. U. T., and Burley, S. K. (2001) Mol. Cell 7, 193-203
15. Marintchev, A. and Wagner, G. (2005) Biochemistry 44, 12265-12272
16. Oberer, M., Marintchev, A., and Wagner, G. (2005) Genes Dev. 19, 2212-2223
17. Bellsolell, L., Cho-Park, P. F., Poulin, F., Sonenberg, N., and Burley, S. K. (2006) Structure. 14, 913-923
18. Schütz, P., Bummann, M., Oberholzer, A. E., Bieniossek, C., Trachsel, H., Altmann, M., and Baumann, U. (2008) Proc. Natl. Acad. Sci. U. S. A 105, 9564-9569
19. Lellis, A. D., Allen, M. L., Aertker, A. W., Tran, J. K., Hillis, D. M., Harbin, C. R., Caldwell, C., Gallie, D. R., and Browning, K. S. (2010) Plant Mol Biol 74, 249-263
20. Browning, K. S., Webster, C., Roberts, J. K. M., and Ravel, J. M. (1992) J. Biol. Chem. 267, 10096-10100
21. Browning, K. S., Humphreys, J., Hobbs, W., Smith, G. B., and Ravel, J. M. (1990) J. Biol. Chem. 265, 17967-17973
22. Van Heerden, A. and Browning, K. S. (1994) J. Biol. Chem. 269, 17454-17457
23. Allen, M. L., Metz, A. M., Timmer, R. T., Rhoads, R. E., and Browning, K. S. (1992) J. Biol. Chem. 267, 23232-23236
24. Robaglia, C. and Caranta, C. (2006) Trends Plant Sci. 11, 40-45
25. Truniger, V. and Aranda, M. A. (2009) Adv. Virus Res. 75, 119-159
26. Duprat, A., Caranta, C., Revers, F., Menand, B., Browning, K. S., and Robaglia, C. (2002) Plant J. 32, 927-934
27. Lellis, A. D., Kasschau, K. D., Whitham, S. A., and Carrington, J. C. (2002) Curr. Biol. 12, 1046-1051
28. Lax, S. R., Lauer, S. J., Browning, K. S., and Ravel, J. M. (1986) Methods Enzymol. 118, 109-128
29. Metz, A. M., Timmer, R. T., and Browning, K. S. (1992) Nucleic Acids Res. 20, 4096
30. Metz, A. M., Wong, K. C. H., Malmström, S. A., and Browning, K. S. (1999) Biochem. Biophys. Res. Commun. 266, 314-321
31. Hebsgaard, S. M., Korning, P. G., Tolstrup, N., Engelbrecht, J., Rouzé, P., and Brunak, S. (1996) Nucleic Acids Res. 24, 3439-3452
32. Lauer, S. J., Browning, K. S., and Ravel, J. M. (1985) Biochemistry 24, 2928-2931
33. Mayberry, L. K., Dennis, M. D., Allen, M. L., Nitka, K. A., Murphy, P. A., Campbell, L., and Browning, K. S. (2007) Methods Enzymol. 430, 397-408
34. Mayberry, L. K., Allen, M. L., Dennis, M. D., and Browning, K. S. (2009) *Plant Physiol* **150**, 1844-1854
35. Serino, G. and Deng, X.-W. (2007) *Cold Spring Harbor Protocols* **2007**, pdb.prot4683. doi: 10.1101/pdb.prot4683.
36. Schwanhausser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., and Selbach, M. (2011) *Nature* **473**, 337-342
37. Freire, M. A., Tourneur, C., Granier, F., Camonis, J., El Amrani, A., Browning, K. S., and Robaglia, C. (2000) *Plant Molecular Biology* **44**, 129-140
38. Freire, M. A. (2005) *Gene* **345**, 271-277
39. Clarkson, B. K., Gilbert, W. V., and Doudna, J. A. (2010) *PLoS. ONE. 5*, e9114
40. Maquat, L. E., Tarn, W. Y., and Isken, O. (2010) *Cell* **142**, 368-374
Table I. Binding Affinities of Correct and Mixed Complexes Measured by SPR.

|               | K_D (nM)     |
|---------------|--------------|
| eIF4G         | eIF4E        |
|               | 0.181 ± 0.002|
| eIFiso4E      | eIF4E        |
|               | 14.3 ± 0.2   |
| eIFiso4G      | eIF4E        |
|               | 11.8 ± 0.1   |
| eIFiso4E      | eIFiso4E     |
|               | 0.080 ± 0.002|
Figure Legends

Figure 1. A. Domain Organization for eIF4G and eIFiso4G. Plant eIF4G and eIFiso4G have similar domain organization, except eIF4G has an extended N-terminal region (19). The eIF4E binding site and HEAT domains are indicated. The HEAT domains interact with eIF4A and eIF3 as indicated. Plant eIF4G and eIFiso4G lack the third HEAT domain present in mammalian eIF4G and yeast eIF4G only has HEAT domain 1. B. Sequence of wheat eIF4G gene. The coding region is shown in capital letters and non-coding regions and introns are in lower case. Differences between the gene sequence and expression clone are indicated (green, silent mutants; red, amino acid changes). The amino terminus of the expression clone was altered to generate an NcoI site as indicated and has a single methionine and the second amino acid was changed to glycine. The amino terminal peptide obtained from Edmund degradation of native protein is indicated by a double underline and internal peptides obtained from tyrocidin digests of native protein are indicated by a single underline. The naturally occurring Ncol and AgeI sites used to generate the expression clone are indicated. The eIF4E binding site (dotted underline) and HEAT domains (yellow, blue) are indicated.

Figure 2. SDS PAGE Analysis of eIF4F, eIFiso4F and Mixed Complexes. SDS PAGE was carried out on a 12.5% acrylamide gel and stained with Coomassie Brilliant Blue. Each lane contains 25 pmol of the indicated complex. Lane 1, native eIF4F; Lane 2, recombinant eIF4F; Lane 3, eIF4G/eIFiso4E; Lane 4, eIFiso4G/eIF4E; Lane 5, recombinant eIFiso4F; Lane 6, native eIFiso4F.

Figure 3. Monoclonal Antibodies to Wheat eIF4F. Mouse monoclonal antibodies were raised to wheat native eIF4F. The ability of individual monoclonal antibodies to react with native (n) eIF4F and recombinant (r) eIF4F were tested by western blotting. Mouse ascites fluid (1/1000) was incubated overnight at 4 °C and the second antibody was goat anti-mouse HRP (1/20,000, Kirkegaard-Perry). The chemiluminescent substrate was Super Signal West Pico (Thermo-Pierce).

Figure 4. Comparison of Recombinant and Native eIF4F and eIFiso4F in Translation. Each 100 μl translation reaction contained 5 pmol of capped rabbit β-hemoglobin mRNA and the indicated amounts of eIF4F or eIFiso4F complexes (■, solid line, native eIF4F; □, dashed line, recombinant eIF4F; ●, solid line, native eIFiso4F; △, dashed line, recombinant eIFiso4F) as described in Experimental Procedures. The amount of [14C]-leucine incorporated in the absence of cap binding complexes was 3.8 pmol. Each point represents the average of three experiments and error bars are indicated.

Figure 5. Translation Assay of eIF4F, eIFiso4F and Mixed Complexes. Each 100 μl translation reaction contained 5 pmol of the indicated mRNA (capped rabbit β-hemoglobin, capped barley α-amylase, capped ArHSP21, STNV RNA (uncapped), capped AMV RNA 4) and the indicated amounts of recombinant eIF4F, eIFiso4F or mixed complex (●, eIF4F; ○, eIF4G/eIFiso4E; ▲, eIFiso4F; △, eIFiso4G/eIF4E) as described in Experimental Procedures. The amount of [14C]-leucine incorporated in the absence of cap binding complexes is as indicated: β-hemoglobin (4.0 pmol); barley α-amylase (4.1 pmol); ArSHSP 21 (6.6 pmol); STNV RNA (16.8 pmol); AMV RNA 4 (5.3 pmol). Each point represents the average of three experiments and error bars are indicated.
**Figure 6. Immunoprecipitation of Wheat Germ Extracts.** Wheat germ extract (1 ml) was immunoprecipitated with rabbit antisera raised to either recombinant eIF4G or eIFiso4G bound to protein A magnetic beads (Genscript). The precipitated proteins were eluted with 50 µl 2X SDS PAGE sample buffer, 20 µl of which was separated on a 10-20% acrylamide gel (BioRad) and transferred to nitrocellulose. Controls of recombinant wheat eIF4E (0.05 µg) and eIFiso4E (0.05 µg) were also separated on the gel. The blots were probed with either mouse monoclonal antibody for eIF4E (8F7) or rabbit polyclonal serum raised to native gel purified eIFiso4E using a mouse or rabbit One Hour Complete IP-Western Kit (Genscript).

**Figure 7. SPR Binding Analysis of Correct and Mixed Complexes.** eIF4E (panels A and D) or eIFiso4E (panels B and C) were tested for binding to the respective immobilized proteins eIF4G (panels A and B) or eIFiso4G (panels C and D). The running buffer contained 20 mM HEPES, 100 mM KCl, 1.0 mM DTT, 0.1 mM EDTA, 100 µM mGTP, 5% glycerol, 0.01% Tween-20, 0.1 mg/ml BSA, pH 7.6. Starting at 50 nM, a three-fold dilution series of eIFiso4E or eIF4E were tested in triplicate. The response data were globally fit using Scrubber2 (Biologic Software Pty Ltd) to a 1:1 interaction model to extract binding constants.

**Figure 8. Displacement Assays.** To determine if the correct cap binding protein is able to displace a mismatched cap binding protein in a mixed complex, the indicated amounts of eIF4E or eIFiso4E were added to 2.5 pmol of mixed complexes, eIF4F or eIFiso4F. Panels A and B were probed with mouse monoclonal antibody (8F7) to eIF4E (1/1000). The blots were stripped and probed with rabbit polyclonal antibody raised to native gel purified eIFiso4E (1/1000) shown in panels C and D. The antibody to native eIFiso4E displays a small amount of cross-reactivity with eIFiso4G (see panel D, eIFiso4G/eIF4E with no eIFiso4E added).
Figure 1A
A · V · Q · H · R · Q · K · D · N · E · V · I · S · G · A · M · V · S · N · K

CCAGTTAGGAGAGGAGCATTAGCTATCCAGAGAGACCTACAGGAAAGGT
P · V · S · E · K · E · S · K · A · P · S · I · P · E · K · H · S · K · E · S

AAAGCACCACATCTGCGGTGAGAGCATCTACGACTTACCTTGTTCAAA
K · A · P · S · A · V · E · K · H · P · T · A · V · T · Q · P · L · P · I · Q

GCTGCAAAGCCAAAGACTGACGTCGGAATTCACCTCTACTCTTGACGGAGCT
A · A · K · P · E · T · A · T · A · N · S · P · S · F · L · T · G · A

GAATGAAAGGAAAGATTTCCTGGGTCAGACCAACACCAGGAgTatcactattcgtttctta
T · R · N · D · T · K · N · L · P · Q · Q · P · Q

intron 1
ttctagcactgtttgctatgttttcttttctgttaacatatctcactcatatcatt
gatccattcagTCGTCTCCCCTGGACAGAGGTGAGGCAAACCTCTCGTGAGATT
· S · A · S · P · A · E · E · L · K · G · Q · T · S · V · K · T

GGTGATGATGTGGTTGGTCACATGGAAACCAAGCTTCGATAGTGAAAAGGTGGATTTA
G · D · D · V · V · G · H · M · E · T · K · S · F · D · S · E · K · V · D · L

ACCAGCAAGGTTCAGCCCTTTACAGCAGAACACATCTGAGGAAAAGGGTATTA
T · S · K · V · S · G · S · A · T · A · T · S · E · S · S · I · S · P · I · L

G
intron 2
GGTAAAAGTAGACTGACGCGACACACTGATAGtgatcactctatattttttgaaagtt
G · K · S · E · A · D · S · T · S · V · N ·

D
aattgaagaatacataaatgtcgttttttttatttttcacatatgttgcattgcccagCTG

NcoI
CTGATGTTCCTCGCAATGTTAATCAGCCTCCTGCAAATTGTCTCTGCGGGAC
A · D · V · F · A · M · V · I · S · S · A · K · L · S · S · A · S · T · G · E ·

CCCAAGCAGTAGAAGGCTTAGGTTGGTCTGCTGGTTAACACCTGTAAGGAAATATCT
P · Q · A · V · E · S · L · G · V · A · V · K · S · K · E · I · E · E · T ·

ACCAAAATTTCTCACTGAATCTAGTGATGGCAAAAATTATGTCTGATTACTGAAATGAAT
H · Q · I · S · P · E · S · S · D · G · K · I · M · S · D · S · T · E · N · E

CACATGACTTCAGGTGGACCTGTGGACTGCGACATGGCAACTTCAAAGCTTGGTA
S · H · D · F · T · V · D · L · A · E · Q · A · S · L · A · T · S · K · P · G ·

ATTTCATGCAAACATCTTTTGTATACCTGGCAAGGGGACTCTTCAAGCTTGGTAA
N · S · D · A · T · S · F · V · T · D · P · Q · E · L · P · K · E · C · T · T ·

CTGTACCAGGACAGGACAGTTTTGATGAAATACATCACATACATAATAGGATACCCAAACTTTAT
S · V · P · E · D · H · S · L · M · N · T · S · H · N · K · D · T · Q · T ·

CAGCTTCTGTGGATGCGCAGCGTGATGTCTGCTGGAGTGCAATCTTCAAGGACCTCATCAGAGTCTA
S · A · S · V · D · A · S · D · V · S · E · V · N · S · G · T · S · S · E · S

CCAGCCAAAGTGACCAACACATTGATGAATAAGATCATGAGATACCATTCAGGAAACTTGAGATTACGT
T · S · Q · S · T · N · D · K · D · I · R · S · S · I · Q · E · T · G · L · A ·

TTTCTGGTATTACCTCTGGGATGTGGCTCTGTAATCATCAGGGATGCTTGGAAGGCGAAG
V · S · G · I · T · P · G · M · L · P · V · N · H · S · V · A · S · E · G · Q ·

TCAAACTGCAGATGGGACAGGATGAGTGCTAGTACTGAGCAATCAATGGCCCTACCAA
V · K · H · A · D · G · A · K · D · E · S · S · T · E · Q · S · S · A · V · P ·

Downloaded from http://www.jbc.org/ by guest on March 23, 2020
CAGTTCTGTAGACCTCTATCAGGGAACCACTAAGCTGAGCTGTGCCCCGGAGAACTGT
T·G·S·V·R·P·L·S·R·E·K·P·T·A·E·L·A·R·T·K·

CTACAGCTGGGAGAAGAGAAGACGGGAGAATGGCTTTCAAGCTGATGCTGGGA
S·T·A·G·R·K·K·K·E·M·L·S·K·A·D·A·A·G·

GCTACAGCTGGTACATCAAGCTCAGGAGGACATGCTGATGTTGCGCCAT
S·D·G·A·D·S·T·V·D·G·T·H·V·L·P·E·E·S·

CAGACGGTGCTGATAGTTCTTCAACAGCTGAGGGAACATGCTGAGGGAATCAG
S·S·D·L·Y·N·A·Y·K·G·P·Q·E·Q·S·E·S·V·A·T·

AAAGGGAGGTGTGATGAGGAGGAGTGAGAAGAAAAAGTTGAGGCCGAGATTTGGGAG
E·R·E·V·M·C·E·D·D·G·K·K·K·V·E·P·D·D·W·E·

ATGCAGCAGCAGTGTACTGCTCAAGCTGACGCTGAGAACCAGGCTAGTG
D·A·A·M·S·S·T·S·S·S·D·S·S·G·N·Q·A·S·

CAGTTCAATTGCCAGATTCGATGAGCTGAAGCTAAATGGCCGAAAGAAATATTCTCGTG
A·V·Q·L·P·D·S·D·M·T·E·A·N·G·E·K·K·Y·S·R·

eIF4E Binding Site

CTGCATATGCTTATCCATCAAGATTCTGCAAGGGTACAGCAGATGAGCTGATGCTGGGA
D·F·L·L·T·F·A·H·Q·Y·S·S·L·P·V·G·I·R·M·D·

CTGCTACCTAGCCTATTCAAAAGATTGTGGCAGAAAAATCTATGTTATGATGCGGAGA
T·V·T·S·T·L·D·L·A·G·K·S·Y·V·I·D·R·E·

CTGCATATGCTTATCCATCAAGATTCTGCAAGGGTACAGCAGATGAGCTGATGCTGGGA
P·A·M·D·D·D·K·W·L·K·S·G·V·P·Y·S·P·N·R·D·

CAGTTCAATTGCCAGATTCGATGAGCTGAAGCTAAATGGCCGAAAGAAATATTCTCGTG
A·H·M·D·L·T·N·G·P·A·I·N·Y·R·G·G·P·G·G·A·

ATGGTGTTCTGAGGAATCCAGCTGCTACCTTCTGATTGGAGACACATCCCATGCTCTCT
H·G·V·L·R·N·P·R·G·A·L·L·V·G·P·Q·S·N·A·P·

AAGTACCCCGCAGGTGCTGAGTGGTGCAGCTGAAAGCCATTCTGAATAACTGACCCCATC
Q·V·P·R·S·G·S·D·E·K·Q·R·Q·L·K·A·I·L·N·K·L·T·P·

CTGTTACACCCCATGCAAGTTAATGCACAAGCCGAGAAAGATGATTGTTGCTGGCAAAAGTT
P·V·T·P·M·Q·V·M·H·K·A·E·K·K·Y·V·V·G·K·V·

CTGATGAGGAGACGAGGACGGCACGCTGAAAGCCCATTTCTGAAATAACTGACCCCATC
S·D·E·E·Q·R·Q·O·L·K·A·I·L·N·K·L·T·P·

AAAACTTTGCAAGCTCTTTTGAACAGAGGAGGTGAACATGTCAGAATTTCTCTCTGTA
Q·N·F·D·K·L·P·E·Q·V·K·E·V·N·I·D·N·V·S·T·

TTACTGGGTTGATTTCCATCAAGATATTTTGACAAAGCATTGTGGAACACTTCTCTGGA
L·T·G·V·I·S·Q·I·F·D·K·A·L·M·E·F·T·F·C·E·

TGATGAGGAGACGAGGACGGCACGCTGAAAGCCCATTTCTGAAATAACTGACCCCATC
N·Y·A·N·F·C·S·H·L·A·G·A·L·P·D·F·L·S·E·D·N·

HEAT DOMAIN 1

AAAAGATTACCCCATGCAAGCTCTTTTGAACAGAGGAGGTGAACATGTCAGAATTTCTCTGTA
K·I·T·F·K·R·L·L·L·N·K·K·C·Q·E·E·F·E·R·G·

AAAAGAGGAGACGAGGACGGCACGCTGAAATAACCGGAGGGAGGTGAGATTTGAGAGGGAG
E·R·E·E·E·A·E·A·D·K·T·E·E·E·G·E·I·K·Q·T·K·
AGCTTCTACCTCTTTGTACATTGTAAGGCAGAAAGATATG
*S··F··Y··P··S··L··V··S··L··W··V··N··D··S··F··E··R··K··D··M·

GAAAGAGAGTTTGCAAGACGCTTCTTTGCGGCTTTACAATGGTGAATAATTATGG
*E··E··T··A··G··A··K··L··F··V··G··L··Y··N··G··G··Y··N··L··L·

HEAT DOMAIN 2
AGCAAGGCTCCAGCTCATTTGAGGGtgagtccttctttgcatgccccattgatgc
*S··K··P··Q··L··I··E··G

intron 4
cactggcgagtgctgcttaactttgacccatattttgtaggctTTCATCCGTTCTTGCTTCA
*S··S··V··L··A··S·

TTGGAGGATGCTCTAAGTGAATTCCTCAAGAAGGCGGGAGTTGCTTGAAGCTTCA
*L··E··D··A··L··S··D··S··P··R··A··E··Y··L··G··R··L··L··A··

AGGTTTTGTTGGAGAAGATACGTGTTTTGCAAGAGCTAGGTAATTGAGAAGAGGC
*R··F··V··E··K··I··L··V··L··Q··D··V··G··K··L··I··E··E··G·

GGAGGAGGAGCTGGACACCTTGTCAGGAAAGCAGCTGATCTTGGCGCAGTC
*G··E··E··F··G··H··L··V··Q··E··G··I··A··A··D··V··L··G··A··V·

TTGGAGTTGATCAAACAGAAAGGCGGGATCTCTCTTCAAGAGGCGAACAGACAGTCC
*L··E··W··I··R··T··E··G··D··S··F··L··K··E··A··K··T··S··S··

AATCTCAAGTTGAGATTCTGAGGCTACAGCTTTCAAGGTTTGGATGCCTTC
*N··L··K··L··E··D··F··R··P··Q··H··L··K··R··S··K··L··D··A··F·

ATGTTGACTTAAgttgtttgtgtagtaagccgtttcagtacaggcagggctttgaa
*M··L··T··*

ttgcatactcagctcttttttctttgtagtagtagtaaatcagcgtagtagcat
gcatgaatgtgaatgaatatcgtggtggtcaattacacaagtccgagtcagccctata
gtgagtcgtattagagc
Figure 2
Figure 3
Figure 4
Figure 5

[Graph showing incorporation of [14C]-Leu into proteins as a function of protein added.]
Figure 6
Figure 7

A  eIF4G/eIF4E

B  eIF4G/eIFiso4E

C  eIFiso4G/eIFiso4E

D  eIFiso4G/eIF4E

Response (RU) vs Time (s)
Figure 8

A. Probed with anti-elF4E

B. Probed with anti-elF4E

C. Probed with anti-elFiso4E

D. Probed with anti-elFiso4E
Plant cap binding complexes eukaryotic initiation factors eIF4F and eIFiso4F: molecular specificity of subunit binding
Laura K. Mayberry, M. Leah Allen, Kelley R. Nitka, Lara Campbell, Patricia A. Murphy and Karen S. Browning

J. Biol. Chem. published online September 30, 2011

Access the most updated version of this article at doi: 10.1074/jbc.M111.280099

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2011/09/30/M111.280099.DC1