The eukaryotic translation initiation factor (eIF) 4B promotes the RNA-dependent ATP hydrolysis activity and ATP-dependent RNA helicase activity of eIF4A and eIF4F during translation initiation. Although this function is conserved among plants, animals, and yeast, eIF4B is one of the least conserved of initiation factors at the sequence level. To gain insight into its functional conservation, the organization of the functional domains of eIF4B from wheat has been investigated. Plant eIF4B contains three RNA binding domains, one more than reported for mammalian or yeast eIF4B, and each domain exhibits a preference for purine-rich RNA. In addition to a conserved RNA recognition motif and a C-terminal RNA binding domain, wheat eIF4B contains a novel N-terminal RNA binding domain that requires a short, lysine-rich containing sequence. Both the lysine-rich motif and an adjacent, C-proximal motif are conserved with an N-proximal sequence in human and yeast eIF4B. The C-proximal motif within the N-terminal RNA binding domain in wheat eIF4B is required for interaction with eIF iso4G, an interaction not reported for other eIF4B proteins. Moreover, each RNA binding domain requires dimerization for binding activity. Two binding sites for the poly(A)-binding protein were mapped to a region within each of two conserved 41-amino acid repeat domains on either side of the C-terminal RNA binding domain. eIF4A bound to an adjacent region within each repeat, supporting a central role for these conserved eIF4B domains in facilitating interaction with other components of the translational machinery. These results support the notion that eIF4B functions by organizing multiple components of the translation initiation machinery and RNA.

Eukaryotic translation initiation differs from that in bacteria in the increased number and complexity of the initiation factors that assist in binding a 40 S ribosomal subunit to an mRNA, in locating the initiation codon, and in promoting binding of a 60 S subunit to assemble the 80 S ribosome. Early in initiation, the 5'-cap structure is bound by the eukaryotic initiation factor (eIF) 4E, the small subunit of eIF4F. eIF4G, the large subunit of eIF4F, recruits several other factors, including eIF4A, eIF3, and the poly(A)-binding protein (PABP) through direct protein-protein interactions (1–6). The binding of these proteins is a prerequisite for the recruitment of a 40 S ribosomal subunit for most mRNAs.

The interaction between PABP and eIF4G is conserved among plants, animals, and yeast (3, 4, 6) and serves to stabilize the binding of eIF4F to the 5'-cap (7). PABP also interacts with eIF4A as was shown first in plants and subsequently in animals (4, 8, 9). The interaction of eIF4G (or its isoform, eIF iso4G) and eIF4B with PABP synergistically increases the binding affinity of PABP for poly(A) RNA, whereas the interaction of PABP with eIF iso4G increases the binding of eIF iso4F (an isoform of eIF4F) to the cap (4, 7). Addition of eIF4B to eIF iso4F or the eIF iso4F-PABP complex lowers the activation energy of binding of each to the 5' cap in plants (10, 11), suggesting that eIF4B and PABP enhance binding by providing a lower energy barrier that may involve a conformational change that is propagated to the cap binding site. Thus, eIF4B, together with PABP, promotes stable recruitment of eIF4F to an mRNA by accelerating its binding to, and reducing its dissociation from, the 5' cap (11). The interaction between PABP and eIF4G, and presumably eIF4B, brings the termini of an mRNA in close physical proximity as demonstrated in vitro (12) to serve as a means test to confirm the integrity of an mRNA as a prerequisite for recruitment of the 40 S subunit (13).

Once recruited, the 40 S subunit scans down the 5'-leader in search of the first initiation codon present in the appropriate context (14). eIF4A serves as an RNA helicase thought to unwind any secondary structure present in the 5'-leader that would otherwise impede scanning of the 40 S subunit. Alone, eIF4A exhibits little RNA helicase activity (15). eIF4B is essential to stimulate the ATPase and RNA helicase activity of eIF4A in mammals (15–23) and increases the ATP affinity of eIF4A and the processivity of its helicase (24, 25). In contrast, wheat eIF4B only moderately stimulates but is not required for the ATP hydrolysis and RNA helicase activities of eIF4A (26–29). PABP also increases the ATPase and the RNA helicase activity of the eIF4F-eIF4A-eIF4B complex in plants (30). In addition to stimulating eIF4A activity, eIF4B mediates mRNA binding to ribosomes (31–35). Translation from mRNAs with a moderately stable secondary structure in the 5' leader is highly stimulated by eIF4B in yeast, whereas the higher basal level of translation from mRNAs with little secondary structure is only moderately stimulated (21) supporting a role for eIF4B in promoting eIF4A RNA helicase activity. In animals, in vitro assembly of 48 S translation initiation complexes on mRNAs with moderately stable secondary structure in the 5' leader also...
Domain Organization of eIF4B

requires eIF4B (36) but only native and not recombinant eIF4B was active in this assay. Thus, eIF4B functions to stabilize eIF4F binding to the 5’ cap, PABP binding to the poly(A) tail, and mRNA binding to ribosomes while stimulating eIF4A activity, demonstrating that it performs multiple functions through interactions with several factors and RNA to facilitate translation initiation.

Mammalian eIF4B contains an N-proximal RNA recognition motif (RRM), a hydrophilic region rich in aspartic acid, arginine, tyrosine, and glycine residues (DRYG) responsible for dimerization and binding to eIF3a, a C-terminal RNA binding domain, and a C-proximal serine-rich region (18, 22, 37, 38). The C-terminal RNA binding domain contains two clusters of arginine-rich sequences that may be involved in RNA binding. Iterative RNA selection in vitro demonstrated that a purine-rich sequence was the preferred substrate for the N-terminal RRM, whereas the C-terminal RNA binding domain showed little specificity (39). A feature of the RNAs selected for binding by the RRM was that they formed a stem-loop structure where a conserved Ala residue was typically part of a bulge in the structure. A poly(A)-rich sequence predominated when eIF4A was included during RNA selection although eIF4A alone did not exhibit any binding preference (39). Moreover, the C-terminal RNA binding domain did not alter the specificity of the RRM or its binding affinity (39). Deletion of the RNP-1 motif within the RRM or mutation of conserved residues within the RNP-1 substantially reduced RNA binding (37, 39). eIF4B also bound 18 S rRNA with high affinity and this binding could be competed by a purine-rich sequence, suggesting that the eIF4B RRM was responsible for the binding and may have bound a purine-rich sequence within the 18 S rRNA (39). Each RNA binding domain functions independently as eIF4B is able to bind two RNA molecules simultaneously (i.e. a purine-rich and nonspecific RNA) (39). Although this ability has been suggested to be the basis for its RNA annealing activity (39), those RNAs bound by eIF4B have not been identified and therefore the purpose of its preferential binding to purine-rich RNA is unknown.

Yeast eIF4B is significantly shorter at the C terminus (21, 40) and lacks the serine-rich domain that is present in mammalian eIF4B although no role has been identified for this domain. Like eIF4B in animals, yeast eIF4B contains an N-terminal RRM and it, together with the N-proximal three of the seven repeat motifs present in yeast eIF4B, were sufficient to complement the cold-sensitive and temperature-sensitive growth phenotypes of eIF4B null yeast, stimulate translation in vitro, and perform RNA strand-exchange (41). However, the C-terminal region and the seven repeat motifs also exhibited some RNA strand-exchange activity and conferred partial complementation of the growth phenotypes. Like mammalian eIF4B, a second region exhibiting RNA binding activity is present in the C-terminal two-thirds of yeast eIF4B, although it was unresolved whether the region containing the repeat motifs or the C-terminal domain was responsible for this (41). The yeast RRM did not bind to 18 S rRNA, 25 S rRNA, or poly(A)-containing RNA, demonstrating a significant difference with mammalian eIF4B (39, 41).

eIF4B from wheat was shown to bind poly(A) and poly(G) RNA (42), indicating that, like the animal and yeast orthologs, plant eIF4B is an RNA-binding protein. Based on the observations with animal and yeast eIF4B, plant eIF4B has been proposed to contain an N-terminal RRM (43) although this has not been experimentally verified. Only limited conservation is observed between eIF4B from wheat and other species: wheat eIF4B shares 29 and 24% identity with human and Saccharomyces cerevisiae eIF4B, respectively (43), making this factor one of the least conserved of the translation factors. The lack of sequence conservation, the apparent lack of interaction between eIF4B and PABP in yeast, and the difference in the degree to which eIF4B is essential for the ATPase and RNA helicase activities of eIF4A between plants and animals raised the question of the extent to which the domain organization of eIF4B was conserved among eukaryotes.

To increase the understanding of the role of eIF4B during translation initiation and investigate the extent to which the functional interactions of eIF4B have been maintained despite the poor conservation of its primary sequence, the domain organization of wheat eIF4B was examined. We observed that whereas plant eIF4B is similar to animal eIF4B in that it contains RNA binding domains, plant eIF4B contains one additional RNA binding domain not reported for other eukaryotic homologs. Each RNA binding domain in wheat eIF4B requires dimerization for RNA binding activity and each exhibits a preference for purine-rich sequence. A functional interaction of eIF4B with eIFiso4G and eIF4A was shown for the first time to involve a physical interaction with conserved sequence elements adjacent to the RNA binding domains. The eIFiso4G binding domain lies immediately C-proximal to the N-proximal RNA binding domain. Two binding sites for PABP and eIF4A were mapped to conserved domains on either side of the C-terminal RNA binding domain that represents two 41-amino acid repeats conserved among plant eIF4B proteins, and to a lesser degree, in human eIF4B. These results support the notion that eIF4B functions by organizing multiple components of the translation initiation machinery and RNA.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Protein Expression—pET3d-eIFiso4G, pET3d-eIF4B, and pET23d-eIF4A were generous gifts from Karen Browning (University of Texas, Austin, TX). pT7-Aso, has been described previously (44). Following PCR amplification of the appropriate region of the eIF4B coding region, the fragments were introduced into the EcoRI and BamHI sites of pGEX-2TK or into the Ndel or NdeI and XhoI sites of pET19b (Novagen) and the eIF4B polypeptides expressed as GST or Hisso-tagged fusions, respectively. For construction of eIF4B-(55–74) and eIF4B-(55–74(K33→A)), sense and antisense oligonucleotides representing the entire wild-type or mutant sequences were annealed and ligated to pGEX-2TK digested with BamHI/EcoRI. pET19b-PABP-(1–651) and pET19b-PABP-(1–393) were made by PCR amplification of the appropriate region and introduced into the BamHI and NdeI/BamHI sites, respectively, of pET19b. All constructs were confirmed by sequencing.

Protein expression was performed in Escherichia coli BL21 following induction with 1 mM isopropyl-β-D-galactopyranoside. Pelleted cells were sonicated in Buffer B-100 (20 mM...
HEPES, 100 mM KCl, 10% glycerol, 1 mM EDTA, 1 mM DTT) and following centrifugation to remove cell debris, crude cell extracts used for analysis or the His$_{10}$-tagged or GST-tagged fusion proteins were purified first using TALON metal affinity resin (Clontech) or glutathione-Sepharose 4B resin (Amersham Biosciences), respectively.

**RNA Binding Analysis**—Poly(A)–Sepharose 4B and poly(C)–, poly(G)–, or poly(U)-agarose (Sigma) were washed three times with Buffer B-100 (supplied with 5 mM DTT, 0.1% Triton X-100). eIF4B proteins were incubated with the resin at 4 °C for 1 h. The resin was washed four times with Buffer B-100 (with 5 mM DTT, 0.1% Triton X-100), re-suspended in SDS sample buffer, and heated. The supernatant was analyzed by SDS-PAGE and the gel stained with Coomassie or used for Western analysis. To determine the affinity of eIF4B for poly(A), poly(U), poly(C) or poly(G) RNA, binding was performed in Buffer B with either 100 or 150 mM KCl.

To test if eIF4B can bind two molecules of RNA simultaneously, purified His-tagged eIF4B was incubated first with poly(A) beads. Radiolabeled poly(A)$_{50}$ was then added and incubated for 2 h. The poly(A) resin was washed four times with Buffer B-40 (containing 40 mM KCl), re-suspended in SDS sample buffer, and the bound protein and poly(A)$_{50}$ RNA resolved on a 12% SDS-PAGE gel. Following electrophoresis, the gel was stained, de-stained, dried, and binding of radiolabeled poly(A) RNA detected by autoradiography. Radiolabeled (A)$_{50}$ RNA was made as previously described (44).

**Protein-Protein Interaction Assay**—GST fusion protein was added to glutathione-Sepharose 4B resin (Amersham Biosciences) washed three times with pre-cooled Buffer B-100 (supplied with 1 mM DTT, 0.1% Triton X-100). Following incubation with shaking at 4 °C for 1 h, the resin was collected by centrifugation and the supernatant removed. Prey protein was added to the resin and incubated for 2 h. The resin was washed four times with Tween-phosphate-buffered saline (TPBS: 0.1% Tween 20, 137 mM NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$, 1.4 mM KH$_2$PO$_4$) with 1 mM DTT. Bound protein was released following the addition of SDS sample buffer to the resin and boiling. Following centrifugation, the protein was resolved by SDS-PAGE and the gel stained or used for Western analysis.

For experiments testing for RNA tethering, micrococcal nuclease was added to the pulldown assay. The nuclease was added to the resin containing the bait protein and incubated in Buffer B-100 (without EDTA) at 30 °C for 1 h. The prey protein was then added and the reaction incubated at 4 °C for an additional 2 h. Identical conditions were used for control reactions in which only the nuclease was lacking.

**Western Analysis**—Protein was transferred to 0.22-mm polyvinylidene difluoride membrane by electroblotting. The membranes were blocked in 5% milk, 1% NaN$_3$ in TBPS followed by incubation with antiserum in TBPS with 1% milk for 1.5 h. Membranes were washed twice with TBPS and incubated with goat anti-rabbit horseradish peroxidase-conjugated antibodies (Southern Biotechnology Associates, Birmingham, AL) diluted from 1:5,000 to 1:10,000 for 1 h. The blots were washed twice with TBPS and the signal detected typically between 1 and 15 min using chemiluminescence (Pierce). His tag antiserum (Santa Cruz Biotechnology) was used as recommended.

**RESULTS**

eIFiso4F and eIF4A antisera (a generous gift of K. Browning) were used at 1:2000 dilution. eIF4B antiserum was used at 1:1000 dilution.

**eIF4B Contains Novel N-terminal and C-terminal RNA Binding Domains**—In previous work, wheat eIF4B was shown to bind poly(A) or poly(G) RNA specifically (42), suggesting that the protein contained at least one RNA binding domain that exhibited a preference for the purine-rich sequence. To identify the number, type, and organization of the eIF4B RNA binding domains and determine their RNA binding requirements, the cDNA encoding eIF4B was introduced into pGEX-2TK and the full-length GST–eIF4B fusion protein (i.e. GST–eIF4B–(1–527)) expressed in E. coli (Fig. 1, lane 1, top panel). GST–eIF4B–(1–527)-containing crude extract was incubated with poly(A)–Sepharose 4B resin, the resin was washed extensively, and bound protein analyzed by SDS-PAGE. GST–eIF4B–(1–527) bound to poly(A) RNA, whereas GST alone did not (Fig. 1, compare lane 1 to lane 9, bottom panel), in good agreement with our earlier observations using native wheat eIF4B (42). eIF4B truncated polypeptides, in which the N-terminal 44 and 68 residues were deleted (i.e. GST–eIF4B–(45–527) and GST–
Identification of RNA binding domains in wheat eIF4B. In A and B, eIF4B polypeptides were expressed as GST fusion proteins in E. coli (top panel) and RNA binding activity was determined by adding crude extract to poly(A)-Sepharose 4B resin. GST-eIF4B-(45–280), was included as a control. Bound proteins was detected by Coomassie staining. GST was employed as a negative control. The region of eIF4B included in each polypeptide is indicated numerically by the residues included. Mutation of four lysines to four alanines within the polypeptide containing residues 55 to 74 is indicated as 55–74(K→A). In C, expressed proteins (top panel) that bound poly(A) RNA were detected by Coomassie staining (middle panel) and Western analysis (bottom panel). In D, the RNA binding of GST-eIF4B-(45–280(K→A)), containing the region corresponding to the RRM of human and yeast eIF4B proteins and the lysine to alanine mutations (residues 58–61) to abolish the RNA binding activity of the N-terminal RNA binding domain, was by adding crude extract to poly(A)-Sepharose 4B resin (Fig. 1, lanes 4 and 5, bottom panel). However, deletion of an additional 20 residues (i.e. GST-eIF4B-(340–527)) abolished RNA binding (Fig. 1, lane 6, bottom panel), which was also observed for larger N-terminal deletions (e.g. GST-eIF4B-(358–527) and GST-eIF4B-(390–527)) (Fig. 1, lanes 7 and 8, bottom panel), suggesting that sequences involved in RNA binding were confined to approximately the region between residues 45 and 340. To delineate further the sequences involved in RNA binding, C-terminal deletions within GST-eIF4B-(45–527) were tested. Truncation to residue 390 (i.e. GST-eIF4B-(45–390)) did not affect RNA binding (Fig. 2A, lane 2, bottom panel) as did truncation to residues 360, 324, or 280 (i.e. GST-eIF4B-(45–360), GST-eIF4B-(45–324), or GST-eIF4B-(45–280), respectively) (Fig. 2A, lanes 3–5, bottom panel). Truncation to residue 76 (i.e. GST-eIF4B-(45–76)) also did not abolish RNA binding (Fig. 2A, lane 8, bottom panel) and residues 55 to 74 (i.e. GST-eIF4B-(55–74)) also exhibited RNA binding activity (Fig. 2A, lane 7, bottom panel). To delineate further the sequences involved in RNA binding, C-terminal deletions within GST-eIF4B-(45–527) were tested. Truncation to residue 390 (i.e. GST-eIF4B-(45–390)) did not affect RNA binding (Fig. 2A, lane 2, bottom panel) as did truncation to residues 360, 324, or 280 (i.e. GST-eIF4B-(45–360), GST-eIF4B-(45–324), or GST-eIF4B-(45–280), respectively) (Fig. 2A, lanes 3–5, bottom panel). Truncation to residue 76 (i.e. GST-eIF4B-(45–76)) also did not abolish RNA binding (Fig. 2A, lane 8, bottom panel) and residues 55 to 74 (i.e. GST-eIF4B-(55–74)) also exhibited RNA binding activity (Fig. 2A, lane 7, bottom panel), suggesting that an RNA binding domain was present within this 20-amino acid region. As this region contains five lysine residues between amino acids 56 and 61 (Fig. 3A) that may interact with the negative charge of the phosphates in RNA, the four lysines residues between amino acids 58 and 61 within GST-eIF4B-(55–74) were mutated to alanine residues. The resulting polypeptide (i.e. GST-eIF4B-(55–74(K→A)) exhibited no RNA binding activity (Fig. 2A, lane 6, bottom panel), demonstrating a requirement for the lysine residues in RNA binding. A lysine-rich sequence is also present in the corresponding location of Arabidopsis eIF4B1 and eIF4B2 and in a more N-proximal position in human and yeast eIF4B (Fig. 3A). In addition, an adjacent C-proximal sequence is conserved in plant and animal eIF4B and to a lesser degree in the yeast homolog (Fig. 3A).

The RNA binding exhibited by GST-eIF4B-(320–527) (Fig. 1, lane 5, bottom panel) implicated another RNA binding domain. To delineate this domain, the N-terminal RNA binding domain (and the RNP-2 of the RRM, see below) was deleted in the GST-eIF4B-(45–390) construct to result in GST-eIF4B-(69–390). As expected from the observations made in Fig. 1, this region exhibited RNA binding activity (Fig. 2B, lane 1, bottom panel). Further truncation to residue 360 (i.e. GST-eIF4B-(69–360)) did not prevent RNA binding although truncation to residue 324 (i.e. GST-eIF4B-(69–324)) reduced but did not eliminate RNA binding (Fig. 2B, lanes 2 and 3, respectively, bottom panel). Truncation to residue 280 (i.e. GST-eIF4B-(69–280)) abolished RNA binding altogether (Fig. 2B, lane 4, bottom panel).
suggesting that this second RNA binding domain required sequence C-terminal to residue 280. To map the C-terminal boundary of this domain more precisely, C-terminal truncations of GST-eIF4B-(69–324) were tested. In addition to Coomassie staining, Western analysis was performed to increase detection of the weak RNA binding activity. Truncation to residue 316 (i.e. GST-eIF4B-(69–316)) exhibited reduced RNA binding activity relative to GST-eIF4B-(69–324), which itself was impaired in RNA binding affinity (Fig. 2C, compare lane 2 to lane 1, bottom panel). Truncation to residue 310 (i.e. GST-eIF4B-(69–310)) exhibited just detectable RNA binding activity, whereas further deletion to amino acid 300 (i.e. GST-eIF4B-(69–300)) abolished RNA binding (Fig. 2C, lanes 3 and 4, respectively, bottom panel). These data suggest that the C-terminal RNA binding domain is present between residues 280 and 360 in which the region from 320 to 360 is particularly important. This region contains several basic residues conserved in eIF4B in higher eukaryotes (Fig. 3C).

Because mammalian and yeast eIF4B contain an RRM, GST-eIF4B-(45–280) containing the corresponding sequence from wheat eIF4B containing the RNP-1 and RNP-2 motifs typical of RRM domains (Fig. 3B) was examined for RNA binding activity. Because this region also contains the lysine motif-containing N-terminal RNA binding domain that is N-proximal to the putative RNP-2 motif, the lysines residues (residues 58–61) were mutated to alanine residues to inactivate the RNA binding activity of this domain and the resulting polypeptide (i.e. GST-eIF4B-(45–280(K3A))) tested. GST-eIF4B-(45–280(K3A)) exhibited RNA binding activity although with lower affinity than GST-eIF4B-(45–280) (Fig. 2D) demonstrating conservation of RNA binding activity within this region with other eukaryotic eIF4B proteins. The observation that GST-eIF4B-(69–280) did not exhibit RNA binding activity demonstrated the sequence N-proximal to residue 69 (Fig. 2B), which contains the putative RNP-2 motif, is required for the activity of this domain.

The RNA binding observed in Figs. 1 and 2 was observed with GST fusions that facilitate protein dimerization through the GST moiety (45). To examine whether dimerization was important for RNA binding, the RNA binding activity of His10-eIF4B, in which the tag did not promote dimerization, was examined. His10-eIF4B bound to poly(A) RNA, although less well than did GST-eIF4B even when the difference in input protein was taken into account (Fig. 4A, lanes 3 and 8, respec-
Domain Organization of eIF4B

![Diagram](image)

**FIGURE 4.** Activity of each RNA binding domain in wheat eIF4B requires dimerization. Full-length or eIF4B polypeptides were expressed as GST or His10-tagged fusion proteins in E. coli (input) and RNA binding activity was determined by binding to poly(A)-Sepharose 4B resin (RNA bound). In A, bound proteins were analyzed by SDS-PAGE and detected using Coomassie staining (lanes 1–12 and 15–18) or Western analysis (lanes 13 and 14). In B, bound proteins were analyzed by SDS-PAGE and detected using Coomassie staining (lanes 1–12) or Western analysis (lanes 13–18). The region of eIF4B included in each polypeptide is indicated numerically by the residues included.

Differently). Adding increasing amounts of GST-eIF4B to a constant amount of His10-eIF4B increased the RNA binding activity of His10-eIF4B (Fig. 4A, lanes 3–7), suggesting an interaction between the two tagged forms of eIF4B.

These results indicated that full-length wheat eIF4B may dimerize to some extent as has been reported for this protein (43) and for mammalian eIF4B (38). Therefore, the activity of GST-tagged eIF4B polypeptides containing each RNA binding domain was compared with His10-tagged versions of the same eIF4B polypeptides. GST-eIF4B-(45–280), in which the lysine-rich and RRM RNA binding domains are present, bound poly(A)-Sepharose (Fig. 4A, lanes 12 and 14, for Coomassie-stained gel and Western analysis, respectively) in good agreement with the data in Fig. 2. In contrast, His10-eIF4B-(45–280) bound poly(A)-Sepharose only poorly despite a higher input level of fusion protein used (Fig. 4A, lanes 11 and 13, for Coomassie-stained gel and Western analysis, respectively). Similar results were obtained when the binding activity of the RRM alone was tested following mutation of the lysine-rich region to alanines (Fig. 4A, lanes 17 and 18).

GST-eIF4B-(69–527), in which only the C-terminal RNA binding domain is present, bound poly(A)-Sepharose (Fig. 4B, lanes 7 and 13, for Coomassie-stained gel and Western analysis, respectively) but no binding was observed for His10-eIF4B-(69–527) (Fig. 4B, lanes 8 and 14, for Coomassie-stained gel and Western analysis, respectively). Similar results were observed when the binding of GST-eIF4B-(280–527) or GST-eIF4B-(69–360) was compared with that of His10-eIF4B-(280–527) or His10-eIF4B-(69–360) in which binding was observed when the RNA binding domain was present as a GST fusion but not as a His-tagged protein. These data suggest that the RNA binding domains of wheat eIF4B require dimerization for their activity.

To examine whether recombinant, full-length eIF4B or the individual RNA binding domains exhibited a preference for purine-rich RNA as observed previously for native wheat eIF4B (42), each was tested for their ability to bind ribonucleic acid homopolymers. GST-eIF4B-(1–527) exhibited a preference for poly(A) and poly(G) RNA although in the presence of 100 mM KCl, significant binding was observed to poly(U) and poly(C) RNA as well (Fig. 5A). Increasing the salt concentration to 150 mM KCl increased the binding specificity for poly(G) RNA (Fig. 5A). GST-eIF4B-(55–74) and GST-eIF4B-(45–280(K–A)) exhibited a strong preference for poly(A) and poly(G) RNA with very little binding to poly(U) and poly(C) RNA as observed previously for GST-eIF4B-(1–527) or native wheat eIF4B (42) in exhibiting a strong preference for purine-rich RNA, whereas the moderate preference for poly(G) exhibited by GST-eIF4B-(280–527) was more similar to recombinant eIF4B.

Because the binding activity of each RNA binding domain in wheat eIF4B required dimerization and three RNA binding domains are present in wheat eIF4B, the ability of eIF4B to bind two RNA molecules simultaneously was investigated as previously described (39). To examine this, His10-eIF4B-(1–527) was employed as it contains all three RNA binding domains and is able to bind poly(A)-Sepharose 4B resin. If dimerization of His10-eIF4B-(1–527) is required to bind poly(A)-Sepharose, this may be accomplished by intramolecular dimerization of two RNA binding domains within the same polypeptide or intermolecular dimerization of RNA binding domains between two polypeptides. His10-eIF4B-(1–527) was first bound through the His tag to the Co2+ resin and radiolabeled poly(A)50 RNA added, incubated, and the resin washed. Radiolabeled poly(A)50 RNA did not bind to the Co2+ resin in the absence of eIF4B (Fig. 5C, lane 2, top panel). Binding of radiolabeled poly(A)50 RNA, however, was observed in the presence of His10-eIF4B-(1–527) (Fig. 5C, lane 3, top panel) demonstrating...
the RNA binding activity of the protein. His\textsubscript{10}-eIF4B-(1–527) was then bound to poly(A)-Sepharose through the RNA binding domains. No binding of eIF4B to Sepharose 4B resin (i.e. in the absence of attached poly(A) RNA) was detected (data not shown), suggesting that the binding of eIF4B to poly(A)-Sepharose was mediated through the RNA binding domain. Following binding of His\textsubscript{10}-eIF4B-(1–527) to poly(A)-Sepharose, the resin was washed to remove unbound protein and radiolabeled poly(A)\textsubscript{50} RNA was added. Radiolabeled poly(A)\textsubscript{50} RNA bound to His\textsubscript{10}-eIF4B-(1–527) that itself was bound to poly(A)-Sepharose (Fig. 5C, lane 5, top panel) but the poly(A)\textsubscript{50} RNA did not bind poly(A)-Sepharose in the absence of His\textsubscript{10}-eIF4B-(1–527) (Fig. 5C, lane 4, top panel), demonstrating that eIF4B could bind two RNA molecules simultaneously.

Wheat eIF4A bound the poly(A) resin weakly but its presence in the poly(A) assay increased the RNA binding activity of GST-eIF4B-(280–527) (Fig. 5D, compare lanes 5–7 to 4). The stimulatory effect of eIF4A on eIF4B RNA binding activity is similar to that reported for the mammalian homologs (22).

**Domain Organization of eIF4B**

A

![Image](image1.png)

**B**

![Image](image2.png)

**C**

![Image](image3.png)

**D**

![Image](image4.png)

**FIGURE 5. Analysis of binding specificity of each RNA binding domain in wheat eIF4B.** In A and B, full-length eIF4B or the N-terminal (eIF4B\textsubscript{55–527}), RRM (eIF4B\textsubscript{520–527}), or C-terminal (eIF4B\textsubscript{280–527}) RNA binding domains expressed as GST fusion proteins in *E. coli* were tested for binding (RNA bound) to poly(A)-Sepharose 4B resin, or poly(U), poly(C), or poly(G)-agarose resins. Bound proteins were analyzed by SDS-PAGE and detected using Coomassie staining. In C, full-length eIF4B expressed as a His\textsubscript{10}-tagged fusion protein was bound to Co\textsuperscript{2+} or poly(A)-Sepharose 4B resin as indicated. Radiolabeled poly(A)\textsubscript{50} RNA was added to the resin in the presence or absence of bound eIF4B protein as indicated. Lane 1 represents the amount of radiolabeled poly(A)\textsubscript{50} RNA that was added to the binding reactions in lanes 2–5. Bound radiolabeled poly(A)\textsubscript{50} RNA was detected following resolution by PAGE and autoradiography. Bound eIF4B was detected by SDS-PAGE and detected using Coomassie staining. Note that a subunit of T7 RNA polymerase with similar molecular weight as eIF4B used to synthesize the poly(A)\textsubscript{50} RNA is present in this region of the gel (i.e. GST-eIF4B-(280–527)) to poly(A)-Sepharose 4B resin was tested in the presence or absence of eIF4A. GST-eIF4B-(280–527) and eIF4A were employed using eIF4B polypeptides and full-length His-tagged PABP (i.e. His\textsubscript{10}-PABP-(1–651)). GST-eIF4B-(1–527) was able to pull down His\textsubscript{10}-PABP-(1–651) (Fig. 6A, lane 1, middle panel) as it could pull down the N-terminal portion of PABP containing the four RRM domains (i.e. His\textsubscript{10}-PABP-(1–393)) (Fig. 6A, lane 1, bottom panel). N-terminal truncation of eIF4B up to residues 45, 69, 280, 320, or 340 did not abolish binding to His\textsubscript{10}-PABP-(1–651) (Fig. 6A, lanes 2–6, middle panel) or His\textsubscript{10}-PABP-(1–393) (Fig. 6A, lanes 2–6, bottom panel). However, truncation to residues 358 or 390, i.e. GST-eIF4B-(358–527) or GST-eIF4B-(390–527), respectively, abolished binding of either His\textsubscript{10}-PABP-(1–651) (Fig. 6A, lanes 7 or 8, middle panel) or His\textsubscript{10}-PABP-(1–393) (Fig. 6A, lanes 7 or 8, bottom panel), suggesting that the sequence N-proximal of residue 358 was required for binding to PABP. C-terminal truncation constructs, GST-eIF4B-(45–390), GST-eIF4B-(45–360), GST-eIF4B-(45–324), and GST-eIF4B-(45–280), bound full-length and His\textsubscript{10}-PABP-(1–393) (Fig. 6B). Similar to these results, GST-eIF4B-(69–390), GST-eIF4B-(69–360), and GST-eIF4B-(69–324) bound both forms of PABP but GST-eIF4B-(69–280) did not (Fig. 6C), suggesting that the sequence between 45 and 69 contained a PABP interaction domain. Although this possibility was supported by the observation that GST-eIF4B-(45–76) and GST-eIF4B-(55–74) could also bind PABP (Fig. 6D, lanes 1 and 2, middle and bottom panels), mutation of the four lysines to alanine that abolished RNA binding also abolished binding to full-length and His\textsubscript{10}-PABP-(1–393) (Fig. 6D, lane 3, middle and bottom panels), suggesting that the binding of PABP to this region is RNA-mediated as PABP is also a RNA-binding protein. This was supported by the observation that micro-
eIF4B Interacts Directly with eIF4A—eIF4B stimulates the ATPase and RNA helicase activity of eIF4A (15–29). Although eIF4B co-purifies with eIF4F (46–48), suggesting an interaction between it and one or more of the subunits in eIF4F, the subunit responsible for interacting with eIF4B has not been identified. A direct physical interaction between mammalian eIF4A and eIF4B using co-immunoprecipitation, yeast two-hybrid, or far Western failed (22). Moreover, despite an observed genetic interaction between eIF4B and eIF4A (40), no direct interaction has been reported for these proteins in yeast.

To examine whether eIF4B and eIF4A interact directly, GST-eIF4B-(1–527) was tested for its ability to bind full-length eIF4A. eIF4A bound to GST-eIF4B-(1–527) as it did to GST-eIF4B-(45–527), GST-eIF4B-(69–527), and GST-eIF4B-(280–527) (Fig. 8A, lanes 1–4, bottom panel). Substantially less binding was observed to GST-eIF4B-(320–527) and GST-eIF4B-(340–527) (Fig. 8A, lanes 5 and 6, bottom panel) and no binding was observed to GST-eIF4B-(358–527) or GST-eIF4B-(390–527) (Fig. 8A, lanes 7 or 8, bottom panel). As with PABP, eIF4A bound GST-eIF4B-(45–280) but not GST-eIF4B-(69–280) and bound to GST-eIF4B-(45–76) and GST-eIF4B-(55–74) (Fig. 8B, lanes 5–6 and 8–9, bottom panel). Binding to GST-eIF4B-(55–74) was not reduced by nuclease treatment (data not shown) but binding to GST-eIF4B-(55–74) was abolished when the four lysines were changed to alanine (Fig. 8B, lane 7, bottom panel), which also abolished RNA binding (Fig. 2A). This suggested that eIF4A binding to the region from residues 55 to 74 was likely RNA-mediated.

To delineate the C-terminal boundary of the remaining region implicated in eIF4A binding, GST-eIF4B-(69–390) was

The observation that the C-terminal half of eIF4B was sufficient to bind PABP (Fig. 6A) suggested a PABP interaction domain within this region. To delineate the C-terminal boundary, truncations of GST-eIF4B-(69–324) were tested in the pulldown assay. GST-eIF4B-(69–324) bound full-length and His10-PABP (Fig. 7A, lane 1, middle and bottom panels, respectively) as shown in Fig. 6C but truncation to residues 316 or 310 (i.e., GST-eIF4B-(69–316) or GST-eIF4B-(69–310), respectively) virtually abolished binding of full-length PABP (Fig. 7A, lanes 2 and 3, respectively, middle panel) and substantially reduced binding of His10-PABP-(1–393) (Fig. 7A, lanes 2 and 3, bottom panel, respectively). Deletion to residue 300 (i.e., GST-eIF4B-(69–300)) abolished binding to both forms of PABP (Fig. 7A, lane 4, middle and bottom panels, respectively). Because GST-eIF4B-(340–527) also demonstrated binding to PABP (Fig. 6A), further deletions to its N-terminal boundary were made. Both GST-eIF4B-(340–527) and GST-eIF4B-(355–527) bound full-length and His10-PABP-(1–393) (Fig. 7B, lanes 1 and 2, middle and bottom panels), whereas truncation to residue 358 (i.e., GST-eIF4B-(358–527)) virtually abolished binding to each form of PABP (Fig. 7B, lane 3, middle and bottom panels). Comparison of these two PABP binding regions revealed that they are part of a 41-amino acid repeat in wheat eIF4B that is also present in Arabidopsis eIF4B1 and eIF4B2 (Fig. 3D). Thus, the results from the deletion analysis of each PABP binding region (Fig. 7C) and their repeat nature (Fig. 3D) suggests that residues from 300 to 324 and 372 to 397 are critical for binding PABP.

coccal nuclease abolished the interaction between eIF4B-(55–74) and PABP (Fig. 6E).
used to eliminate the N-terminal RNA binding domain. GST-eIF4B-(69–390) bound eIF4A as did truncation to residues 360 or 324 (Fig. 8C, lanes 1–3, bottom panel). Note that the molecular weight of GST-eIF4B-(69–324) is approximately the same as eIF4A, which displaced the migration of the latter in the gel (Fig. 8C, lane 3, bottom panel). GST-eIF4B-(69–280) failed to bind eIF4A (Fig. 8C, lane 4, bottom panel), suggesting that the eIF4A binding domain involved a sequence between residues 280 and 324. Truncation of GST-eIF4B-(69–324) to residues 316, 310, or 300 did not impair eIF4A binding (Fig. 8D, lanes 1–4, bottom panel). Because GST-eIF4B-(340–527) also demonstrated binding to eIF4A, albeit with reduced affinity (Fig. 8A, lane 6, bottom panel), further N-terminal truncations were made. Both GST-eIF4B-(340–527) and GST-eIF4B-(355–527) bound eIF4A (Fig. 8E, lanes 1 and 2), whereas deletion to residue 358 abolished eIF4A binding (Fig. 8E, lane 3). These data indicate that sequences affecting eIF4A binding map to a region within the 41-amino acid repeat that also contains the PABP binding site (Fig. 3D). The results from the deletion analysis of each eIF4A binding region (Fig. 8F) and their repeat nature (Fig. 3D) suggests that residues 283–300 in the first repeat and residues 355–372 in the second repeat are important for binding eIF4A. The observation that eIF4A stimulates the RNA binding activity of eIF4B containing the two repeats (Fig. 5D) is consistent with the location of eIF4A binding sites in each repeat.

**eIF4B Interacts Directly with eIFiso4G**—In addition to eIF4G, plants contain an isoform, eIFiso4G, which differs in molecular mass (165 and 86 kDa, respectively) and shares only 30% identity with eIFiso4G (49, 50). eIF4G and eIFiso4G contain the central domain responsible for interaction with eIF4A and eIF3 (2, 5, 51, 52) and exhibit the highest level of conservation with animal and yeast eIF4G proteins (50).

The observation that wheat eIF4B binds directly to wheat eIF4A raised the possibility that eIF4B may bind directly to eIFiso4G, the subunit of eIFiso4F that contains the eIF4A binding site. To examine this, GST-eIF4B-(1–527) was tested for its ability to bind full-length eIFiso4G. eIFiso4G bound to GST-eIF4B-(1–527) (Fig. 9A, lane 1, bottom panel) but its binding to GST-eIF4B-(280–527) was reduced to a level similar to that observed for the GST control alone (Fig. 9A, compare lanes 2 to 7, bottom panel). eIFiso4G bound to GST-eIF4B-(45–280), GST-eIF4B-(55–280), and eIF4B-(55–74) above that observed for GST (Fig. 9A, lanes 3–5, bottom panel). The apparent binding of PABP and eIF4A to eIF4B-(55–74) was abolished when the four lysine residues were changed to alanine residues, suggesting that the binding was likely RNA-mediated. Therefore, the lysine to alanine mutant of eIF4B-(55–74) was tested for its ability to bind eIFiso4G. In contrast to the effect that this mutation had on the binding of PABP and eIF4A, eIF4G bound the eIF4B-(55–74(K→A)) mutant with nearly the same affinity...
as it did with eIF4B-(55–74) (Fig. 9A, compare lane 5 to 6, bottom panel), suggesting that the eIFiso4G binding site is within the sequence C-proximal to the lysine stretch. Binding to this mutant also demonstrated that the mutation of the lysines did not disrupt the structure of this domain.

DISCUSSION

We show here that wheat eIF4B contains three RNA binding domains, two binding sites for eIF4A and PABP, and one binding site for eIFiso4G (Fig. 10) that provides for the first time a physical basis for the known functional interactions between eIF4B and other components of the translational machinery. The organization of the RNA binding domains in wheat eIF4B is similar to mammalian eIF4B with one corresponding to the RRM and another in the central region of the protein. Interestingly, wheat eIF4B contains a third, N-terminal RNA binding domain that requires a lysine-rich region (i.e. residues 58–61) for activity (Fig. 3A). A lysine-rich region is also present in the corresponding region of Arabidopsis eIF4B1 and eIF4B2 (Fig. 3A). Within this same domain is a C-proximal sequence (i.e. \( \text{TLSLSEFT}^{1 \text{a}} \)) that is highly conserved among plant eIF4B proteins and contains the putative RNP-2 motif. Interestingly, a similar lysine-rich region with an adjacent C-proximal sequence is present in human eIF4B close to the N terminus (Fig. 3A). Moreover, the C-proximal motif in the N-terminal RNA binding domain of wheat eIF4B exhibits greater similarity to this adjacent C-proximal sequence of human eIF4B than it does with the RNP-2 motif of human eIF4B (Fig. 3, A and B). Whether the N-terminal lysine-rich region in human eIF4B possesses RNA binding activity is unknown. A lysine-rich region with a less conserved C-proximal sequence is also present in the N-terminal region of yeast eIF4B (Fig. 3A). The C-terminal RNA binding domain in wheat eIF4B between residues 280 and 360 in which residues from 320 to 423 in which residues from 385 to 423 are critical for binding activity (22). Although the wheat eIF4B C-terminal RNA binding domain shares homology with the corresponding region in Arabidopsis eIF4B1 and eIF4B2, it exhibits little conservation with the human C-terminal RNA binding domain (Fig. 3C) and of the residues that are conserved, most are basic amino acids previously suggested to be possible candidates for mediating RNA binding (22). An RNA binding domain is also
activity of each RNA binding domain was observed only when the domain was fused to GST, which is known to dimerize (45) and not when fused to a His tag, which does not promote dimerization. This suggested that dimerization of each RNA binding domain may be required for binding activity. This requirement for dimerization and the observation that His-tagged, full-length wheat eIF4B exhibits RNA binding activity supports the notion that wheat eIF4B may self-associate, consistent with the apparent dimerization reported for this protein (43). Mammalian eIF4B also undergoes dimerization facilitated by a DRYG domain (residues 164–356) rich in aspartic acid-arginine dipeptide repeats near glycine or tyrosine residues (38). Although the corresponding domain in wheat eIF4B is also rich in aspartic acid, arginine, and glycine residues (but only few tyrosine residues) (43), attempts to detect dimerization between His-tagged and GST-tagged wheat eIF4B were unsuccessful using either full-length or truncated polypeptides (data not shown), suggesting that dimerization of recombinant protein is weak at best. Lack of RNA binding activity exhibited by His\textsubscript{10}-eIF4B-(45–280) (Fig. 4A), which contains the domain corresponding to the DRYG dimerization domain of mammalian eIF4B, supported the conclusion that substantial dimerization of recombinant eIF4B does not occur in vitro. Subsequent work with wheat and *Arabidopsis* eIF4B suggested that the bulk of the protein is present as a monomer and that the initial conclusion that eIF4B is a dimer based on analysis by gel filtration could also be explained by an extended conformation of the protein. The observation that GST-tagged, full-length wheat eIF4B increased RNA binding activity of His-tagged, full-length wheat eIF4B (Fig. 4A), however, does support the notion that some degree of self-association does occur. We conclude, therefore, that if recombinant wheat eIF4B dimerizes, it may do so weakly or may require either post-translational modification or interaction with partner proteins for stronger self-association. It is also possible that weak dimerization is required for initial RNA binding, which is enhanced following RNA binding.

Dimerization was not required for the RNA binding activity of mammalian eIF4B although this was based on the failure of a truncated, dimerization-competent eIF4B polypeptide to exert a trans-dominant negative effect in the eIF4A RNA helicase assay and a truncated polypeptide may not be as competent for self-association as the full-length protein (38). The DRYG-rich domain of mammalian eIF4B, required for dimerization, is also responsible for binding the eIF3a subunit (38). The corresponding domain in *Arabidopsis* eIF4B interacts with eIF3g (53), the same eIF3 subunit that interacts with *S. cerevisiae* eIF4B (54). Therefore, although the function of the DRYG domain as the binding site for eIF3 has been conserved among eukaryotes, the eIF3 subunit interaction has not been conserved.

The presence of three RNA binding domains in eIF4B raised the possibility that the domains are required to bind one molecule of RNA. However, the ability of His\textsubscript{10}-eIF4B to bind two independent molecules of poly(A) RNA (Fig. 5C) did not support this possibility: if intramolecular dimerization of RNA binding domains within the same polypeptide was required to
bind poly(A)-Sepharose, it could not also bind a second molecule of poly(A) RNA, whereas intermolecular dimerization of RNA binding domains between two polypeptides would be expected to bind both.

Native wheat eIF4B binds poly(A) and poly(G) RNA but not poly(U) or poly(C) RNA (42). It also did not bind RNA of random sequence or RNA that was 50% adenosine residues (42). Recombinant eIF4B also exhibited a preference for purine-rich RNA but this was not exclusive as binding to poly(U) or poly(C) RNA was also observed, particularly under conditions of reduced stringency. When each RNA binding domain was tested separately, the binding characteristics of the N-terminal RNA binding domain and the RRM were similar to native wheat eIF4B in that they bound exclusively to poly(A) and poly(G) RNA, whereas the binding characteristics of the C-terminal RNA binding domain was similar to recombinant wheat eIF4B in that it bound to all four RNAs with a moderate preference for poly(G) RNA. Despite the suggestion that eIF4B may bind a purine-rich region of 18 S rRNA (39), the relevance of the binding specificity of eIF4B remains unknown. Although the binding specificity of the RRM was similar to the N-terminal RNA binding domain, its apparent affinity for RNA was lower, which may account for the presence of the third RNA binding domain in wheat eIF4B.

The yeast N-terminal RRM does not bind 18 S rRNA or poly(A)-containing RNA (41). The N-terminal RNA binding domain and the RRM of wheat eIF4B exhibit a preference for a purine-rich sequence that is similar to that of animal eIF4B but differs from yeast eIF4B in this respect. The reduced specificity of the yeast C-terminal RNA binding domain is similar to the nonspecific binding observed for the C-terminal RNA binding domain of mammalian eIF4B although it did exhibit a preference for the purine-rich sequence. These results are consistent with our previous observations concerning the RNA binding specificity of wheat eIF4B (42).

In addition to binding RNA, the region containing the N-terminal RNA binding domain (i.e. residues 55–74) also contains the eIFiso4G binding site. The eIFiso4G binding site may not overlap with the site for RNA binding as mutation of the four lysines within this domain (residues 58–61) that abolished RNA binding did not affect eIFiso4G binding, data indicating that the eIFiso4G binding site may reside between residues 62 and 74, which contains a sequence element (i.e. $^{64}$TLSLSEFT$^{71}$) conserved among plant and animal eIF4B (Fig. 3A). The observation that eIF4B-69–280) virtually failed to bind eIFiso4G suggested that the eIFiso4G binding site involves the region N-proximal to residue 69. The $^{64}$TLSLSEFT$^{71}$ element contains the putative RNP-2 motif, which is more conserved to an N-terminal sequence element in human and yeast eIF4B than it is to the RNP-2 motif in these homologs (Fig. 3, A and B).

An interaction between eIF4B and eIF4G has not been reported in other eukaryotes. Although the interaction between wheat eIF4B and eIFiso4G was observed using recombinant proteins, which demonstrated that the interaction is direct, the multiple interactions among several of the factors, e.g. eIF4B-eIF4A, eIF4A-eIF4G, eIF4B-PABP, PABP-eIF4G, eIF4B-eIF3, and eIF3-eIF4G in addition to the interaction between eIF4B and the eIF4G isoform, precluded confirmation of this direct interaction using crude wheat cell extracts. To reduce the possibility of a nonspecific interaction, however, crude bacterial extracts containing the recombinant proteins were used for the interaction analysis to increase the complexity of the proteins present in the binding assay. Moreover, the delineation of the eIFiso4G binding site within eIF4B, in addition to the observed interaction between full-length eIFiso4G and eIF4B, provides additional evidence supporting this interaction.

The N-terminal 80-amino acid region of mammalian eIF4B was sufficient to bind PABP and removal of the N-terminal 45 amino acids following caspase-3 cleavage abolished PABP binding (9). This N-terminal region of eIF4B was reported to interact with the PABP C-terminal domain (9) but a sequence in eIF4G similar to eIF4B interacts with the N-terminal PABP RRM and not with the PABP C-terminal domain, an apparent conflict. PABP binding to the N terminus of wheat eIF4B was observed but likely was RNA-mediated as mutations abolishing RNA binding activity of the N-terminal domain or nuclease treatment abolished PABP binding. The N terminus of eIF4B is the least conserved region of the protein in plants and animals that may account for the observed difference in PABP binding. Instead of binding to the N terminus, two PABP interaction domains were mapped to either side of the C-terminal RNA binding domain in wheat eIF4B. The binding sites mapped to regions within two 41-amino acid repeats in wheat eIF4B that are conserved in Arabidopsis eIF4B1 and eIF4B2 (Fig. 3D). The corresponding region in human eIF4B shows only a limited repeat nature (21% similar) and little conservation with the repeats in plant eIF4B proteins that may account for the difference in PABP binding in plant and animal eIF4B.

N-proximal to each PABP binding site is an eIF4A binding site that is conserved in wheat and Arabidopsis eIF4B proteins (Fig. 3D). An interaction between eIF4B and eIF4A is consistent with the observation that wheat eIF4B stimulates the ATP hydrolysis and RNA helicase activities of eIF4A (26–29). Interestingly, wheat eIF4A increased the RNA binding activity of the
C-terminal region of eIF4B (i.e. residues 280–527) that contains the two eIF4A binding sites and the C-terminal RNA binding domain, suggesting that binding of eIF4A to these sites functions to enhance the activity of the C-terminal RNA binding domain. A direct interaction between eIF4B and eIF4A has not been reported in animals despite the observation that eIF4B is essential to stimulate the ATPase and RNA helicase activity of eIF4A (15–23). Those residues within the repeats in human eIF4B that are conserved with plant eIF4B, however, are largely localized to the eIF4B binding region. Binding of eIF4A to the corresponding region in human eIF4B would be consistent with the observation that the C-terminal half of mammalian eIF4B enhances the eIF4A RNA helicase activity (22). Moreover, eIF4A also stimulated the RNA binding activity of the C-terminal RNA binding domain of mammalian eIF4B and those eIF4B sequences required for the eIF4A enhancement included the repeat corresponding to the N-terminal eIF4A binding site (22), suggesting that mammalian eIF4A may interact with eIF4B in the region corresponding to the eIF4A binding sites of wheat eIF4B.

The position of an eIF4A binding site on either side of the C-terminal RNA binding domain supports the notion that their proximity to this RNA binding domain is necessary for the observed stimulation of eIF4B RNA binding activity mediated by eIF4A. The binding of eIF4A proximal to PABP also suggests a possible functional interaction. The presence of PABP increases the ATPase and the RNA helicase activity of the eIF4F-eIF4A-eIF4B complex in plants (30). Thus, the proximity of PABP to eIF4A when bound to eIF4B may enable PABP to interact directly with eIF4A and facilitate its activities.

The importance of eIF4G in translation is in the number of its interactions with other components of the translation initiation machinery and is thought to function as a scaffold protein that assembles eIF4E, eIF4A, PABP, and eIF3 to facilitate initiation. eIF4B, like eIF4G, interacts with eIF4A, PABP, and eIF3. Both bind RNA and eIF4B and eIFiso4G interact with each other. Neither have been observed to contain catalytic activity. Whether eIF4B interacts with RNA and its partner proteins simultaneously or sequentially is unknown. However, such extensive interactions suggests an organizing role for this factor during translation initiation. Thus, the function of eIF4B may be similar to eIF4G to the extent that its role may be primarily to aid the assembly of its partner proteins onto mRNA, thereby increasing their function.

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Acknowledgment—We thank Dr. Karen Browning for pET3d-C-terminal region of eIF4B (i.e. residues 280–527) that contains the two eIF4A binding sites and the C-terminal RNA binding domain, suggesting that binding of eIF4A to these sites functions to enhance the activity of the C-terminal RNA binding domain. A direct interaction between eIF4B and eIF4A has not been reported in animals despite the observation that eIF4B is essential to stimulate the ATPase and RNA helicase activity of eIF4A (15–23). Those residues within the repeats in human eIF4B that are conserved with plant eIF4B, however, are largely localized to the eIF4B binding region. Binding of eIF4A to the corresponding region in human eIF4B would be consistent with the observation that the C-terminal half of mammalian eIF4B enhances the eIF4A RNA helicase activity (22). Moreover, eIF4A also stimulated the RNA binding activity of the C-terminal RNA binding domain of mammalian eIF4B and those eIF4B sequences required for the eIF4A enhancement included the repeat corresponding to the N-terminal eIF4A binding site (22), suggesting that mammalian eIF4A may interact with eIF4B in the region corresponding to the eIF4A binding sites of wheat eIF4B.

The position of an eIF4A binding site on either side of the C-terminal RNA binding domain supports the notion that their proximity to this RNA binding domain is necessary for the observed stimulation of eIF4B RNA binding activity mediated by eIF4A. The binding of eIF4A proximal to PABP also suggests a possible functional interaction. The presence of PABP increases the ATPase and the RNA helicase activity of the eIF4F-eIF4A-eIF4B complex in plants (30). Thus, the proximity of PABP to eIF4A when bound to eIF4B may enable PABP to interact directly with eIF4A and facilitate its activities.

The importance of eIF4G in translation is in the number of its interactions with other components of the translation initiation machinery and is thought to function as a scaffold protein that assembles eIF4E, eIF4A, PABP, and eIF3 to facilitate initiation. eIF4B, like eIF4G, interacts with eIF4A, PABP, and eIF3. Both bind RNA and eIF4B and eIFiso4G interact with each other. Neither have been observed to contain catalytic activity. Whether eIF4B interacts with RNA and its partner proteins simultaneously or sequentially is unknown. However, such extensive interactions suggests an organizing role for this factor during translation initiation. Thus, the function of eIF4B may be similar to eIF4G to the extent that its role may be primarily to aid the assembly of its partner proteins onto mRNA, thereby increasing their function.
Domain Organization of eIF4B

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