Ribosomal Association of Poly(A)-binding Protein in Poly(A)-deficient Saccharomyces cerevisiae*

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Poly(A)-binding protein, the most abundant eukaryotic mRNP protein, is known primarily for its association with polyadenylate tails of mRNA. In the yeast, Saccharomyces cerevisiae, this protein (Pabp) was found to be essential for viability and has been implicated in mechanisms featuring roles in mRNA stability and as an enhancer of translation initiation. Although the mechanism of action is unknown, it is thought to require an activity to bind poly(A) tails and an additional capacity for an interaction with 60S ribosomal subunits, perhaps via ribosomal protein L46 (Rpl46). We have found that a significant amount of Pabp in wild-type cells is not associated with polyribosome complexes. The remaining majority, which is found in these complexes, maintains its association even in yeast cells deficient in polyadenylated mRNA and/or Rpl46. These observations suggest that Pabp may not require interaction with poly(A) tails during translation. Further treatment of polyribosomes lysates with agents known to differentially disrupt components of polyribosomes indicated that Pabp may require contact with some RNA component of the polyribosome, which could be either non-poly(A)-rich sequences of the translated mRNA or possibly a component of the ribosome. These findings suggest that Pabp may possess the ability to bind to ribosomes independently of its interaction with poly(A). We discuss these conclusions with respect to current models suggesting a multifunctional binding capacity of Pabp.

Eukaryotic mRNA associates with a variety of proteins, both in the nucleus as heterogeneous nuclear ribonuclear proteins and in the cytoplasm as messenger ribonuclear proteins. Both of these complexes have been implicated in various cellular activities including RNA processing, nuclear transport, mRNA stability, and translation (1–3). The most abundant RNA-binding protein found in a functional messenger ribonuclear protein complex is the poly(A)-binding protein (Pabp),1 which can also be found within nuclei as an heterogeneous nuclear ribonuclear proteins in some cell types (1). Early photo-cross-linking and nuclease protection experiments revealed that Pabp is typically found in association with polyadenylated mRNA and more specifically with the poly(A) tail (4–7). Subsequent isolation and disruption of the Pabp gene (PAB1) in Saccharomyces cerevisiae demonstrated that yeast Pabp is essential for cell viability, suggesting that the critical role of Pabp involves its binding interaction with mRNA poly(A) tails (8, 9). However, the mechanism for conveying the major function or functions of Pabp is unknown.

Several studies in vitro suggested that Pabp acts in concert with the poly(A) tail to enhance the translational efficiency of mRNA. For example, experiments in rabbit reticulocytes lysates showed that while excess poly(A) can inhibit translation of capped and polyadenylated mRNA, purified Pabp added in trans can reverse the inhibition (10–12). Interestingly, these results also led to a model suggesting an interaction between the 5′ and 3′ termini of mRNA because the effect of added poly(A) and Pabp was cap-dependent (13).

The function of S. cerevisiae Pabp in vivo was initially assessed by utilizing a temperature-sensitive allele of PAB1 (pab1-f364l), which at the non-permissive temperature resulted in a translation defect in addition to yielding longer than average poly(A) tails (14). Moreover, the resultant lethality could be suppressed by a mutation (spb2) in ribosomal protein L46 (Rpl46) without restoration of normal poly(A) tail lengths (14). This genetic result led to the appealing suggestion that the Pabp and the 60S ribosome (perhaps via Rpl46) interact, thereby implicating a role for Pabp in translation initiation. Taken together, these studies supported a model in which the Pabp facilitates translation by improving the re-initiation efficiency of a terminating ribosome in cis via interaction with the poly(A) tail.

Pabp from Xenopus laevis was also shown to be associated with translational activity during frog development (15). Analysis, beginning with stage VI oocytes and ending at the tadpole stage, revealed an increased synthesis of Pabp mRNA and protein that paralleled an increase in poly(A) content, even though total mRNA synthesis decreased during this time. In addition, Pabp was preferentially found in tissues with active protein synthesis, thereby supporting earlier observations that both poly(A) and Pabp are associated with the translational apparatus. Interestingly, Pabp levels are extremely low prior to, and during oocyte maturation, a time when discrimination between poly(A)− and poly(A)+ mRNAs plays a key role in regulating maternal mRNA translation (15). The limited amount of Pabp present during this time could amplify the relative translational advantage of poly(A)+ compared to poly(A)− mRNAs if Pabp played a key role in initiation.

Analysis of Pabp amino acid sequences from both vertebrate and invertebrate organisms revealed a highly homologous N terminus containing four RNA binding domains (I, II, III, and IV), each approximately 90–200 amino acids long and containing both RNP I- and II-type RNA recognition motifs (reviewed

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1 The abbreviations used are: Pabp, poly(A)-binding protein; UTR, untranslated region; RBD, RNA binding domain.
in Ref. 16). Extensive analyses using truncated Pabp peptides containing individual and/or combinations of RNA binding domains revealed that the domains do not harbor redundant functions as shown by differential binding to homoribopolymers and by the ability for specific domains to rescue the lethality of a PAB1 chromosomal deletion (9, 17, 18). Curiously, individual domains are more closely related to the corresponding domain among different organisms than to the remaining domains from the same protein supporting the notion that each domain has evolved distinct functions. In yeast, only domain IV can maintain viability, but it has relatively low specificity for physiological salt conditions (9, 18); in fact, domains I and II, together, generate the greatest specificity and affinity for poly(A) under physiological salt conditions (−100 mM NaCl or KCl), yet are dispensable for cell growth (14, 18). Moreover, the temperature-sensitive PAB1 allele, pab1-f364l, actually encodes a truncated, 66-amino acid peptide containing only a mutated domain IV and C-terminal residues, and as such lacks the strong poly(A) binding capability (14). Suppression of this allelic variant by a 60 S ribosomal protein (Rpl46) may therefore establish a critical involvement of Pabp in the translational apparatus, independent of a role in binding poly(A) tails (14).

In previous studies, we showed that poly(A)-deficient mRNA was efficiently translated in vivo after inactivation of poly(A) polymerase activity in a temperature-sensitive pap1-1 strain of S. cerevisiae (19, 20). This suggested that poly(A) tails are not required for efficient translation under these conditions and thereby questioned the necessity for a Pabp-poly(A) tail interaction to facilitate translation. In this report, we present evidence that the Pabp of the yeast S. cerevisiae remains associated with polyribosomes in the presence or absence of polyadenylated mRNA in vivo. Furthermore, the non-lethal deletion of RPL46 does not alter the amount nor ribosomal association of Pabp along polyribosome gradients. Taken together, these findings suggest that the essential function of Pabp might not be exerted via direct interactions with poly(A) tails nor Rpl46, adding further complexity to the mechanism of Pabp action. The apparent association of Pabp with ribosomes suggests an undetermined mechanism whereby Pabp might directly interact with another ribosomal protein, a ribosome-associated protein, or with RNA.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Media**—The experiments reported here were carried out in the following strains: T481 (MATa, ura3-52, ade2-101, lys2-801) and UR31481B (MATa, ade2/ade2, lys2, gal17, ura3-52, pab1-1). Construction of T481 and UR31481B strains harboring a chromosomal knockout of ribosomal protein L46 (rpl46) was done by electrottransformation of haploid cells with a BglII-EcoRI deletion fragment from pAS195 containing the URA3 marker flanked by RPL46 sequence. The resultant URA+ transformants were screened for cold sensitivity, and subsequent polyribosome profile analysis (see below) revealed the expected phenotype showing a decreased ratio of 60 S to 40 S subunits (14). All strains were propagated in YEPD media.

Preparation of Total Protein Lysates—Cells were grown to an 

**RESULTS**

The current model for Pabp function depicts the protein in a RNP complex, linking mRNA to ribosomes by binding to poly(A) tails and by binding to the 60 S ribosomal subunit through contact with ribosomal protein L46 (Rpl46) (reviewed by J. Jacobson in Ref. 22). Such interactions are predicted to enhance the rate of re-initiation of ribosomes in cis, thereby increasing the translational efficiency of a mRNA molecule. To test various features of this model, we performed a series of experiments that measure relative cellular and polyribosome-associated Pabp by monitoring its sedimentation profile in polyribosome gradients from S. cerevisiae deficient in poly(A) and/or Rpl46.

Total Amount of Cellular Pabp Is Similar in Yeast Strains Harboring the pap1-1 or rpl46 Mutation—In an early characterization of the Pabp of S. cerevisiae, it was predicted that the concentration of Pabp might be autoregulated at the translational level by the capacity for Pabp to bind A-rich sequences within the PAB1 mRNA 5′-untranslated region (9). Presumably, under conditions where Pabp is in excess of poly(A) tail substrate, the protein would bind to its 5′-untranslated region inhibiting further synthesis. Indeed, Pabp represses translation of its own mRNA in rabbit reticulocyte lysates in a manner dependent on its own poly(A)-rich 5′-untranslated region (23). We have shown previously that, when a yeast strain harboring a temperature-sensitive allele of poly(A) polymerase (pap1-1) is shifted to the non-permissive temperature (35 °C) for 1 h, there is an 80% loss of poly(A) content as well as a 2-fold reduction in mRNA levels (20). Based on these findings, we predicted that the loss of mRNA or poly(A) content might result in a reduction in total Pabp levels if an autoregulatory mechanism operates.
Equal numbers of harvested, and the remaining half was shifted to 35°C and harvested from strains containing either a normal (Pabp levels under conditions where both mRNA and poly(A) alterations or autoregulation leads to a new steady-state amount of protein. We constructed PAP1, rpl46 strains (24, 25). Taken together, these findings demonstrate that sudden reductions in poly(A) content or the absence of Rpl46 do not significantly alter total levels of Pabp.

Levels of Polyribozyme-associated Pabp Are Unaffected by Changes in Poly(A) Content—Although total cellular Pabp levels remain unchanged in the absence of poly(A) tails, a more significant measure of Pabp's functional activity might result from an investigation of the levels of this protein associated with polyribosomes under conditions that alter the content of poly(A). As mentioned above, when pap1-1 cells are shifted to the non-permissive temperature (35°C) for 1 h, poly(A) content decreases by 80% and mRNA levels fall by 50%. Therefore, we predicted that if Pabp binds primarily to poly(A) or other mRNA sequences, then the amount of polyribozyme-associated Pabp might be reduced by 80% or 50%, respectively, in shifted pap1-1 cells. Polyribosome lysates from wild-type (PAP1) and pap1-1 cells grown at both 25°C and 35°C were fractionated after sedimentation on low salt sucrose gradients. As shown in Fig. 2, the poly(A) profiles generated by the PAB1 gene were similar for the two temperatures. Furthermore, the poly(A) content of each fraction was separated by 3% polyacrylamide gel electrophoresis, transferred to nitrocellulose, and analyzed by Western blot. The relative amount of poly(A) was visualized using anti-PAB1 monoclonal antibody coupled to a fluorescein-conjugated secondary antibody. The results of a typical experiment shown in Fig. 1 and Table I indicate that inactivation of poly(A) polymerase and the ensuing loss of poly(A) from the cell have little impact on the total level of Pabp.

We also monitored Pabp levels in a strain containing a deletion of RPL46, since a mutation (pap1-1, rpl46) were prepared as above and analyzed by Western blot (Fig. 1). Table I shows the relative amounts of Pabp in RPL46 deletion strains grown at 25°C and after shifting to 35°C for 1 h and indicates that Pabp levels do not change significantly, even in the absence of Rpl46. In addition, we also measured relative amounts of the 60 S ribosomal protein, Tcm1p, and found a decrease in its level in RPL46 deletion strains, consistent with a response to a reduction in 60 S ribosomal subunit assembly as observed by deletions of other large subunit ribosomal proteins (24, 25). These findings demonstrate that sudden reductions in poly(A) content or the absence of Rpl46 do not significantly alter total levels of Pabp.

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Table I. Relative levels of Pabp and Tcm1p in various strains

| Genotype | Temperature | Pabp 35°C/25°C | Tcm1p | Pabp/Tcm1p |
|----------|-------------|----------------|-------|------------|
|          |             | rfu            |       |            |            |
| PAP1, RPL46 | 25          | 3,834,005      | 1,464,573 | 3          |
| PAP1, RPL46 | 35          | 3,765,950      | 1,795,140 | 2          |
| PAP1, rpl46  | 25          | 2,916,070      | 844,217  | 4          |
| PAP1, rpl46  | 35          | 2,838,142      | 796,505  | 4          |
| pap1-1, RPL46 | 25          | 2,263,319      | 1,090,635 | 2          |
| pap1-1, RPL46 | 35          | 2,387,025      | 1,107,429 | 2          |
| pap1-1, rpl46 | 25          | 1,777,759      | 617,029  | 3          |
| pap1-1, rpl46 | 35          | 2,272,737      | 842,730  | 3          |

Fig. 1. Amounts of Pabp and Tcm1p in pap1-1 and rpl46 strains grown at 25°C and 35°C. Strains with the indicated genotype were grown at 25°C to similar densities. Half the culture was harvested, and the remaining half was shifted to 35°C and harvested after 1 h. Polyribosome lysates were prepared as described under “Experimental Procedures.” Equal numbers of poly(A) units were separated by SDS-polyacrylamide gel electrophoresis, and the proteins were electro- phoretically transferred to nitrocellulose. Protein levels were determined by immunoblotting with monoclonal antibodies to each protein and detection with a common fluorochrome-conjugated secondary antibody and quantitated using a Molecular Dynamics FluorImager 575. Pilot experiments demonstrated that the levels of protein detected here fall within the linear range of detection.

We performed an initial experiment to determine the fate of Pabp levels under conditions where both mRNA and poly(A) changes are differentially reduced. Total cellular protein was isolated from strains containing either a normal (PAP1) or mutant (pap1-1) allele of poly(A) polymerase during growth at 25°C and after a 1-h shift to 35°C. Proteins were separated by 13% polyacrylamide gel electrophoresis and transferred to nitrocellulose for Western blotting. To quantitate the relative amount of Pabp under these conditions, the blot was probed with a monoclonal antibody against yeast Pabp followed by a fluorescein-conjugated secondary antibody. Relative amounts of Pabp were calculated by ImageQuant software after scanning the blots in a FluorImager 575™ (Molecular Dynamics). The results of a typical experiment shown in Fig. 1 and Table I indicate that inactivation of poly(A) polymerase and the ensuing loss of poly(A) from the cell have little impact on the total level of Pabp.
some profiles obtained from cell lysates harvested at 25 °C reveals a decrease in the number of large polyribosomes and an increase in the number of small polyribosomes (Fig. 2). This apparent change in polyribosome density results most likely from a decrease in the number of 60 S subunits available for 40 S subunit joining caused by the deletion of RPL46 (14). The polyribosome profiles appear to reveal an interesting difference between the polyribosome density after shift of pap1-1 to 35 °C, depending on the presence or absence of Rpl46. The possible significance of this difference will be treated elsewhere.2

To determine the impact of a deletion of RPL46 on the levels of polyribosome-associated Pabp, we fractionated polyribosome lysates obtained from PAP1, RPL46; Pap1-1, RPL46; and pap1-1, rpl46 strains grown either at 25 °C or following a 1-h shift to 35 °C. Again, proteins from each fraction were separated by electrophoresis and transferred to nitrocellulose for Western analysis. Fig. 3 shows the distribution of Pabp along the polyribosome gradients of each strain. We find that a deletion of RPL46 does not change the relative amount of polyribosome-associated Pabp (Table II); however, there is a shift in Pabp distribution coincident with the redistribution of polyribosomes due to the translation initiation defect seen in PAP1, rpl46 and pap1-1, rpl46 strains at 25 °C. At 35 °C in a pap1-1, rpl46 strain, the Pabp and polyribosome distributions are restored to that observed in strains with a normal RPL46 allele present, paralleling the restoration of a normal ratio of mRNA to ribosomes (Fig. 2). These data indicate that Pabp sediments in association with polyribosomes in the absence of Rpl46.

Co-localization of Pabp with Ribosomes following Disruption of the Polyribosome Complex—Our experiments thus far support the conclusion that Pabp sediments along low salt sucrose gradients as a protein component of active polyribosomes and not as a homogeneous aggregate that may be engaged nonspecifically with RNA. To further clarify the apparent interaction between Pabp and polyribosomes, we treated identical poly(A)-deficient polyribosome lysates from pap1-1, rpl46 cells incubated at 35 °C for 1 h with various agents known to differentially disrupt polyribosome complexes. We employed three types of established chemical treatments. First, treatment of the lysate with high salt (0.5M KCl) does not alter the salt-resistant polyribosome complexes (data not shown), but can release associated factors by disrupting relatively weak ionic interactions (21). Second, treatment with RNase A results in hydrolysis of RNA in polyribosomes, thereby releasing 80 S couples together with their associated factors. Finally, incubation of lysates with EDTA releases ribosomal subunits from mRNA and possibly disrupts other Mg2+-dependent protein interactions (13). Treated lysates were fractionated on sucrose gradients.

2 A. Proweller and J. S. Butler, manuscript in preparation.
gradients and protein fractions separated by electrophoresis and transferred to nitrocellulose. For Western blotting, we again employed the anti-Pabp antibody used in previous experiments as well as the antibody against ribosomal protein L3 (Tcm1p) to monitor the sedimentation behavior of 60 S ribosomal subunits. Fig. 4 shows the results of these treatments on the sedimentation pattern of Pabp and Tcm1p in a pap1-1, rpl46 strain shifted to 35°C for 1 h. The results show that inclusion of EDTA releases polyribosome-associated Pabp causing about half of the released Pabp to sediment with ribosomal subunits, while the remaining released Pabp sediments at the top of the gradient (Figs. 4A and 5, Table III). This contrasts with the effect of high salt and RNase A treatment, each of which releases Pabp to the lightest, ribosome-free portions of the gradient. The sedimentation behavior of Pabp in RNase A-treated samples suggests that the association of Pabp with polyribosomes does not result from a nonspecific, ionic interaction between ribosomes and Pabp since RNase A completely disrupts this interaction without changing the ionic strength of the solution and without removing Tcm1p from 60 S subunits (Fig. 4A). Accordingly, we consider it unlikely that Pabp merely sticks to ribosomes to yield the sedimentation patterns shown in Fig. 3.

About half of the Pabp in EDTA-treated lysates from poly(A)-deficient cells sediments in the portion of the gradients containing free 60 S subunits (Figs. 4A and 5, Table III), suggesting that Pabp may have an affinity for ribosomes independent of its ability to bind poly(A). Alternatively, Pabp may sediment in this portion of the gradient by virtue of binding to mRNA. We monitored the sedimentation behavior of a representative mRNA (TCM1, 1200 nucleotides) encoding ribosomal protein L3 in an effort to differentiate between these two possibilities. After inactivation of polyadenylation, RNase protection analysis reveals that this mRNA accumulates as a poly(A) + species (TCM1 A+) and a poly(A)-deficient species (TCM1 A−10) that has poly(A) tails between about 10 and 20 nucleotides (Ref. 20; Fig. 4B). The majority of each TCM1 mRNA species sediments in a peak in fractions 13–16, which partially overlaps the fractions containing 60 S ribosomes (revealed by Tcm1p; Figs. 4A and 5). Since the majority of TCM1 mRNA sediments in the portion of the gradient expected of ribosome-free mRNA after EDTA treatment, we suggest that the Pabp sedimenting in heavier fractions (fractions 9–13) could be doing so by virtue of its ability to interact with ribosomes independently of mRNA.
Ribosome Association of Poly(A)-binding Protein

The fact that a significant fraction of Pabp sediments with poly(A)-deficient polyribosomes is consistent with the possibility that Pabp may contact ribosomes independently of mRNA. Nevertheless, the sedimentation behavior of Pabp with ribosomal subunits observed in Fig. 4A could result from binding to small amounts of mRNA still associated with ribosomes under these conditions.

**DISCUSSION**

The poly(A)-binding protein from several organisms has been the subject of many studies attempting to elucidate the critical function or functions of this molecule. Its early detection by UV cross-linking and nuclease protection experiments showed that Pabp bound to mRNA via the polyadenylate tail and that the protein was extremely abundant (4–7). These primary observations established the notion that Pabp exerts an important function in association with the poly(A) tail, and early studies suggested that the function was to control 3'-exonucleolytic activity by establishing a physical barrier to such enzymes, thereby regulating stability of the mRNA (27, 28). This proposed function was consistent with models generated for the decapping and decay of poly(A).

In this report, we present evidence that the polyribosome binding function of *S. cerevisiae* Pabp apparently does not require mRNA with normal length poly(A) tails. In addition, we propose that a fraction of Pabp may associate with ribosomes independently of mRNA and that this association may involve an RNA or protein component of the ribosome. Our experiments demonstrated comparable distributions of Pabp along polyribosome gradients from both *PAP1* and *pap1-1* strains shifted to 35 °C, suggesting that Pabp may not require poly(A) tails for its association with polyribosomes. We also determined that Pabp does not require *RPL46* for its ribosomal association, since deletion of the *RPL46* gene in either *PAP1* or *pap1-1* backgrounds does not interfere with the co-sedimentation of Pabp and polyribosomes. From these results, we conclude that Pabp may not require poly(A) tails or *RPL46* for its essential function or functions.

The Pabps from several organisms ranging from invertebrates to mammals display many similarities and extensive homology in the N-terminal portion of the protein, which contains four RNA binding domains (RBDs) approximately 100 amino acids long, each containing the conserved RNP I (8 amino acids, "octamer") and II (6 amino acids) motifs, as well as additional conserved hydrophobic residues found throughout each domain. The N terminus was therefore predicted to harbor the activity necessary for binding to, for example, the poly(A) tails of mRNA. Structure/function studies using deletion mutants of yeast and amphibian Pabp have revealed that each RNA binding domain is not functionally equivalent within a single protein but may have comparable activity to the analogous domain in Pabps from different organisms (17, 18). The Pabp from *X. laevis* binds specifically to poly(A) and with significant affinity to poly(G), while having a lesser capacity to bind poly(U) in vitro. Truncated Pabps from *X. laevis* revealed that Domains I and II are necessary for specific RNA binding to poly(A) (17). Domain IV, alone, can bind poly(A) and poly(U) but with little discrimination; in fact, this domain binds better to poly(U) and more poorly to poly(A) compared to the full-length Pabp. Domains II and III together were found to have normal discrimination and binding efficiencies whereas domain I alone could not bind RNA.

In *S. cerevisiae*, domain IV joined with C-terminal residues was sufficient to maintain viability in a strain lacking a functional chromosomal copy of Pabp, suggesting that the critical function lies within this sequence (9, 18). Interestingly, domain IV is the most conserved RNA binding domain among Pabps from *S. cerevisiae*, Schizosaccharomyces pombe, *X. laevis*, and humans (18). In addition, this truncated peptide was shown to bind poly(A) in vitro, but in the absence of competitor RNA (9).
In contrast, binding studies of all four RNA binding domains from S. cerevisiae demonstrated a lack of poly(A)-binding specificity for individual domains, while only domains I and II together displayed the strongest and most specific poly(A)-binding activity at physiological salt concentrations (~0.1 M KCl or 0.1 M NaCl) in the presence of heparin (18). Under these conditions, domain IV bound better to poly(U) and poly(G) than to poly(A). Finally, this study also confirmed that domains I and II are not necessary for cell viability. The SPB (suppressor of poly(A)-binding protein) mutations, including rpl46 (spb2), were selected to suppress the defective function of a truncated Pabp containing only the essential domain IV and C-terminal sequences lacking the strong poly(A) binding activity (14). Moreover, the allele of PA1B used for suppression analysis contained an additional point mutation in domain IV, suggesting that the suppressors may bypass a non-poly(A) binding function of Pabp.

A recent study of HeLa cell Pabp showed that the protein was extremely abundant in the cytoplasm with a concentration of approximately 4 μM and with only 30% of Pabp binding with high affinity (K_d ~ 7 nm) to available poly(A) tails (31). This suggested that the majority of Pabp in HeLa cells could bind to alternative, non-poly(A), RNA sequences having lower affinities (K_d > 0.5 μM) for Pabp. Evidence for such a conclusion was obtained from a “Selex”-type experiment predicting Pabp binding to non-poly(A)-rich heptameric sequence. In addition, a previous study demonstrated that HeLa cell Pabp can be photo-cross-linked to RNA in cells labeled with either [3H]adenosine or [3H]juridine, suggesting that the protein can bind RNA sequences in addition to the poly(A) tail (32). Finally, Drawbridge et al. (33) reported that at least 90% of sea urchin Pabp was uncomplexed to poly(A). Taken together, these findings support the possibility that the essential function of HeLa cell Pabp might be carried out by RNA contacts other than the poly(A) tail.

The data presented here support the possibility that Pabp may have multiple functions that do not require a high affinity for mRNA poly(A) tails. An interaction with poly(A) may facilitate some function of Pabp, but lack of this sequence apparently does not inhibit the vital cellular role of Pabp. We demonstrated that Pabp remains associated with polyribosomes in the absence of poly(A) tails. This association may result from binding to a non-poly(A) RNA sequence, which might be a component of the ribosome, or it may result from a protein-protein interaction such as an undetermined ribosomal protein or associated factor. We have also shown by quantitation of Western blots derived from fractionated polyribosome gradients that a large fraction (approximately 40% see Table II of Pabp does not sediment with polyribosomes, arguing that, similar to the situation found in HeLa cells and sea urchins, Pabp exists in excess of poly(A) binding substrate (31, 33). These results support the idea that the mechanism through which Pabp may enhance translation initiation is more complicated than an interaction with ribosomes via the poly(A) tail and offers the intriguing possibility of other potential Pabp contacts in the polyribosome RNP.

We have also provided evidence that polyribosome-associated Pabp does not require the presence of Rpl46 in the 60 S ribosomal subunit. This finding is somewhat surprising, since a mutant of Rpl46 (spb2) suppresses the lethality of a temperature-sensitive allele of Pabp (pab1-f364l) containing only the essential RBD IV and C-terminal sequence (14). In this case, suppression may be through an indirect interaction with Pabp or perhaps a bypass mechanism. Consistent with a bypass mechanism, suppression of pab1-f364l by rpl46 mutations does not lead to restoration of abnormally long cytoplasmic poly(A) tails found in cells at non-permissive temperature (14), and structure-function studies demonstrate that the RBD IV and C-terminal sequence comprising Pabp-f364l lack an efficient poly(A) binding capacity (18). These considerations imply that if Pabp's ribosomal association conveys a critical role in translation initiation without the need for poly(A) binding, then we might also expect that the poly(A) status of an mRNA may not significantly alter this function of Pabp.

Finally, some RNA-binding proteins containing multiple RNP I and II motifs bind simultaneously to more than one RNA. For example, U1A binds pre-mRNA through RBD domain I and U1 small nuclear RNA through domain I, supporting the idea that the individual RNA binding domains have evolved distinct functions (26). The same may hold true for Pabp, whereby domains I and II bind to poly(A) tails while domain IV engages in crucial contacts with other RNA sequences associated with ribosomes.

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