Wheat Germ Poly(A)-binding Protein Increases the ATPase and the RNA Helicase Activity of Translation Initiation Factors eIF4A, eIF4B, and eIF-iso4F

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Recent studies demonstrated that wheat germ poly(A)-binding protein (PABP) interacted with translation eukaryotic initiation factor (eIF)-iso4G and eIF4B, and these interactions increased the poly(A) binding activity of PABP (Le, H., Tanguay, R. L., Balasta, M. L., Wei, C. C., Browning, K. S., Metz, A. M., Goss, D. J., and Gallie, D. R. (1997) J. Biol. Chem. 272, 16247–16255) and the cap binding activity of eIF-iso4F (Wei, C. C., Balasta, M. L., Ren, J., and Goss, D. J. (1998) Biochemistry 37, 1910–1916). We report here that the interaction between PABP and eIF-iso4G has a substantial effect on the ATPase activity and RNA helicase activity of (eIF4A + eIF4B + eIF-iso4F) complex. ATPase kinetic assays show, in the presence of poly(U), PABP can increase the parameter ($k_{cat}/K_m$) by 3.5-fold with a 2-fold decrease of $K_m$ for the (eIF4A + eIF-iso4F) complex. In the presence of globin messenger RNA, the ATPase activity of the complex (eIF4A + eIF-iso4F) was increased 2-fold by the presence of PABP. RNA helicase assays demonstrated that the presence of PABP enhanced the RNA duplex unwinding activity of the initiation factor complex. These results suggest that, in terms of the scanning model of translation initiation, PABP may enhance the mRNA scanning rate of the complex formed by eIF4A, eIF4B, and eIF4F or eIF-iso4F and increase the rate of translation.

Most eukaryotic mRNAs contain both a 5' cap (m7GpppN) and a 3' poly(A) tail. The cap-dependent binding of mRNA to the 40 S ribosome is mediated by at least three eukaryotic initiation factors (eIF), eIF4F, eIF4A, and eIF4B, and requires energy derived from ATP hydrolysis (1, 2). Early studies have shown that the cap structure and poly(A) tail act in a cooperative manner to regulate the translation of mRNAs (3, 4). Recent studies indicated that the cooperative effect is related to the interaction among eIF-iso4G, eIF4B, and PABP, which increases the poly(A) binding affinity of PABP and the m7G-cap binding affinity of eIF4F and eIF-iso4F (5, 6).

In wheat germ and other plants, an isozyme form of eIF4F called eIF-iso4F has been found (7). eIF-iso4F shows an indistinguishable function from eIF4F in supporting in vitro translation (8). It contains two subunits, a 28-kDa eIF-iso4E and an 86-kDa eIF-iso4G. The eIF-iso4E acts as the binding site of m7GpppN cap. The function of eIF-iso4G is not well characterized. It binds to mRNA in an ATP-dependent manner, may interact with eIF4A and eIF4B (9), and, more interestingly, may interact with PABP (5, 6). cDNA analysis indicated that the amino acid sequence of eIF-iso4G contained possible motifs for ATP binding, metal binding, and phosphorylation (10). Both eIF4F and eIF-iso4F show RNA-dependent ATPase activity, which is stimulated by the presence of eIF4A (11), and mRNA-dependent helicase activity in the presence of eIF4A (12, 13). Recently, four initiation factors of wheat germ, eIF4A, eIF4B, eIF-iso4E, and eIF-iso4G, were expressed in Escherichia coli (14). In vitro translation measurements indicated that expressed initiation factors had the same capacity to support translation in wheat germ extracts as native factors purified from wheat germ (14).

In eukaryotic protein biosynthesis, the binding of mRNA to the small (40 S) ribosomal subunit is the rate-limiting step and is a key target for regulation (15–18). Two models were proposed for the pathway of binding of mRNA to the 40 S ribosomal subunit. In the first model (1), the first step in mRNA binding is the recognition of m7GpppN cap by eIF4F or eIF-iso4F, and then unwinding of secondary structure in combination with eIF4A and eIF4B in the 5'-untranslated region, to create a single-strand RNA, which serves as the binding site for the 43 S preinitiation complex. In the second model (19), eIF4E binds to m7GpppN cap first and then associates with eIF4G, which is already bound to the 43 S complex. Despite the differences in the two models, both models require that, once bound to the cap, the eIF4F, eIF4A, and eIF4B complex must scan along mRNA to unwind secondary structure before reaching the initiation codon AUG. Therefore, the scanning step is most likely the rate-limiting step for mRNA binding to the 43 S preinitiation complex, and a potential target for regulation. The communication between 5' cap and 3' poly(A) tail is a key regulation pathway for mRNA translation (reviewed in Ref. 4). However, the role of PABP in the scanning step has not been determined. In order to begin to answer this question, in this investigation, we have studied the effect of PABP on ATPase and RNA helicase activity of wheat germ initiation factors. PABP stimulates the ATPase activity of initiation factors in a poly(U)- or mRNA-dependent manner. The helicase activity is also enhanced, suggesting a role for PABP in the rate of scanning by the initiation factor complex.

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The ATPase activity was determined by measuring the release of $^{32}$Pi as described previously (23). The standard assay solution (20 $\mu$l) contained 0.1 $\mu$g of poly(U), 3.0 $\mu$g of eIF4A, 2.5 $\mu$g of eIF-iso4E, 8.0 $\mu$g of eIF-iso4F, and indicated amount of PABP.

**Experimental Procedures**

Purification of Proteins—eIF4A, eIF4B, eIF-iso4E, and eIF-iso4F were expressed in E. coli containing the constructed pET3d or pET23d (for eIF4A only) vector in BL21(DE3) pLYS as described elsewhere (14). A HiTrap SP column from Amersham Pharmacia Biotech was used to purify eIF-4B, eIF-iso4E, and eIF-iso4F by the following procedure. E. coli cells were disrupted by alumina, suspended in buffer B (20 mM HEPES/KOH, pH 7.6, 0.1 mM EDTA, 1.0 mM DTT, and 10% glycerol) containing 600 mM KCl (B-600), and centrifuged at 45,000 rpm for 2 h. The supernatant was diluted with B-0 to a final concentration of 60 mM KCl, and loaded onto a 2 x 5 ml Hitrap SP column and washed by B-60 buffer to base line. A 60–1000 mM KCl linear gradient of a total volume 80 ml was used to elute the proteins. 1.0-mL fractions were collected and analyzed by 10% SDS-gel electrophoresis. All steps were carried out in a cold box at approximately 5 °C. The pH of buffer B used for eIF-iso4E and eIF-iso4F purification was 7.0; for eIF4B it was 7.5. The proteins appeared in the 200–300 mM KCl fractions. eIF4A was purified by using a 10-mL column and His-Bind kits from Novagen. The binding and elution of the 50-g wheat germ extract was loaded onto a 100-ml Affi-Blue gel column. The column was washed with 4 M NaCl in buffer A, and subsequently dialyzed against buffer A. Protein concentrations were measured by the Bradford method, with bovine serum albumin as a standard (22).

**ATPase Assay—**The ATPase activity was determined by measuring the release of $^{32}$P, as described previously (23). The standard assay was carried out in a reaction volume of 20 $\mu$l, which contained 25 mM HEPES/KOH, pH 7.5, 100 mM KCl, 2.0 mM MgOAc, 1.0 mM DTT, and 1.0 mM phenylmethylsulfonyl fluoride, and 1.0 mM DTT), and PABP was eluted with 2.0 $\mu$g guanidine hydrochloride in buffer A. The eluted protein was dialyzed in buffer A containing 10% glycerol, then 0.2 mg/mL poly(C) was added, and the sample was loaded onto a 2.0-mL poly(A)-Sepharose 4B column, and washed to baseline by buffer A, and subsequently dialyzed against buffer A. Protein concentrations were measured by the Bradford method, with bovine serum albumin as a standard (22).

**Helicase Substrate—**The RNA duplex used in the helicase reaction was made by combining the top strand (12-mer) oligonucleotides in a 1.5:1 ratio. The complementary strands were annealed (20 mM Tris-HCl, pH 7.5, 80 mM KCl, 1.0 mM EDTA) in a water bath heated to 98 °C for 5.0 min, and cooled slowly in a cold box (about 5 °C) with gentle stirring. Under these conditions, about 75% of the labeled oligonucleotide was hybridized.

**Helicase Assay—**The assay was performed as described previously (24) in a buffer that contained 20 mM HEPES/KOH, pH 7.5, 75 mM KCl, 2.0 mM DTT, 2.0 mM magnesium acetate, and 2.0 units/µl RNAse inhibitor RNAsin (Promega). The concentration of ATP was 2.0 mM, duplex concentration was about 2.5 nM, and the concentrations of eIF4A, eIF4B, eIF-iso4F, and PABP were 1.0 µM. The final reaction volume was 10 µl. The reaction was started by addition of eIF4A or the initiation factor complex (as indicated in Fig. 6), and incubated at 37 °C. Reactions were terminated by adding 2.5 µl of a solution containing 50% glycerol, 2% SDS, 20 mM EDTA, and 0.01% bromphenol blue. Unwinding reaction products were analyzed by separating the displaced labeled bottom strand from the duplex by electrophoresis on 12% native polyacrylamide gel in 1× TBE buffer at ambient temperature (about 25 °C). Autoradiographs were generated by exposing the gels to Fuji RX X-ray film and developed by standard procedures. The bands containing the unwound labeled bottom strand and the duplex were cut and purified by a denaturing (8.0 mM urea) 20% polyacrylamide (19:1 bisacrylamide:acrylamide) gel.

**Transcription of Top Strand of RNA Duplex—**The top strand of RNA duplex used in RNA helicase assay, 5'-GGGGAGA(A4C)5UAGCACCG-UAAAGACGCG (a 50-mer RNA oligonucleotide), was synthesized by in vitro transcription using T7 RNA polymerase. The template for transcription was composed of the following synthetic DNA oligonucleotides: T7 polymerase promoter, 5'-GAATTTAATAGCTACCTAT (for eIF4A only) vector in BL21(DE3) pLyS as described elsewhere (14). The transcription reaction was performed using Ambion’s Megashortscript™ transcription kit following the manufacturer’s instructions. After the reaction, the transcription solution was mixed with 10 µl urea, 0.01% bromphenol blue, and loaded on to a denaturing (8.0 mM urea) 20% polyacrylamide (19:1 bisacrylamide:acrylamide) gel. The bands containing the transcription product were cut out and extracted with 0.5 mM ammonium acetate, 2.0 mM EDTA, 0.1% SDS at 37 °C. Synthesized RNA oligonucleotide was precipitated from the extraction buffer with 2.5 volumes of ethanol, washed with ethanol, lyophilized, and resuspended in RNase-free water. Concentrations of RNA oligonucleotides were measured by UV spectroscopy. A value of 33 µg/1.0 $\mu$l was used to determine the concentration.

**2P 5' End-labeling of RNA Oligonucleotide—**The 5'-end of RNA duplex (12-mer) was $^{32}$P-labeled at the 5'-end with T4 polynucleotide kinase from Amersham Pharmacia Biotech. Forty picomoles of bottom strand RNA oligonucleotide and 10 pmol of $[^{32}P]ATP$ (specific activity 3000 Ci/mmol, 10 mCi/ml) were combined with 20 units of T4 polynucleotide kinase (20 µl final volume), and the reaction was performed according to the manufacturer’s instructions. The labeled RNA oligonucleotide was purified by a denaturing (8.0 mM urea) 20% polyacrylamide (19:1 bisacrylamide:acrylamide) gel as described above.

**DNA and RNA Oligonucleotides—**DNA oligonucleotides were synthesized by Genosys, and were purified by precipitation with 2 volumes of ethanol in TES (10 mM Tris, pH 7.5, 10 mM NaCl, 1.0 mM EDTA) adjusted to 0.5 $\mu$g ammonium acetate, chilling at −20 °C for 2 h, centrifuging and then washing with cold 70% ethanol. A 12-mer RNA oligonucleotide (5'-GCUUACGUGUC), the bottom strand of the RNA duplex used in the helicase assay, was synthesized by Cybergen and purified by a denaturing (8.0 mM urea) 20% polyacrylamide (19:1 bisacrylamide:acrylamide) gel.
and counted. The percentage of unwound duplex was calculated by the following formula: \( \% \text{ unwound} = \frac{\text{cpm of bottom band} - \text{cpm of top band}}{\text{cpm of background of bottom band}} \times 100 \).

The ATPase activities of initiation factors and their complexes in the standard assay solution containing 0.1 \( \mu \text{g} \) of poly(U) were about 5-10% (1 sd). Variation among replicate experiments was about 25% for different protein preparations; however, the magnitude of the PABP stimulation varied less than 10% among replicate experiments.

| No. | 4A | 4B | iso4E | iso4G | PABP | \( P_i \) released |
|-----|----|----|------|------|------|------------------|
| 1   | 1.5| 6  | 2.5  | 8    | 26   | 3                |
| 2   | 1  | 6  | 8    | 6    | 6    | 1                |
| 3   | 1.5| 6  | 2.5  | 8    | 6    | 58               |
| 4   | 1  | 6  | 2.5  | 8    | 91   |                  |
| 5   | 1.5| 6  | 2.5  | 8    | 91   |                  |
| 6   | 1  | 6  | 2.5  | 8    | 91   |                  |
| 7   | 1  | 6  | 2.5  | 8    | 91   |                  |
| 8   | 1  | 6  | 2.5  | 8    | 91   |                  |
| 9   | 1  | 6  | 2.5  | 8    | 91   |                  |
| 10  | 1  | 6  | 2.5  | 8    | 91   |                  |
| 11  | 1  | 6  | 2.5  | 8    | 91   |                  |
| 12  | 1  | 6  | 2.5  | 8    | 91   |                  |
| 13  | 1  | 6  | 2.5  | 8    | 91   |                  |
| 14  | 1  | 6  | 2.5  | 8    | 91   |                  |
| 15  | 1  | 6  | 2.5  | 8    | 91   |                  |
| 16  | 1  | 6  | 2.5  | 8    | 91   |                  |

RESULTS

According to the scanning model of translation initiation, the scanning step, i.e. scanning along mRNA and unwinding secondary structure in the 5’-untranslated region of mRNA by the complex formed by eIF4A, eIF4B, and eIF4F, requires energy derived from ATP hydrolysis (1, 19, 25). Recent studies have shown that, in the wheat germ system, the communication between cap and poly(A) tail involved the interaction between PABP and eIF-iso4G and eIF4B. The interaction increased the poly(A) binding affinity of PABP and m7GTP binding affinity of eIF-iso4F and eIF4F (5, 6). These results suggest PABP may also affect ATPase and helicase activity.

Fig. 1 shows the effect of PABP on the ATPase activity of (eIF4A + eIF-iso4F) complex. Clearly, PABP stimulates substantially the ATPase activity of the complex. The effect of PABP on ATPase activities of initiation factors and their complexes are summarized in Table I. The ATPase activity of eIF4B or PABP alone, if any, is hardly detectable. eIF4A or eIF-iso4G alone exhibits ATPase activity, consistent with previous observations (11, 23). A mixture of equimolar concentrations of eIF-iso4E and eIF-iso4G has the same ATPase activity as eIF-iso4G alone. The ATPase activity of expressed eIF-iso4G or (eIF4A + eIF-iso4G) is higher than eIF-iso4F purified directly from wheat germ (11). In addition, from Table I we can see that the ATPase activity of (eIF4A + eIF-iso4E + eIF-iso4G) is higher than the ATPase activity of eIF4A and (eIF-iso4E + eIF-iso4G) (Table I, 201 pmol > 26 + 91 pmol), indicating eIF4A has a synergistic stimulatory effect on eIF-iso4F ATPase activity. However, the extent of stimulation of eIF4A on ATPase activity of eIF-iso4F is much lower than its effect on eIF-iso4F purified from wheat germ (11). This may be due to the fact that expressed eIF-iso4F is more active than the native eIF-iso4F purified from wheat germ. One possible interpretation of this difference is that the phosphorylation state of expressed eIF-iso4F is different from that of native eIF-iso4F purified from wheat germ. CDNA analysis indicated that eIF-iso4G has two potential protein kinase C phosphorylation sites (amino acids 396–399 and 501–504) and two potential casein kinase phosphorylation sites (amino acids 627–631 and 703–708) (10), both of which may be involved in translational regulation. Phosphorylation of eIF4E has been shown to affect its binding affinity for the cap structure (26). It was suggested that the function of eIF-iso4F might be regulated by phosphorylation, which is a post-translational phenomena (27). Despite the difference in ATPase activity, expressed eIF-iso4F exhibited the same capacity to support in vitro translation as native eIF-iso4F purified from wheat germ when satellite tobacco necrosis viral RNA was used as the messenger (14).

PABP exhibits a strong stimulation of ATPase activity of (eIF4A + eIF-iso4F) complex (Fig. 1 and Table I). The ATPase activity of (eIF4A + eIF-iso4E + eIF-iso4G + PABP) complex is 3 times the activity of the complex without PABP (Table I, 647 pmol versus 201 pmol). The presence of other initiation factors, such as eIF4B and eIF-iso4G, has no substantial effect on the stimulatory effect of PABP (Table I). However, 0.1 mM m7GTP (same as the concentration of ATP in the assay solution) does produce inhibition of the ATPase activity of (eIF4A + eIF-iso4E + eIF-iso4G) complex (Table I, nos. 9 and 14). The inhibitory effect of m7GTP is mediated by its interaction with eIF-iso4E,
since no inhibition was observed in the mixture without adding eIF-iso4E.

The stimulatory effect of PABP is shown more clearly in Fig. 2. The ATPase activity of eIF4A is enhanced 2-fold by the presence of eIF4B, and is further stimulated by the presence of PABP (Fig. 2A). The interaction between PABP and eIF-iso4G greatly enhances the ATPase activity of the initiation factor mixture (Fig. 2B). The ATPase activity of eIF-iso4G alone is enhanced more than 2-fold by the presence of PABP (Table I, nos. 15 and 16). But the ATPase activity of eIF-iso4G in the presence of PABP accounts for less than one third of the ATPase activity of (eIF4A + eIF-iso4E) or (eIF4A + eIF-iso4F) complex in the presence of PABP (Table I, comparing no. 16 to no. 9 or 11). PABP enhances the ATPase activity of (eIF4A + eIF-iso4F) by stimulating the synergistic effect of the two factors.

The above results were obtained in the presence of poly(U). It is known that the ATPase activity of the initiation factor complex is dependent on the properties of the RNA present in the assay solution (11, 23). We therefore investigated the effect of PABP on the ATPase activity of the initiation factor complex in the presence of globin mRNA. Fig. 3 shows the effect of PABP on the ATPase activity of (eIF4A + eIF-iso4F) complex in the presence of poly(U).
presence of globin messenger RNA. PABP increased the ATPase activity of the complex about 2-fold. However, the ATPase activity of the complex in the presence of globin mRNA is about half the activity in the presence of poly(U).

An unexpected observation is that the stimulatory effect of PABP is dependent on poly(U) concentration. Poly(U) at higher concentration abolishes much of the effect of PABP on the ATPase activity of the (eIF4A + eIF-iso4F) mixture (Fig. 4A). In the absence of PABP, increasing the poly(U) concentration leads to slight enhancement of ATPase activity (Fig. 4B). It has been shown that (eIF4A + eIF-iso4F) or (eIF4A + eIF-iso4F + PABP) complexes have different ATPase activity when different RNA were present in the assay solution (11, 23). The reason for this is not clear since the mechanism of (eIF4A + eIF4F) complex unwinding of RNA structure with energy derived from ATP hydrolysis is not well understood. An interpre-

Fig. 6. RNA helicase activity of eIF4A, (eIF4A + eIF4B), and (eIF4A + eIF4B + eIF-iso4F). In autoradiograph in A, lane 1 is RNA duplex + PABP; lane 2 is RNA duplex incubated for 6 min with (PABP + ATP); lane 3 is the RNA duplex heated for 5 min at 98 °C and then quenched in dry ice/ethanol. Lanes 5 and 6 show the ATP requirement for unwinding the RNA duplex. Lanes 8 and 9 show that nonspecific proteins do not affect the helicase reaction. In autoradiograph in B, lanes 1–5 are the time course of unwinding of RNA duplex by (eIF4A + eIF4B + eIF-iso4F); lanes 6–10 are the time course of unwinding of RNA duplex by (eIF4A + eIF4B + eIF-iso4F + PABP). The concentration of eIFs and PABP was 1.0 μM. Concentration of ATP/Mg2+ was 2.0 mM. Concentration of RNA duplex was 2.5 nM. All assays were performed at 37 °C. Graph in C shows the kinetic plots of unwinding of RNA duplex by eIF4A (filled circle), (eIF4A + eIF4B) complex (open triangle), (eIF4A + eIF4B + eIF-iso4F) complex (open square), and (eIF4A + eIF4B + eIF-iso4F + PABP) complex (filled square). The data were obtained by counting the bands in autoradiographs (B and data not shown) and calculating according the formula indicated under “Experimental Procedures.”
PABP Stimulates the ATPase and RNA Helicase Activity

The parameters $K_m$ and $k_{cat}/K_m$ were determined for the ATPase activity of (eIF4A + eIF-iso4F + eIF-iso4G) complex in the presence or absence PABP (Fig. 5, A and B). The $K_m$ value for the mixture in the presence of PABP is 40 ± 5 µM in the absence of PABP, $K_m$ is 93 ± 22 µM. The presence of PABP induces a 2-fold decrease in $K_m$ value. In addition, PABP leads to a 3-fold increase in $k_{cat}/K_m$ value. There is no unique interpretation for the effect of PABP on the $K_m$ or $k_{cat}/K_m$ value of (eIF4A + eIF-iso4F) complex since the ATPase activity of the complex is enhanced synergistically by the presence of the both factors, while the mechanism of this factor effect is unknown.

The above results clearly demonstrate that PABP increased the ATPase activity of the initiation factor complex. The effect of PABP on the RNA helicase activity of the initiation factor complex therefore was investigated. Fig. 6 shows the autoradiography results of the RNA helicase assay. Fig. 6A shows that PABP alone has no helicase activity. ATP hydrolysis is required (lanes 5 and 6). Two nonspecific RNA-binding proteins: MAX, which also binds DNA, and T7 RNA polymerase, had no effect on the unwinding reaction (lanes 7–9). Wheat germ eIF4A has no significant unwinding activity of the RNA duplex (radiograph not shown; Fig. 6C). eIF4B has some stimulation effect on the unwinding activity of eIF4A (Fig. 6C; radiograph not shown), whereas the same RNA duplex can be unwind successfully by mammalian eIF4A and (eIF4A + eIF4B) complex (24). However, the (eIF4A + eIF4B + eIF-iso4F) complex exhibits full RNA helicase activity of the RNA duplex (Fig. 6B, lanes 1–5). The RNA helicase activity of the complex is ATP-dependent. In the absence of ATP, no detectable unwinding of the RNA duplex is observed for the (eIF4A + eIF4B + eIF-iso4F) complex (Fig. 6A, lane 5; data not shown). Additionally, PABP increases the RNA duplex unwinding activity of (eIF4A + eIF4B + eIF-iso4F) complex (Fig. 6B, lanes 6–10 and c). These results suggest that the RNA helicase activity of the (eIF4A + eIF4B + eIF-iso4F) complex is coupled with the ATPase activity of the complex.

**DISCUSSION**

PABPs have been found in all eukaryotic cells studied thus far. Sequence analysis has revealed the extensive evolutionary conservation of the yeast, plant, and human PABP-encoding genes (20, 21). Early studies have suggested that PABP may be the mediator of the role of poly(A) tail (reviewed in Ref. 3). This suggestion has been confirmed by recent studies, which demonstrated that interactions among eIF4G, eIF4B, and PABP enhance the cap binding affinity of eIF-iso4F and poly(A) binding affinity of PABP (5, 6). These observations suggest that the interaction of PABP with eIF4G and eIF4B can increase the ability of the initiation factor complex to discriminate between capped and polyadenylated mRNA from capped nonpolyadenylated mRNA, or uncapped and polyadenylated mRNA, and therefore provide a competitive advantage for the efficient translation of capped and polyadenylated mRNA. Mutagenesis studies in the yeast system demonstrated that the interaction between eIF4G and PABP is a prerequisite for the stimulatory effect of poly(A) tail and the synergistic stimulatory effect of cap and poly(A) tail on in vitro translation (4, 28–30). These observations indicate that PABP is involved in translation initiation as part of the initiation factor complex.

In terms of the scanning model, the initial recognition of cap and poly(A) tail by the eIF4E and PABP in the initiation factor complex is the first step for the binding of 40 S ribosome to mRNA, which is followed by the unwinding of secondary structure in the 5′-untranslated region of mRNA with the energy derived from the hydrolysis of ATP (1, 19, 25). The previous studies indicated that the interaction between PABP and eIF4G enhances the initial recognition of cap and poly(A) tail by eIF4E and PABP (5, 6). Our present results show that the interaction between PABP and eIF4G and eIF4B also promotes the unwinding of secondary structure in the 5′-untranslated region of mRNA by the (eIF4A + eIF4B + eIF-iso4F) complex. In the scanning model of translation initiation, it is likely the scanning step, which seems to be a multiple step process (24), is the rate-limiting step of translation initiation, and a key target for translation regulation. The present results demonstrate that the interaction between PABP and eIF-iso4G increases both the ATPase activity and RNA helicase activity of the (eIF4A + eIF4B + eIF-iso4F) complex. Clearly, these results implicate PABP as an important component in the regulation of translation.

The mechanism of how the initiation factor complex unwinds RNA secondary structure in the 5′-untranslated region of mRNA during translation initiation is not well understood. Our present results and the results of previous assays demonstrate that the full RNA helicase activity of eIF4A needs the concerted action of two other factors, eIF4B and eIF4F or eIF-iso4F (24, 12). It has been suggested recently that eIF4A might undergo a cycle of conformational changes during ATP hydrolysis and that such conformational changes might be used by eIF4A to transduce the energy derived from ATP hydrolysis to physical work to unwind RNA secondary structure (31). Since there is no direct interaction between PABP and eIF4A (5), the role of PABP in the RNA helicase activity might be to stimulate the actions of eIF4B and eIF4F or eIF-iso4F in promoting the conformational change of eIF4A. Another possibility, suggested by the concentration dependence of poly(U) stimulation of ATPase activity (Fig. 4), is that PABP is involved in the release step of mRNA binding, which allows movement along the mRNA. A simple mechanism would involve binding of eIF4A to mRNA, stimulated by the presence of eIF4F or eIF-iso4F, to unwind the secondary structure of the mRNA. eIF4B subsequently stimulates the ATPase activity by increasing the release of ADP, possibly through a cycle of conformational changes. PABP, interacting with eIF4B, stabilizes the complex and allows movement along the mRNA by promoting release from one binding site, which has now become single-stranded, and progression further along the mRNA to continue unwinding activity.

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