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# The role of the poly(A) binding protein in the assembly of the Cap-binding complex during translation initiation in plants

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**Keywords:** eIF4G, eIF4B, eIF4A, eIF4E, initiation factors, poly(A) binding protein, translation initiation

**Abbreviations:** eIF, eukaryotic initiation factors; HEAT, Huntington, Elongation Factor 3, PR65/A, TOR ; PABP, poly(A) binding protein; RRM, RNA recognition motif; 5′-UTR, 5′-untranslated region

Translation initiation in eukaryotes requires the involvement of multiple initiation factors (eIFs) that facilitate the binding of the 40 S ribosomal subunit to an mRNA and assemble the 80 S ribosome at the correct initiation codon. eIF4F, composed of eIF4E, eIF4A, and eIF4G, binds to the 5′-cap structure of an mRNA and prepares an mRNA for recruitment of a 40 S subunit. eIF4B promotes the ATP-dependent RNA helicase activity of eIF4A and eIF4F needed to unwind secondary structure present in a 5′-leader that would otherwise impede scanning of the 40 S subunit during initiation. The poly(A) binding protein (PABP), which binds the poly(A) tail, interacts with eIF4G and eIF4B to promote circularization of an mRNA and stimulates translation by promoting 40 S subunit recruitment. Thus, these factors serve essential functions in the early steps of protein synthesis. Their assembly and function requires multiple interactions that are competitive in nature and determine the nature of interactions between the termini of an mRNA. In this review, the domain organization and partner protein interactions are presented for the factors in plants which share similarities with those in animals and yeast but differ in several important respects. The functional consequences of their interactions on factor activity are also discussed.

## Introduction

Protein synthesis involves 3 phases: initiation, elongation, and termination. Because the 18 S rRNA of the eukaryotic 40 S ribosomal subunit lacks the region corresponding to the Shine-Dalgarno sequence of the bacterial 16 S rRNA that enables the latter to bind near an initiation codon in a prokaryotic mRNA, the recruitment of the 40 S subunit depends on the action of multiple initiation factors. These factors facilitate 40 S subunit binding at the 5′-cap structure ($m^7$GpppN) of an mRNA and assist in the 5′ to 3′ directional scanning of the 5′-leader to identify the correct initiation codon. Recruitment of the 40 S subunit requires the functions of the cap-binding complex, a group of initiation factors that include eIF4E, which binds to the 5′-cap structure; eIF4A, a DEAD/H-box, ATP-dependent, RNA helicase that uses energy from ATP hydrolysis to unwind secondary structure in the 5′-leader that could inhibit 40 S subunit scanning; and eIF4G, a modular scaffolding protein.[1-4] Together, these 3 factors are known as eIF4F. eIF4G stimulates the ATPase activity of eIF4A[5] as does eIF4B which is also associated the cap-binding complex.[6] Other factors associated with the cap-binding complex include the poly(A) binding protein (PABP), which is bound to the poly(A) tail, and eIF3, which through its interaction with eIF4G and the 40 S subunit, recruits the latter to the mRNA. Thus, the interactions among these factors are essential for their assembly at the 5′-cap and for the translation of most cellular mRNAs. In this review, the interactions among eIF4E, eIF4G, eIF4A, eIF4B, and PABP and the functional consequences of their interactions in plants are examined in detail and are compared with those in animals and yeast.

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## The Scaffolding Protein eIF4G Organizes the Assembly of the Cap-Binding Factors

### Plants express a novel eIF4G isoform not found in other eukaryotes

The scaffolding function of eIF4G is central to the cap-binding complex in that, through its interactions with most of the other components, it maintains the integrity of the complex. eIF4G is the largest subunit of eIF4F and 2 similar isoforms are expressed in most eukaryotes. For example, in animals and yeast, the 2 eIF4G proteins are expressed from distinct genes but are similar in mass and sequence[7,8] whereas the 2 isoforms in plants, referred to as eIF4G and eIFiso4G, differ substantially in mass and sequence.[9] eIFiso4G in wheat is only 30% identical to eIF4G from the same species[9] compared with 53% identity between the 2 isoforms in yeast and 46% identity between the 2 isoforms in humans.[7,8] At 86 kDa, eIFiso4G is substantially smaller than eIF4G in plants or other eukaryotes but, like plant eIF4G, it contains 2 HEAT domains that are composed of

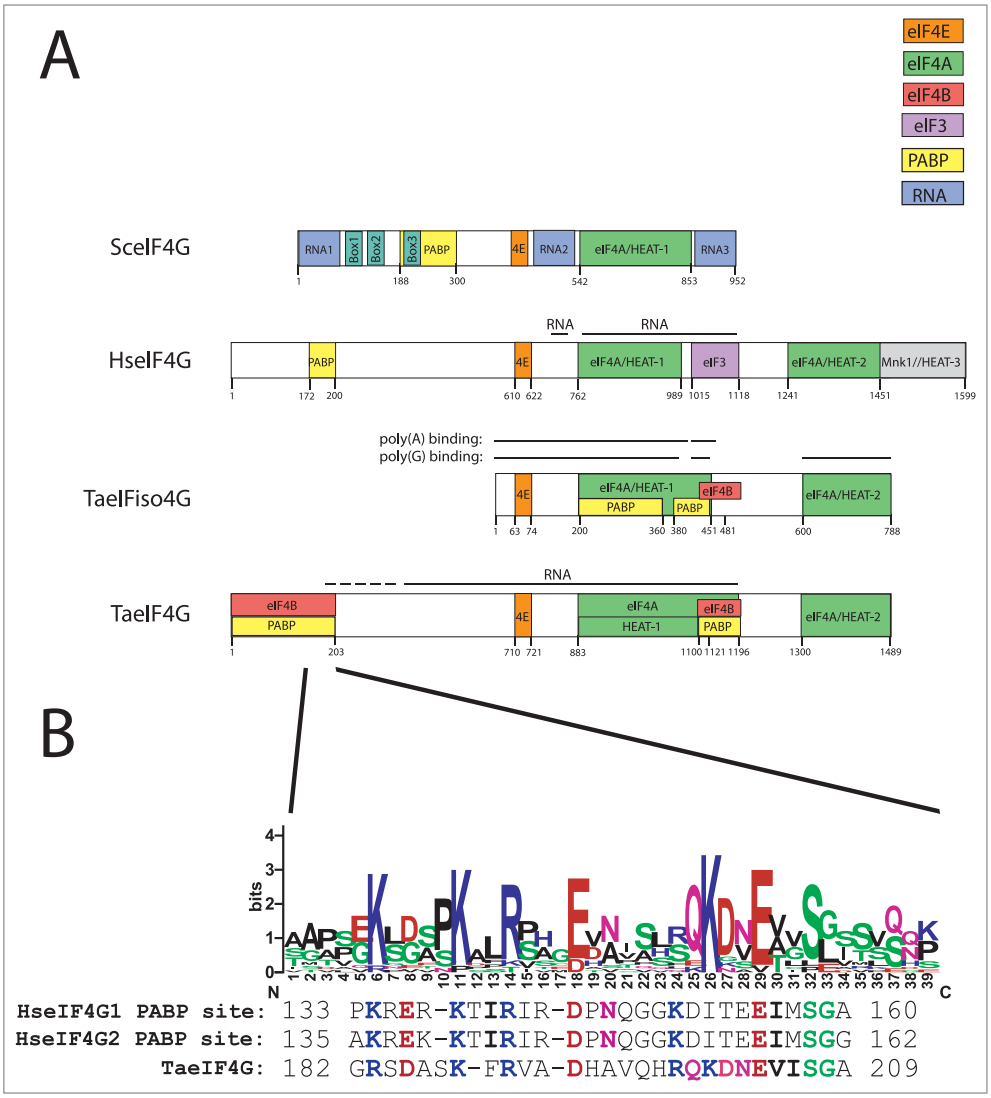


**Figure 1.** Partner protein interactions of eIF4G in higher eukaryotes. (**A**) The RNA and protein binding domains in wheat eIF4G (TaeIF4G), eIFiso4G (TaeIFiso4G), human eIF4G (HseIF4G), and yeast eIF4G (ScIF4G) are shown. Interaction domains for partner proteins are indicated by color with a key included. Domains for yeast eIF4G are defined as described previously.[37] (**B**) Below the N-terminal PABP binding site of wheat eIF4G is a sequence logo for the region corresponding to the PABP interaction site in human eIF4G. For this analysis, a sequence alignment from 6 monocot and 7 dicot eIF4G homologs was generated using EBI MUSCLE. The aligned sequences were submitted to WebLogo Berkeley to generate a sequence logo, in which the most conserved one, 2, or sometimes 3 residues at each position are shown as the consensus. Below the protein sequence logo are the sequences of the PABP interaction sites for human eIF4G1 and eIF4G2 as well as the corresponding sequence for wheat eIF4G. In each case, residues conserved with the consensus sequence are colored as in the sequence logo. The logo was constructed from eIF4G homologs from Triticum aestivum (wheat, JN091779), Brachypodium distachyon (Bd1g25002), Oryza sativa (Os07 g36940), Setaria italica (Si028648), Panicum virgatum (Pv00019592 and Pv00063738), Phaseolus vulgaris (010G043700), Ricinus communis (29709), Brassica rapa Chiifu-401 (Bra014505), Thellungiella halophila (Thhalv10005736), Capsella rubella (Cr10016570), Arabidopsis thaliana (AEE80028), and Arabidopsis lyrata (939058).

antiparallel α-helical hairpins known as HEAT repeats[10,11] and these account for most of the shared similarity between the 2 plant isoforms (**Fig. 1A**). The eIF4E interaction domain lies N-proximal to the first of these HEAT domains and its position as well as its sequence is conserved among animal, plant, and yeast eIF4G proteins. Together, eIF4E and eIF4G comprise plant eIF4F while eIFiso4G and eIFiso4E (the isoform of eIF4E in plants) comprise eIFiso4F.

Although all eIF4G proteins contain one or more HEAT domains, they differ in number. Animal eIF4G proteins contain 3 HEAT domains, yeast eIF4G proteins contain one, while, as mentioned, plant eIF4G and eIFiso4G contain 2. The HEAT-1l/MIF4G domain, which contains 5 HEAT repeats in a helical stack, is present in animal, plant, and yeast eIF4G[10] whereas the HEAT-2/MA3 domain, also composed of 5 HEAT repeats,[11] is found in animal and plant eIF4G.[12,13] The HEAT-3 domain, composed of 3 and one half HEAT repeats, is present only in animal eIF4G and functions to bind Mnk eIF4E kinases 1/2.[11]

eIF4A binds the first 2 HEAT domains of animal eIF4G as well as the region between these domains but each HEAT domain contacts separate surfaces of eIF4A in order to contribute to its stable binding.[14] Binding to HEAT-1 promotes eIF4A helicase activity while HEAT-2 serves a modulatory role.[6,15-20] The binding of eIF4A to the HEAT domains positions the 2 eIF4A domains containing the ATP- and RNA-binding sites in a partially closed state that facilitates interaction with substrates and the release of reaction products.[19,21,22] Binding of eIF4E to human eIF4G also stimulates eIF4A helicase activity by overcoming the autoinhibitory function of the eIF4E-binding site in eIF4G and the ability of eIF4E to do so is independent of its cap-binding function.[23] As yeast eIF4G lacks the HEAT-2 domain, eIF4A must bind through the HEAT-1 domain alone which eIF4B may facilitate.[24] As the HEAT-1 and HEAT-2 domains of plant eIF4G and eIFiso4G bind eIF4A, they are more similar to the animal homologs than to yeast.[12,13] However, like yeast eIF4G, plant eIF4G and eIFiso4G lack the HEAT-3 domain.

Although eIF4A interacts with the eIFiso4G HEAT-1 domain, its binding is abolished if the N-terminal 30–40 residues

of HEAT-1 are deleted.[12] Moreover, the N-terminal region of the HEAT-1 domain is sufficient to support interaction with eIF4A, suggesting that this region is essential for interaction with eIF4A while the remainder of the HEAT-1 domain serves to stabilize eIF4A binding.[12] A 7 amino acid sequence, i.e., ILNKLTP, within this region is important for eIF4A binding as mutations within the sequence abolish eIF4A binding and the asparagine, lysine, and threonine residues in this highly conserved element make direct contacts with specific residues in eIF4A.[16,19,25] Although eIF4A binds the HEAT-1 and HEAT-2 domains of plant eIFiso4G and eIF4G, they differ in that the eIFiso4G HEAT-1 domain is sufficient to bind eIF4A whereas a short region immediately C-proximal to the HEAT-1 domain of eIF4G is needed in addition to the HEAT-1 domain,[12,13] consistent with observations made with animal eIF4G in which the HEAT-1 domain alone bound eIF4A with lower affinity than did a longer region of eIF4G.[26]

In animal eIF4G, eIF3 binds to a 90 amino acid region just C-proximal to the HEAT-1 domain through its c, d, and e subunits[27,28] which was reported to bind in a cooperative manner with eIF4A[29] although this was not confirmed in a subsequent study.[28] Whether eIF3 binds to the corresponding region in plant eIF4G or eIFiso4G remains to be determined.

eIF1 and eIF5 bind yeast eIF4G within an arginine and serine rich domain that lies just N-proximal to the HEAT-1 domain and their interaction with eIF4G is competitive.[30,31] eIF1 interacts with eIF3 and is involved in AUG selection whereas eIF5 interacts with eIF2 and eIF3 and promotes their binding to a 40 S subunit as well as their function.[32] Whether these factors affect eIF4A binding to the HEAT-1 domain of eIF4G is unknown and whether these factors interact directly with plant and animal eIF4G remains to be determined.

**eIF4G and eIFiso4G bind PABP and eIF4B in distinct ways**

An interaction domain for PABP lies N-proximal to the eIF4E interaction domain in eIF4G and the position of this interaction domain is conserved in animals, plants, and yeast (**Fig. 1A**).[13,17,33-36] The N-terminal PABP interaction domain in wheat eIF4G is contained within the first 200 amino acids.[13] In human eIF4G1 and eIF4GII, the 2 isoforms present in this species, the PABP interaction domain lies within a discrete 28–29 amino acid region in the N-terminal portion of each protein.[17] To identify the positions that might be conserved among plant eIF4G orthologs in the corresponding region, a graphical logo showing the conservation of sequence at each residue was generated from a sequence alignment of the corresponding region from eIF4G orthologs of 6 monocot and 7 dicot species (**Fig. 1B**). Several residues were observed to be nearly invariant throughout the region including several basic and acidic residues. Comparing the PABP interaction domain of human eIF4G1 and eIF4GII as well as the corresponding region from wheat eIF4G shows that these exhibit comparable similarity to the consensus sequence (**Fig. 1B**). Deletion of this region in wheat eIF4G results in loss of PABP binding,[13] suggesting that this region exhibiting conservation with animal eIF4G homologs is important for interaction with PABP. Although specific residues within this region required for PABP binding have not been identified for either animal or plant eIF4G, the conservation suggests that some of these residues may be functionally important.

The presence of an N-terminal RNA-binding domain (referred to as RNA1) in yeast eIF4G1 can functionally substitute for the adjacent PABP-binding domain as can a region between RNA1 and the PABP-binding domain which contains 2 elements, Box1 and Box2 (**Fig. 1A**).[37] RNA1 and Box1 assist in the direct binding of PABP to eIF4G1 while RNA1, Box1, Box2, and the PABP-binding domain all contribute to the formation of the eIF4G:PABP:mRNA complex.[37] These data suggest that the interaction of PABP with eIF4G is only one of several interactions that stabilizes eIF4G binding to an mRNA, at least in yeast. These findings support the earlier observation that the interaction between eIF4G and PABP in yeast is RNA-dependent[33] as the RNA-binding domain RNA1 assists the interaction between PABP and eIF4G.[37] Whether functionally analogous sequence elements to RNA1, Box1, and Box2 identified in yeast eIF4G1 are present in plant or animal eIF4G is unknown although no obvious sequences exhibiting conservation with these yeast elements are apparent.

Although only one PABP interaction domain has been identified in animal and yeast eIF4G proteins, a second binding domain is present in plant eIF4G which lies C-proximal to its HEAT-1 domain (**Fig. 1A**)[13] which represents a significant difference among these homologs. The observation that the interaction between eIF4G and PABP is RNA-dependent in yeast[33] but not in plants[13,34,38] might indicate that the PABP-eIF4G interaction mediated by 2 domains obviates the need for RNA to stabilize the interaction. That RNA is not required for PABP binding to eIF4G in animals[17] as well as in plants suggests that the interaction between eIF4G and PABP in higher eukaryotes may differ fundamentally from that in yeast. However, whether a second PABP binding site is present in animal eIF4G is a question worth re-examining in light of the findings with plant eIF4G.

eIFiso4G differs from eIF4G in plants in that it lacks most of the region that lies N-terminal of the HEAT-1 domain (**Fig. 1A**). Therefore, the eIFiso4E interaction site lies closer to the eIFiso4G N-terminus than does the eIF4E interaction site in eIF4G and the N-terminal PABP interaction domain present in eIF4G is absent in eIFiso4G. Indeed, as eIFiso4G lacks any sequence analogous to the N-terminal region of eIF4G of plant, animal, or yeast eIF4G proteins, there is no possibility of even functionally analogous sequences for the RNA1, Box1, and Box2 elements of yeast eIF4G1.[37] Instead, PABP binds eIFiso4G within the HEAT-1 domain at 2 contiguous sites that overlap extensively with the eIF4A binding site in this same domain (**Fig. 1A**).[12] Because the 2 PABP binding sites within the HEAT-1 domain are contiguous, they may be considered as 2 subregions composing a single PABP interaction domain. As the PABP binding site in HEAT-1 lies C-proximal to the eIF4E binding site, eIFiso4G differs substantially from animal and yeast eIF4G where PABP binds to the N-proximal side of the eIF4E binding domain. eIFiso4G differs also from plant eIF4G in that it effectively has one PABP interaction domain in contrast to the 2 present in plant eIF4G (**Fig. 1A**).[12]

Despite its role in promoting eIF4A function, there has been no report of a direct interaction between eIF4B and eIF4G in animals or yeast although eIF4B is associated with the cap-binding complex.[24,26] In contrast, an eIF4B interaction domain was identified in the N-terminal region of plant eIF4G which maps to the same region responsible for binding PABP (**Fig. 1A**).[13] Similarly a second eIF4B binding site lies C-proximal to the HEAT-1 domain of plant eIF4G that extensively overlaps with the PABP binding site in this same region (**Fig. 1A**).[13] Interestingly, eIF4H, a close homolog of eIF4B, partially displaces eIF4A from animal eIF4G that is bound to a region corresponding to the eIF4B binding site in plant eIF4G.[14] As with PABP, eIF4B also binds eIFiso4G at a single site but it differs from PABP in that its binding domain overlaps just the C-terminal end of the HEAT-1 domain (**Fig. 1A**).[12] The eIF4B binding domain also overlaps the C-terminal end of the PABP interaction domain in eIFiso4G.

### eIF4B and PABP compete for binding plant eIF4G and eIFiso4G

Because eIF4B and PABP bind to 2 similar regions in plant eIF4G and to a similar region in eIFiso4G (**Fig. 1A**), it was possible that they bind either cooperatively or competitively to each region. Assays to determine the nature of their binding revealed that eIF4B and PABP bind competitively to each region of wheat eIF4G and eIFiso4G,[12,13] suggesting that the binding of PABP and eIF4B to these sites is mutually exclusive. When PABP and either region of eIF4G were present in equal molar amounts in a competition assay, eIF4B was able to compete with PABP noticeably at substoichiometric molar ratios, suggesting that eIF4B binds wheat eIF4G at each site more strongly than does PABP. Because eIF4B competed with PABP at a lower molar ratio at the HEAT-1-proximal eIF4B/PABP interaction site than at the N-proximal eIF4B/PABP interaction site, it likely binds relatively more strongly than PABP to the HEAT-1-proximal site than it does to the N-terminal site. These findings suggest that the binding of PABP and eIF4B to each of the 2 sites in eIF4G is mutually exclusive but this does not exclude the possibility that PABP and eIF4B could bind simultaneously to eIF4G (**Fig. 2**).

The binding sites for PABP and eIF4B overlap extensively in eIFiso4G (**Fig. 1A**) and, as with eIF4G, PABP and eIF4B compete for binding eIFiso4G.[12] Because eIFiso4G differs from eIF4G in that it lacks the region corresponding to the N-terminal binding site for PABP or eIF4B, the competition between PABP and eIF4B suggests their binding to eIFiso4G is mutually exclusive (**Fig. 2**) and they would not be expected to bind the same molecule of eIFiso4G. This has significant implications for the interactions between the 5′ and 3′ ends of an mRNA as discussed below.

### eIF4A does not compete with either eIF4B or PABP for binding plant eIF4G and eIFiso4G

Because the coincident eIF4B and PABP interaction domains in plant eIF4G result in their competitive binding to eIF4G, the observation that the HEAT-1-proximal eIF4B/PABP binding sites partially overlap with the eIF4A binding domain that includes the eIF4G HEAT-1 domain raised the possibility that eIF4B and/or PABP might bind cooperatively or competitively with eIF4A to this region of eIF4G. Binding assays revealed, however, that neither eIF4B or PABP had any effect, either cooperatively or competitively, on eIF4A binding to the HEAT-1 domain,[13] suggesting that the eIF4B or PABP binding sites do not overlap sufficiently with the eIF4A interaction domain to prevent eIF4A binding. Similarly, the presence of eIF4A had no detectable effect on the binding of eIF4B or PABP to the HEAT-1-containing region of plant eIF4G. These observations indicate that eIF4A and either eIF4B or PABP can bind plant eIF4G simultaneously but that eIF4B and PABP compete to bind each of the 2 sites in eIF4G. In animals, the binding of eIF4G and eIF4B to eIF4A may be mutually exclusive[26] but this has not been examined for plants.

Because of the extensive overlap between the PABP and eIF4A binding sites in the wheat eIFiso4G HEAT-1 domain, PABP competes with eIF4A for binding to this domain in the absence of the HEAT-2 domain.[12] However, when the region of eIFiso4G containing the HEAT-1 and HEAT-2 domains is tested, PABP no longer competes with eIF4A for binding to eIFiso4G.[12] Thus, in the absence of the HEAT-2 domain, binding of eIF4A to the eIFiso4G HEAT-1 domain may be weaker and more likely to be displaced by PABP. The presence of the HEAT-2 domain, which also binds eIF4A, likely stabilizes the binding of eIF4A while accommodating binding of PABP to eIFiso4G.

The eIF4B binding site in eIFiso4G overlaps just the C-terminal end of the HEAT-1 domain[12] (**Fig. 1A**). Nevertheless, as with PABP, eIF4B competes with eIF4A for

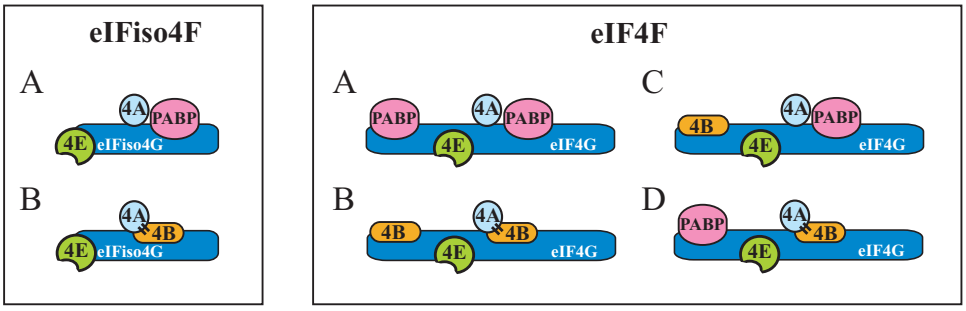


**Figure 2.** Mutually exclusive binding of PABP and eIF4B to eIFiso4G and eIF4G in plants. For eIFiso4F: (**A**) binding of PABP or (**B**) binding of eIF4B is depicted. For eIF4F: (**A**) binding of PABP to both PABP binding sites, (**B**) binding of eIF4B to both eIF4B binding sites, (**C**) binding of eIF4B and PABP to the N- and C-terminal binding sites, respectively, or (**D**) binding of PABP and eIF4B to the N- and C-terminal binding sites, respectively, is depicted. eIF4B is shown interacting with eIF4A (indicated with bars) but whether eIF4B can bind eIF4A when bound to eIFiso4G or eIF4G is unknown.

binding to the eIFiso4G HEAT-1 domain in the absence of the HEAT-2 domain. However, like PABP, eIF4B does not compete with eIF4A when both the HEAT-1 and HEAT-2 domains of eIFiso4G are present.[12] This suggests that, as with PABP, the HEAT-2 domain alleviates the competition between eIF4B and eIF4A, perhaps through stabilizing the binding of eIF4A to eIFiso4G in the presence of eIF4B.

The competition that exists between PABP and eIF4A or between eIF4B and eIF4A in binding wheat eIFiso4G may be explained by the greater overlap between the binding sites for PABP and eIF4A or for eIF4B and eIF4A in the eIFiso4G HEAT-1 domain compared with the minimal overlap with the eIF4G HEAT-1 region. Although this would appear to be a significant difference between eIFiso4G and eIF4G in plants, the observation that PABP and eIF4A or eIF4B and eIF4A no longer compete when the HEAT-2 domain is also present suggests that intact eIFiso4G and eIF4G would not exhibit differences in their interaction with these partner proteins.

### eIF4B, a second bridge between the 5′ and 3′ termini of an mRNA

eIF4B stimulates the RNA-dependent ATP hydrolysis activity and the ATP-dependent RNA helicase activity of animal eIF4A,[6] the helicase activity of yeast eIF4A[39] and the activity of plant eIF4A in wheat germ extract.[40-43] eIF4B increases the affinity of eIF4A for ATP and its helicase processivity[6,44,45] in part through coupling ATP hydrolysis to RNA unwinding.[46] Together, eIF4B and eIF4G synergistically increase the ATPase activity of eIF4A,[26] suggesting a functional interaction between the 2 factors. eIF4B may mediate mRNA binding to ribosomes[47-52] or bridge an interaction between eIF4F bound to mRNA and eIF3.[53] In yeast, eIF4B promotes eIF4A binding to eIF4G perhaps through a conformational change in the single HEAT domain present in the yeast factor.[24] Yeast eIF4B preferentially stimulates translation from mRNAs with moderately stable secondary structure in the 5′ UTR while it stimulates the higher basal level of translation from mRNAs that lack secondary structure only moderately.[54] Formation of a 48 S translation initiation complex (i.e., the binding of a 40 S subunit to an mRNA) with animal mRNAs containing a structured 5′ UTR was dependent on eIF4B,[55] suggesting that, although the ability of eIF4B to promote eIF4A helicase activity is likely involved in the translation of mRNAs in general, it may be of particular importance for certain types of mRNAs.

Despite its functional conservation among plants, animals, and yeast, only limited conservation is observed for eIF4B from wheat and other species: wheat eIF4B shares just 29% and 24% identity with human and yeast eIF4B, respectively,[56] making this factor one of the least conserved of the translation initiation factors. As mentioned above, wheat eIF4B interacts with wheat eIF4G and eIFiso4G and each of these bind to the N-terminal region of eIF4B. For example, eIFiso4G binds at a single site proximal to the N-terminal RNA binding domain of eIF4B (**Fig. 3A**).[57]

Although a complex between mammalian eIF4B and eIF4A is observed in the presence of RNA and ATP,[14,26,58] no direct interaction between the 2 factors has been reported nor has the eIF4A binding site in animal eIF4B been identified. NMR studies with human eIF4H, a factor related to eIF4B that lacks sequence corresponding to the N- and C-terminal ends of eIF4B, revealed binding to the C-terminal domain of eIF4A.[14] The C-terminal 72 amino acids of eIF4H were sufficient for this interaction with eIF4A. A direct interaction between eIF4B and eIF4A (as well as PABP), however, has been shown for the plant homologs (**Fig. 3A**).[57] eIF4A binds wheat eIF4B at 2 conserved repeats approximately 18 amino acids long that lie on either side of a C-terminal RNA binding domain[57] (**Fig. 3**). PABP also binds immediately C-proximal to each eIF4A-binding site to a conserved 24 amino acid long repeat. As these repeats are conserved, this suggests that plant eIF4B may bind 2 molecules of eIF4A and 2 of PABP.

Despite the fact that eIF4B is poorly conserved, even among plants, the region containing the eIF4A and PABP binding sites represents the most conserved region of the protein in plants. The conservation within this region can be seen with a sequence logo generated from an alignment representing 16 monocot and 11 dicot eIF4B homologs (**Fig. 3B**). The eIF4A binding repeats exhibit the greatest conservation and significant conservation is observed in the PABP binding sites and the RNA binding domain. Interestingly, the C-terminal 72 amino acid region of human eIF4H sufficient for interaction with eIF4A contains the region corresponding to the eIF4A-binding repeat of plant eIF4B and exhibits significant homology with the plant eIF4A-binding site. Repeats with some similarity are also present in yeast eIF4B.[59] Whether eIF4B or PABP bind to such elements in animal or yeast eIF4B, however, is unknown. The position of the 2 PABP binding sites in the central region of plant eIF4B differs with the position of the reported PABP binding site in mammalian eIF4B that lies within the N-terminal 80 amino acids of the protein (**Fig. 3A**).[60] The lack of conservation in the position of the PABP binding site between mammalian and plant eIF4B is a significant difference between the homologs but may be a result of the poorly conserved nature of eIF4B in eukaryotes. However, the fact that an interaction between eIF4B and PABP has been conserved, at least in higher eukaryotes, suggests that the interaction between these 2 factors is important.

These findings support the notion that plant eIF4B organizes interactions among translation factors to a greater extent than was previously appreciated. Evidence in yeast suggests that the interaction of eIF4B with eIF4G prepares the latter for binding eIF4A.[24] As in yeast,[61,62] eIF4A is typically lost from preparations of wheat eIF4F (or eIFiso4F),[63] suggesting the binding of eIF4A to eIF4G may be transient. Therefore, eIF4B may enhance binding between eIF4A and eIF4G in plants as it does in yeast although no stimulatory effect of eIF4B on eIF4A binding to either eIF4G or eIFiso4G was observed in vitro.[12,13]

### eIF4G, eIFiso4G, and eIF4B bind competitively within the PABP RRM1 domain

PABP contains 4, N-terminal RNA recognition motifs (RRMs) that are tandemly arranged and a C-terminal region that is required for its self-association (**Fig. 4A**).[64,65] As each RRM

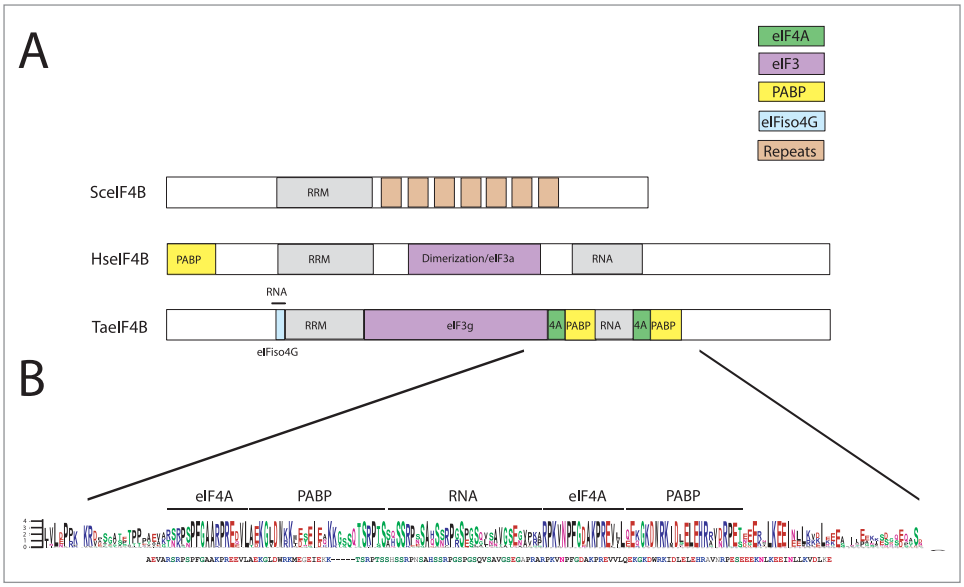


**Figure 3.** Partner protein interactions of eIF4B in higher eukaryotes. (**A**) The RNA and protein binding domains in wheat eIF4B (TaeIF4B), human eIF4B (HseIF4B), and yeast eIF4B (SceIF4B) are shown. Interaction domains for partner proteins are indicated by color with a key included. The 7 repeats in yeast eIF4B represent 26 amino acid repeats implicated in promoting translation.[59] (**B**) Below the wheat eIF4B is a sequence logo for the region encompassing the PABP and eIF4A binding site repeats and the RNA binding domain that separates the repeats. A sequence alignment from 16 monocot and 11 dicot eIF4B homologs was generated using EBI MUSCLE. The aligned sequences were submitted to WebLogo Berkeley to generate a sequence logo, in which the most conserved one, 2, or sometimes 3 residues at each position are shown as the consensus. Below the protein sequence logo is the consensus sequence for the region in which residues conserved with the sequence logo are similarly colored. The logo was constructed from eIF4B homologs from Triticum aestivum, Brachypodium distachyon (Bradi5g13500 and Bradi3g47700), Oryza sativa (Os04 g40400 and Os02 g38220), Setaria italica (Si009805 and Si016924), Sorghum bicolor (Sb06 g020170 and Sb04 g024860), Zea mays (GRMZM2G163471, GRMZM2G066815, and GRMZM2G139614), Panicum virgatum (Pavirv00022707 and Pavirv00022708, Pavirv00021798, and Pavirv00059810), Phaseolus vulgaris (l002G259400), Ricinus communis (29792), Brassica rapa Chiifu-401 (Bra026941), Thellungiella halophila (Thhalv10007279 and Thhalv10003946), Capsella rubella (Carubv10008811 and Carubv10016953), Arabidopsis thaliana (AT1G13020 and AT3G26400), and Arabidopsis lyrata (920166 and 484402).

contacts approximately 3 adenosine residues,[66] the tandem arrangement allows the protein to bind a continuous stretch of 12 adenosine residues which is the minimum length that PABP requires in order to bind poly(A) RNA.[64,67] In animals, the region encompassing RRM1 to RRM2 within PABP is required for interaction with eIF4G whereas in yeast, PABP RRM2 is principally required for eIF4G binding with PABP RRM1 playing a contributing role (**Fig. 4A**).[17,68,69] In plants, eIF4G binds PABP within its RRM1 and eIFiso4G binds a region encompassing the C-terminal end of RRM1 and the N-terminal end of RRM2[70] (**Fig. 4A**), suggesting that eIF4G proteins interact with PABP within a similar if not quite identical region of PABP across eukaryotes. If the RRM1 of plant PABP is the only domain involved in the interaction with eIF4G, the presence of 2 distinct PABP interaction domains in eIF4G[13] suggests that the RRM1 of PABP interacts with both eIF4G sites although presumably with different subsequences as the 2 PABP binding sites in eIF4G do not share any obvious similarity.

In addition to its principal binding site in PABP RRM1–2, wheat eIFiso4G interacts at a second site within PABP RRM3–4[70] (**Fig. 4A**). eIFiso4G binding to the site in RRM1–2 is considerably stronger than its binding to RRM3–4.[70] As mentioned above, PABP binds to 2 distinct subregions within the eIFiso4G HEAT-1 domain[12] (**Fig. 1A**). The region of PABP encompassing the C-terminal end of RRM1 and the N-terminal end of RRM2 binds the C-terminal subregion within the eIFiso4G HEAT-1 domain whereas PABP RRM3–4 binds the N-terminal subregion of the HEAT-1 domain.[12]

As with eIF4G and eIFiso4G, plant eIF4B binds within the RRM1 of PABP at a site located within the C-terminal end of this domain (**Fig. 4A**). To identify the residues in this region that might be conserved among eukaryotes, a sequence logo showing the conservation of sequence at each residue was generated from an alignment of the corresponding region from PABP orthologs of yeast, animals, and vascular and non-vascular plants (**Fig. 4B**). The RNP2 motif of RRM2 within this region that makes direct contact with poly(A) RNA is highly conserved as expected. In addition to this motif, several residues are invariant across kingdoms including several present within the linker between RRM1 and RRM2 (**Fig. 4B**). Whether these conserved residues are important for binding eIF4G (as well as eIFiso4G and eIF4B for plant PABP) remains to be determined.

As the binding sites of eIF4B and PABP in plant eIF4G and eIFiso4G overlap causing their binding to eIF4G and eIFiso4G to be mutually exclusive (**Fig. 2**), it is not surprising that eIF4B and eIFiso4G (and presumably eIF4B and eIF4G although this has not been tested) compete in their binding to PABP.[70] These observations indicate that the binding of eIF4B and eIFiso4G to PABP is mutually exclusive as is the binding of eIF4B and PABP to eIFiso4G, suggesting that a molecule of PABP can bind either eIF4B or eIFiso4G but not both simultaneously just as a molecule of eIFiso4G can bind either eIF4B or PABP but not both simultaneously.

eIF4B has been reported to bind mammalian PABP within its C-terminal region (**Fig. 4A**) that also interacts with eRF3, Paip1, Paip2.[60,71-75] Although the ability of proteins to bind the PABP C-terminal region requires a PAM2 motif that is present among these partner proteins, interestingly, this motif is not present in mammalian eIF4B.

## Functional Consequences of the Interactions Between the Cap-Associated Factors and PABP

### PABP and the cap-binding proteins mutually stabilize binding to an mRNA

Given the interactions among the cap-associated initiation factors and PABP, what impact might these interactions have on their function? The 5′-cap and the poly (A) tail synergistically stimulate translation in plants, animals, and yeast which is a consequence of the physical interactions between PABP bound to the poly(A) tail and the cap-associated initiation factors bound at the 5′-cap.[76,77] One functional consequence of the physical interaction between plant PABP and eIFiso4G is that PABP increases the binding of eIFiso4F to the 5′-cap by 40-fold and PABP stimulates the ATPase and RNA helicase activity of the eIFiso4F:eIF4A:eIF4B complex.[38,78] The interaction of PABP with eIF4G also increases eIF4F binding to the 5′-cap in yeast.[79] In mammals, the results have been mixed with depletion of PABP from cell extracts impairing the interaction of eIF4E with the mRNA 5′-cap[80] but loss of the N-terminal PABP-binding site in eIF4G having no effect on the interaction of eIF4G with eIF4E, at least when tested in vitro.[81]

Addition of wheat eIF4B to eIFiso4F or the eIFiso4F:PABP complex lowers the activation energy of binding of each to the 5′-cap in plants.[82,83] Fluorescence stopped-flow studies of plant PABP:eIFiso4F complex binding to a 5′-cap showed that the PABP-mediated increase in the eIFiso4F affinity for a 5′-cap results from a concentration-independent conformational change and a reduced dissociation rate that enables eIFiso4F to more easily achieve a stable conformation with the 5′-cap.[82] This can also explain how binding of PABP increases the ATPase and RNA helicase activity of the cap-associated complex.[78] The interaction between PABP and eIF4G stimulates eIF4F binding to an mRNA in mammalian extracts[80] and in yeast,[84] perhaps by assisting eIF4G to out-compete general RNA-binding proteins that are unable to interact with PABP.[85] 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.[83]

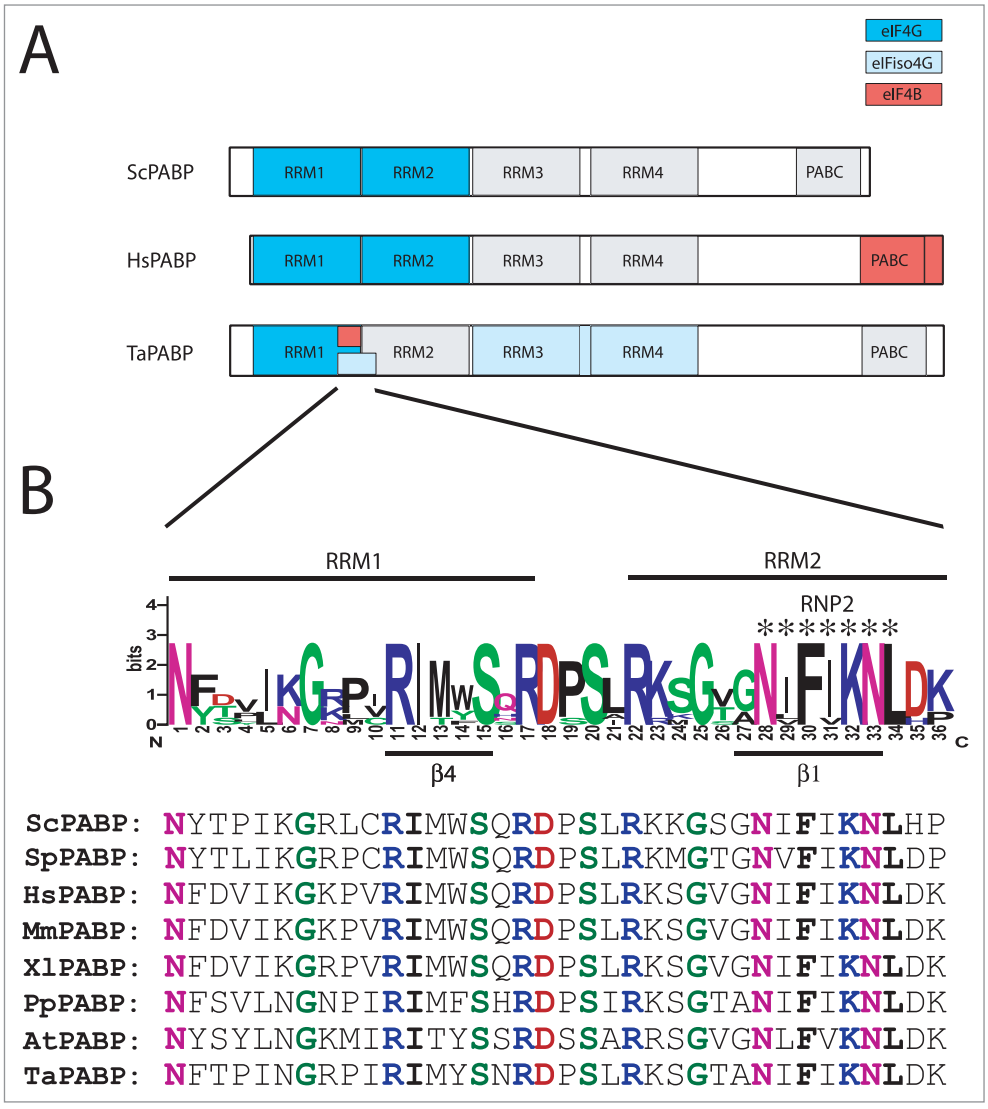


**Figure 4.** Partner protein interactions of PABP in higher eukaryotes. (**A**) The RNA and protein binding domains in wheat PABP (TaPABP), human PABP (HsPABP), and yeast PABP (ScPABP). are shown. Interaction domains for partner proteins are indicated by color with a key included. (**B**) Below the wheat PABP is a sequence logo for higher and lower eukaryotic PABP proteins encompassing the eIF4G, eIFiso4G, and eIF4B binding sites of the wheat PABP. For this analysis, a sequence alignment from yeast, animal, and plant PABP homologs was generated using EBI MUSCLE. A sequence logo was generated from the aligned sequences in which the most conserved one or residues at each position are shown as the consensus. The sequences part of RRM1 or RRM2 domains are indicated by the labels above the logo. The RNP2 motif of RRM2 is indicated by the asterisks. The fourth β sheet (β4) of RRM1 and the first β sheet (β1) of RRM2 are indicated by the labels below the logo. The logo was constructed from PABP homologs from Saccharomyces cerevisiae (ScPABP, NM_001179055), Schizosaccharomyces pombe (SpPABP, NM_001018809), Homo sapiens (HsPABP, NM_002568), Xenopus laevis (XlPABP, NM_001086735), Mus musculus (MmPABP, NM_008774), Physcomitrella patens (PpPABP, Pp1s257_72V6), Arabidopsis thaliana (AtPABP, At2g23350), and Triticum aestivum (TaPABP, TAU81318). Below the logo are the sequences from each species used in which invariant residues are colored as in the sequence logo.

The interaction of plant eIFiso4G, eIF4G, or eIF4B with PABP increases binding of the latter to poly(A) RNA by decreasing its dissociation rate.[34,86] Moreover, eIF4F (or eIFiso4F) and eIF4B exert a synergistic effect on the poly(A) RNA binding activity of PABP,[34] suggesting a functional interaction between all 3 proteins. Therefore, the physical interaction between eIF4G (or eIFiso4G) and PABP mutually stabilizes their association with their respective binding sites while bringing the termini of an mRNA into physical proximity.[34,82,86,87] An additional consequence of the physical interaction between PABP and the cap-binding complex is that it serves as a means test to confirm the integrity of an mRNA prior to 40 S subunit recruitment to prevent translation initiation on an mRNA undergoing degradation.[88] The interaction between PABP and eIF4G promotes formation of the 48 S pre-initiation complex as well as the assembly of the 80 S ribosome and increases translational efficiency.[80,81] That PABP increases 40 S subunit binding to an mRNA demonstrates its stimulatory role in translation initiation,[89,90] likely in part through its ability to increase the binding of the cap-associated complex to an mRNA which in turn more efficiently recruits 40 S subunits.

## The Mutually Exclusive Binding Between eIF4G and eIF4B to PABP Increases The Number of Interactions Between The Termini of An mRNA

As mentioned, the binding of eIF4B and PABP to eIF4G (or eIFiso4G) in plants is mutually exclusive as is the binding of eIF4B and eIF4G (or eIFiso4G) to PABP. Therefore, because the average length of a poly(A) tail is sufficiently long to support the binding of multiple molecules of PABP, the mutually exclusive nature of eIF4G (or eIFiso4G) and eIF4B binding to PABP suggests they interact with separate molecules of PABP on a poly(A) tail, providing increased stability to the complex through an increase in the number of protein interactions (**Fig. 5**). Moreover, as PABP and eIF4G (or eIFiso4G) bind to eIF4B at well separated sites and eIF4B can dimerize,[91] plant eIF4B likely can bind to PABP and eIF4G (or eIFiso4G) simultaneously, providing yet another means to increase and therefore stabilize the physical interactions between the termini of an mRNA (**Fig. 5**). Finally, the observation that the combination of eIF4G (or eIFiso4G) and eIF4B synergistically promotes PABP binding to poly(A) RNA in plants and does so in part through promoting multimeric PABP binding[34] suggests that eIF4G (or eIFiso4G) and eIF4B cooperate to interact with PABP and increase the stability of the complex.

## Regulation of the Interactions Between the Cap-Associated Factors and PABP

### The phosphorylation state of PABP determines its type of binding and affinity to poly(A) RNA

Several components of the cap-binding complex and PABP are phosphoproteins and their phosphorylation state can affect the nature and strength of their interaction. For example, PABP is differentially phosphorylated in plants, yeast, and sea urchin.[86,92-94] In plants, the phosphorylation state of PABP affects its affinity and type of binding to poly(A) RNA as well as its specificity of its interaction with eIF4G, eIFiso4G, and eIF4B,[86] the result of which is to prevent competition between eIFiso4G and eIF4B binding.

Self-interaction of PABP in *Xenopus* is mediated through its C-terminal domain.[65] When phosphorylated, plant PABP binds poly(A) RNA cooperatively while PABP in a hypophosphorylated state does not bind poly(A) RNA cooperatively but does bind with substantially greater affinity.[86] Only under saturating conditions does more than one molecule of hypophosphorylated PABP bind to poly(A) RNA if the RNA is long enough to bind multiple PABP molecules. However, the combination of PABP isoforms of opposite phosphorylation states exhibited the greatest cooperative binding.[86] As diverse PABP phosphoisoforms are observed in all plant tissues examined[86,95] and the average poly (A) tail of a typical mRNA sufficiently long to bind multiple molecules of PABP,[64,67] the bound PABP is likely be a heterogeneous population of phosphorylated states, consistent with what is observed for polysome-bound PABP.[86] Differentially phosphorylated PABP species have been reported in sea urchin[92] and the in vivo phosphorylation of a *Xenopus laevis* embryonic poly (A)-binding protein (known as ePABP or PABPc1-like) at a 4 residue cluster is required for oocyte maturation.[96] Although the main cytoplasmic human PABP appears to be extensively post-translationally modified,[97] no evidence of its phosphorylation has been detected but not all members of the gene family have been examined to date. Whether the phosphorylated state is important for the interaction between the cap-associated factors and PABP in animals remains to be determined.

### The phosphorylation state of PABP determines its binding to eIF4G, eIFiso4G, and eIF4B

The PABP phosphorylation state also affects PABP's binding with its partner proteins.[86] The interaction of wheat eIF4G with PABP increases its RNA binding activity and does so by promoting its cooperative binding.[86] As this effect is specific to the hypophosphorylated form of PABP, this suggests that eIF4G interacts preferentially with hypophosphorylated PABP. In contrast, the phosphorylation state of plant PABP does not appear to affect the interaction with eIFiso4G.[86] eIF4B exhibits a third type of specificity and preferentially increases the RNA binding activity of phosphorylated PABP in plants by increasing its affinity to RNA.[86]

The phosphorylation state of plant eIF4B also affects the PABP-eIF4G interaction. The presence of eIF4B in its phosphorylated form increased the RNA binding activity of phosphorylated PABP while recombinant eIF4B, lacking phosphorylation, had little effect.[86] Only when substantially more recombinant eIF4B was present did it increase the PABP binding to poly(A) RNA but this was specific to the hypophosphorylated form of PABP.[86] As wheat eIF4B has a greater effect on PABP's binding activity than does eIF4F or eIFiso4F,[34] eIF4B may be particularly important in the interaction between cap-associated factors and

PABP during plant translation. Whether this is true in animals where the interaction between eIF4B and PABP also occurs[60] has not been examined.

The observation that the phosphorylation state of plant PABP affects its binding with partner proteins suggests that eIF4G and eIF4B (or eIFiso4G and eIF4B) would interact with independent molecules of PABP (**Fig. 5**). As eIF4B binds eIF4G (or eIFiso4G) in plants,[12,13,57,91] such a possibility would involve the interaction of 4 molecules (i.e., eIF4G, eIF4B, and 2 molecules of PABP). In addition, as PABP cooperatively binds to poly(A) RNA, the interactions among the 4 molecules may yield a more stable complex. The observation that the combinatorial effect of eIF4G and eIF4B (or eIFiso4G and eIF4B) on the multimeric binding of PABP to poly(A) RNA is synergistic supports this notion.[34,86] Because the binding sites for eIF4G, eIFiso4G, and eIF4B overlap in PABP, this also dictates that these components of the cap-binding complex interact with independent molecules of PABP, thereby avoiding competition in their binding.

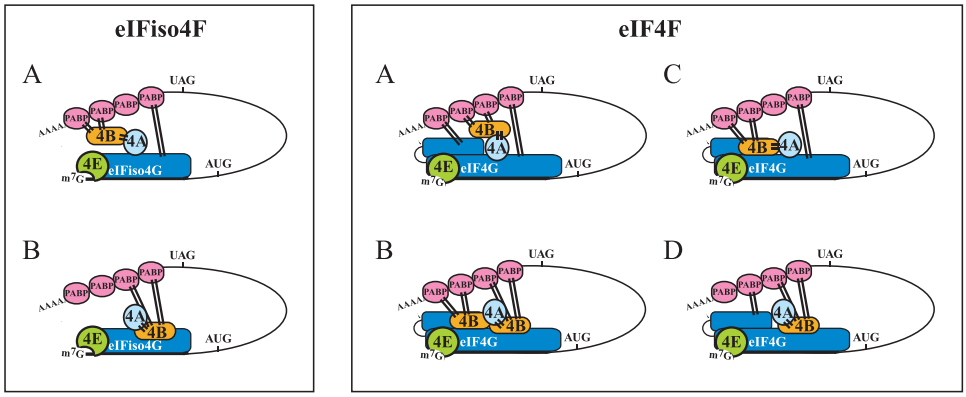


**Figure 5.** Models for possible interactions between PABP and eIFiso4G, eIF4G, and eIF4B during translation initiation in plants. For eIFiso4F: (**A**) The interaction between the termini of a translating mRNA is facilitated by interactions between PABP to eIFiso4G and eIF4B (indicated with bars). eIFiso4G contains a single binding site for PABP and this interaction is depicted. However, because eIF4A binds eIF4B (which contains 2 eIF4A binding sites), an interaction between eIF4B and 2 molecules of PABP is also depicted. Whether eIF4B can bind 2 molecules of PABP simultaneously and whether eIF4A can bind eIFiso4G and eIF4B simultaneously is unknown. (**B**) The interaction between the termini of a translating mRNA is facilitated solely by the binding of PABP to eIF4B which in turn is bound to eIFiso4G which contains a single eIF4B binding site. However, because eIF4B contains 2 binding sites for PABP, it is depicted as interacting with 2 molecules of PABP. For eIF4F: (**A**) The interaction between the termini of a translating mRNA is facilitated through the binding of PABP at the N- and C-terminal PABP binding sites in eIF4G. Because eIF4A binds eIF4B, an interaction between eIF4B and 2 molecules of PABP is also depicted. (**B**) The interaction between the termini of a translating mRNA is facilitated solely through the binding of PABP to eIF4B which is bound at the N- and C-terminal eIF4B binding sites in eIF4G. Because eIF4B can dimerize, the 2 molecules of eIF4B are shown as a dimer but whether dimerization occurs during binding to eIF4G is unknown. (**C**) The interaction between the termini of a translating mRNA is facilitated through the binding of 2 molecules of PABP to eIF4B bound at the N-terminal eIF4B binding site in eIF4G and the binding of PABP to the C-terminal PABP binding site in eIF4G. (**D**) The interaction between the termini of a translating mRNA is facilitated through the binding of PABP to the N-terminal PABP binding site in eIF4G and the binding of 2 molecules of PABP to eIF4B bound at the C-terminal eIF4B binding site in eIF4G.

**Regulation of the phosphorylation state of eIF4B correlates with the strength of its interaction with PABP and with active translation**

Although the specificity and strength of the PABP-eIF4B interaction is determined by their phosphorylation state in plants, is their phosphorylation regulated either developmentally or in response to environmental changes? Whereas the array of PABP phosphorylated isoforms is fully represented in polysomes and does not substantially change during development or following stress in plants, eIF4B is largely present in its most phosphorylated species in actively growing tissues when protein synthetic rates are highest and it is the phosphorylated species of eIF4B that are preferentially recruited into polysomes.[86,93,95] In contrast, eIF4B is present mostly as dephosphorylated species during late seed development or following a heat shock when protein synthesis is repressed.[93] Thus, the regulation of eIF4B phosphorylation by developmental or stress-related cues could serve to regulate global protein synthesis in plants. In mammalian cells, eIF4B phosphorylation is regulated by growth factors or stress: it is phosphorylated under conditions of active translation (e.g., following insulin stimulation) but is dephosphorylated following serum depletion or during a heat shock.[98-100] Whether the interaction between mammalian eIF4B and PABP is regulated by the phosphorylation state of eIF4B has not been examined in animals.

## Concluding Remarks and Future Directions

The presence of multiple interaction domains among the components of the cap-binding complex and PABP and the mutually exclusive binding observed for some partner proteins suggests that more than one set of interactions may be possible during initiation in plants. As mentioned, eIF4G and eIF4B or eIFiso4G and eIF4B likely interact with independent molecules of PABP (**Fig. 5**). Their interaction with PABP may be direct and simultaneous if multiple PABPs of differing phosphorylation states are bound along a poly(A) tail. Alternatively, it is possible that PABP could bind eIF4B while the latter is bound to eIF4G (or eIFiso4G), so that eIF4B acts as a bridge linking eIF4G (or eIFiso4G) with PABP (**Fig. 5**). PABP may also bind eIF4B while the latter is bound to eIF4A which in turn is bound to eIF4G (or eIFiso4G), so that eIF4B, through eIF4A, bridges eIF4G (or eIFiso4G) with PABP (**Fig. 5**). Because eIF4B can dimerize,[91] it is possible that each monomer of the eIF4B dimer could contact

a PABP while eIF4G (or eIFiso4G) binds to a third PABP. Moreover, the presence of 2 conserved PABP binding sites in eIF4B suggests that the latter may bind 2 molecules of PABP. Alternatively, only one molecule of PABP may bind eIF4B at any one time but the presence of 2 PABP interaction sites in eIF4B may increase the likelihood of interaction between PABP and eIF4B. Given the number of possible interactions among these factors, it is unlikely that the interactions are static but rather are likely to be dynamic and alternate between the examples depicted in **Figure 5** during the progression of initiation or in the presence of additional factors that will influence the interactions that predominate. These models will require experimental confirmation before a complete understanding of the possibilities of interaction between the termini of an RNA can be fully appreciated.

Although eIF4B can interact with eIF4G (or eIFiso4G) in plants, it also binds eIF4A directly.[12,13,57,91] As an RNA helicase, eIF4A may not only be present as a subunit of eIF4F (or eIFiso4F), it may also be bound to RNA independently of its interaction with eIF4G.[4] Therefore, it will be important to determine whether eIF4B can interact with PABP when the former is bound to eIF4G or eIF4A or to both and whether there are different functional consequences between them.

eIF4A is not the sole RNA helicase involved in translation. Ded1 is a DExD/H-box RNA helicase in yeast that binds directly to eIF4G1 to form a translationally repressed Ded1-eIF4F-mRNA intermediate which accumulates in stress granules.[101] Ded1 binds to the RNA3 domain of yeast eIF4G which is distinctly different than the interaction of eIF4A with the HEAT-1 domain of eIF4G. Whether DDX3, the Ded1 homolog in humans, is involved in regulating protein synthesis has been controversial.[102] The *Arabidopsis thaliana* gene, At5g14610, encodes a likely Ded1 homolog (80.2%/82.9% amino acid identity/similarity) but whether this binds plant eIF4G or eIFiso4G or exhibits a similar function has not been examined.

Another RNA helicase, DHX29, a member of the SF2 DEAH/RHA family, promotes translation in animals through its helicase activity.[103] DHX29 mainly binds around helix 16 of the 40 S subunit and likely functions to induce conformational changes in the small ribosomal subunit that open and close the mRNA entrance to aid 43S complexes to unwind structured sequences in an mRNA.[104] However, DHX29 is not known to be a component of the cap-binding complex. Proteins encoded by the *A. thaliana* genes, At1g48650 and At3g26560, exhibit limited homology with DHX29 (49.2%/54.9% and 50.7%/56.3% amino acid identity/similarity, respectively) but any potential role in translation has not been examined.

Plant eIF4G contains 2 binding sites each for eIF4B and PABP.[13] Whether this results in the binding of one or 2 molecules of each partner protein is also an outstanding question but this represents a significant difference between the eIF4G and eIFiso4G isoforms in plants,[12,13] a difference that does not exist between eIF4G isoforms in other eukaryotes. The binding of eIF4B and PABP to eIFiso4G is mutually exclusive but the fact that plant eIF4G contains 2 binding sites each for eIF4B and PABP raises the possibility that eIF4B and PABP could bind simultaneously to eIF4G (**Fig. 2**) although this has not been demonstrated. How such differences between the 2 eIF4G isoforms may affect their function is not known although preferences between plant eIF4G and eIFiso4G in the mRNAs they translate have been reported.[9,105-107] Future work will need to address whether PABP and eIF4B can bind the same molecule of eIF4G simultaneously in plants, and if not, whether PABP and eIF4B cycle through eIF4G during translation initiation as has been reported for eIF4A.[108]

The interaction between PABP and the cap-binding complex raises the question of how important is the interaction to protein synthesis? The observation that many mRNAs naturally lacking a 5′-cap or a poly(A) tail, but which have evolved functional equivalents, still retain a requirement for an interaction between the termini of the mRNA,[109-114] suggesting that the interaction between the termini of an mRNA is a common feature among mRNAs and that such an interaction provides a benefit to the synthesis of protein from an mRNA.

Depletion of PABP from mammalian extracts reduced eIF4E binding to a cap, 48 S complex formation, and translation, all of which could be restored by the addition of wild-type PABP but not by a mutant of PABP unable to interact with eIF4G.[80] In contrast, analysis in vivo suggested that deletion of the PABP binding site from mammalian eIF4GI had little effect on the latter's ability to support translation.[81] However, mammalian eIF4G lacking the binding sites for eIF4A or eIF3 also had a minimal effect on the ability of eIF4G to support translation in the same study.[81] As eIF4G lacking the binding sites for eIF4A or eIF3 showed a marked reduction in activity during translation in vitro and eIF4G lacking the PABP binding site was less effective than native eIF4G,[81] the siRNA knockdown approach used to suppress endogenous eIF4G expression in vivo may not have been stringent enough to assay fully the contribution that the interaction between PABP and eIF4G has on translation. When hypertonic shock was used to disassemble polysomes and eIF4F in mammalian cells, eIF4G lacking the PABP binding site was incorporated into eIF4F as efficiently as native eIF4G but was less efficiently incorporated into polysomes.[81] In *Xenopus* oocytes, eIF4G lacking the PABP binding site inhibited translation of coinjected mRNA suggesting it had a dominant negative effect.[115]

Although these studies might suggest that the interaction between PABP and eIF4G is not essential but may contribute to optimal translation in animals, the interaction between PABP and eIF4B was not considered. In the context of a translating mRNA, this second interaction may stabilize the association of PABP with the cap-binding complex even if the PABP binding site in eIF4G is deleted. Conservation of this interaction in animals and plants suggests it serves a function, despite the fact that eIF4B is the least conserved of all the initiation factors. Until all means for the interaction between PABP and components of the cap-binding complex have been removed, it is difficult to estimate the magnitude that the interaction makes to translation. It is also possible that the interaction is more important in one species than another so its contribution will need to be accurately assessed in animals, plants, and yeast individually.

Although the factors providing the necessary specificity for binding mRNA are eIF4E and PABP, the RNA binding domains in plant eIF4G, eIFiso4G, eIF4A, and eIF4B suggest that these likely contribute to stabilizing the complex to an mRNA as observed for animal and yeast eIF4G,[37,116,117] while the presence of protein interaction domains in each factor serves to bring together the activities required to promote the binding of 40 S subunits and their subsequent scanning. The interaction of PABP with the cap-associated factors not only increases these activities and stabilizes the binding of the complex to the 5′-cap, but may promote the re-recruitment of 40 S subunits following translation termination, thereby increasing the yield of protein from a given mRNA before its eventual destruction.

The interaction between PABP and eIF4G may be of particular importance under conditions of competitive translation. Thus, in some translation lysates, the abundance of unengaged eIF4F or PABP may mask competitive translation.[105,106] Such a possibility is supported by the observation that the degree to which translation is cap-dependent in wheat germ lysate is inversely proportional to the concentration of PABP or eIF4G/eIFiso4G.[9] The interaction between PABP and eIF4G during competitive translation in animals may be particularly important in the presence of general ribonucleoproteins (e.g., YB-1) as their presence permits only those proteins with sufficient strength and specificity to compete for binding.[85] In the case of YB-1 for example, the interaction of PABP with eIF4G overcomes the inhibition imposed by this ribonucleoprotein on the eIF4E-5′-cap interaction.[85] Thus, the combinatorial strength provided by the multiple interactions among PABP, eIF4G, eIF4E, eIF4A, eIF4B, and eIF3 likely come into greatest play when presented with the full array of RNA binding proteins in a cell as well as a diverse population of mRNAs that are competing for their binding.

The numerous interactions among PABP, eIF4G, eIF4E, eIF4A, eIF4B, and eIF3 support the notion of functional redundancy within the complex. Such functional redundancy does not mean that individual interactions are of no importance as each likely contributes to the rate of assembly and overall stability of the complex. Functional redundancy also means that individual interactions can be targets for regulation in order to modulate the rate of assembly and complex stability as a means to regulate global protein synthesis in response to developmental or environmental signals. For example, developmental cues during late embryo development resulting in a translationally quiescent state by seed maturity correlate with changes in the phosphorylation state of eIF4B that would reduce its interaction with PABP.[93] Global repression of protein synthesis in wheat following an exposure to elevated temperature similarly results in the rapid dephosphorylation of eIF4B[93] and a reduction in its binding to PABP and to eIF4F and eIFiso4F.[86] Although exposure to heat-stress does not alter the distribution of plant PABP isoforms, a reduction in the poly(A)-binding activity of PABP and its binding to eIF4F and eIFiso4F is observed which may be a consequence of the reduced eIF4B interaction that would normally increase the poly(A)-binding activity of PABP and its association with eIF4F and eIFiso4F.[86] Whether the regulation of global protein synthesis involves changes in other components of the plant cap-binding complex, such as eIF4E, eIFiso4E, eIF4G, eIFiso4G, or eIF4A remains an outstanding question. However, the apparent lack of a plant homolog of 4E-BP (4E-binding protein), which in animals and yeast binds to eIF4E to block the interaction between eIF4E and eIF4G and repress global protein synthesis,[118,119] or a plant homolog of Paip2 (PABP interacting protein 2), which in animals binds to PABP and represses its poly(A) binding activity,[71] suggests that the regulation of interactions among the cap-associated factors and PABP in plants may rely to a greater extent on post-translational modifications than observed in other species. Future research focusing on these questions in plants will reveal those aspects common among eukaryotes but also those features that are unique to a very distinct eukaryotic kingdom.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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