Protein family review

Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression

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Summary

Most eukaryotic mRNAs are subject to considerable post-transcriptional modification, including capping, splicing, and polyadenylation. The process of polyadenylation adds a 3′ poly(A) tail and provides the mRNA with a binding site for a major class of regulatory factors, the poly(A)-binding proteins (PABPs). These highly conserved polypeptides are found only in eukaryotes; single-celled eukaryotes each have a single PABP, whereas humans have five and *Arabidopis* has eight. They typically bind poly(A) using one or more RNA-recognition motifs, globular domains common to numerous other eukaryotic RNA-binding proteins. Although they lack catalytic activity, PABPs have several roles in mediating gene expression. Nuclear PABPs are necessary for the synthesis of the poly(A) tail, regulating its ultimate length and stimulating maturation of the mRNA. Association with PABP is also a requirement for some mRNAs to be exported from the nucleus. In the cytoplasm, PABPs facilitate the formation of the 'closed loop' structure of the messenger ribonucleoprotein particle that is crucial for additional PABP activities that promote translation initiation and termination, recycling of ribosomes, and stability of the mRNA. Collectively, these sequential nuclear and cytoplasmic contributions comprise a cycle in which PABPs and the poly(A) tail first create and then eliminate a network of *cis*-acting interactions that control mRNA function.

Gene organization and evolutionary history

RNA-binding proteins are often purified and classified on the basis of the RNA sequences with which they interact [1]. One class of these factors comprises proteins recognizing the homopolymeric polyadenylate tracts that are added to the 3′ end of most mRNAs. Poly(A)-binding proteins have been identified in many eukaryotes, but appear to be absent from prokaryotes. PABP genes have been cloned from a number of organisms, and their sequences are available in several databases; a current list with database links is available as an additional data file with the online version of this article and on our website [2]. Typically, only one gene encoding cytoplasmic PABP (PABPC) is present in the single-cell eukaryotes, whereas multiple PAPBC genes are present in metazoans and plants (Table 1, Figure 1). A single gene encoding a nuclear PABP (PABPN) has also been identified in cow, frog, human, mouse, fly, worm, and yeasts (Figure 1). A phylogenetic analysis comparing all known PABP protein sequences groups PABPs by organism type (such as metazoans, yeast, and plants) and also identifies similarities among the PABP family members (Figure 1). To date, genes encoding a single nuclear PABP and four cytoplasmic PABPs, as well as four pseudogenes, have been identified in human cells, and their chromosomal locations have been mapped (Table 2). In humans, three lineages of PABP proteins are observed: cytoplasmic PABPs (PABPC1, PABPC3, and iPABP); nuclear PABP (PABPN1); and X-linked PABP (PABPC5). Within the PABPC group, PABPC1 and PABPC3 are most closely related. Interestingly, the mouse gene encoding the alternate PABP,

Table 1

Genes encoding cytoplasmic PABPs in various organisms

Organism	Number of PABPC genes
Arabidopsis thaliana	8
Caenorhabditis elegans	2
Candida albicans	1
Drosophila melanogaster	1
Homo sapiens	4
Mus musculus	2
Saccharomyces cerevisiae	1
Schizosaccharomyces pombe	1
Xenopus laevis	3

mPABPC2, seems to be a retroposon, as it has no introns and its promoter is distinct from that of *mPABPC1* [3]; *mPABPC2* is most closely related to *hPABPC3*, which also lacks introns [4]. Similarly, all the characterized *PABPC5* genes lack introns [5], suggesting that they too may be derived from retrotransposition events.

A comparable evolutionary analysis was reported for the eight *PAB* genes identified in the plant *Arabidopsis thaliana* [6]. Phylogenetic comparisons coupled with expression analyses identified four classes of PABP proteins. In class I (*PAB3* and *PAB5*), expression is limited to reproductive tissue; class II members (*PAB2*, *PAB4* and *PAB8*) are highly and broadly expressed; class III PABPs (*PAB6* and *PAB7*) have a restricted, weak expression pattern; and the sole member of class IV (*PAB1*) has low, tissue-specific expression. Comparison of the *Arabidopsis* PABPs with those from rice indicates that the duplication events which gave rise to classes I-III in flowering plants occurred prior to the divergence of monocots and dicots, more than 200 million years ago [6]. By analyzing the conservation and loss of introns within the PABP gene family, an evolutionary model has been derived in which an ancestral PABP independently gave rise to classes II, III and IV, with class I subsequently derived from class II [6]. Although all eight of the *Arabidopsis* PABPs are more closely related to the set of nuclear PABPs than to the PABPs of most other eukaryotes (Figure 1), none of these proteins appears to be an authentic PABPN1 species**.**

One interesting characteristic conserved among the *PABPC1* genes is an adenylate-rich region in the 5′ untranslated region (UTR). Several studies have suggested that PABP regulates its own expression by binding to these sequences [7-9].

Characteristic structural features

The association of PABPs with poly(A) requires a minimal binding site of 12 adenosines, and multiple PABP molecules can bind to the same poly(A) tract, forming a repeating unit covering approximately 27 nucleotides [10-13]. *In vitro* binding affinities of PABP for poly(A) are of the order of 2-7 nM [13-15]. PABPs interact with poly(A) via RNA-recognition motifs (RRMs; Figure 2).

The RRM is the most prevalent domain used in the recognition of RNA, as shown by its presence in hundreds of different proteins [16]. RRMs, which are typically 90-100 amino acids in length, appear to be present in proteins in all types of organisms, suggesting that this is an ancestral motif with important functions in RNA biology. Solution nuclear magnetic resonance (NMR) and X-ray crystallographic studies have determined that the RRM is a globular domain composed of a four-stranded anti-parallel β sheet backed by two α helices (Figure 3a) [17]. The central two β strands of each RRM include two highly conserved sequence motifs, octameric RNP1 ((K/R)-G-(F/Y)-(G/A)-F-V-X-(F/Y), where X is any amino acid) and hexameric RNP2 ((L/I)-(F/Y)-(V/I)-(G/K)-(N/G)-(L/M)) (Figure 3a). The electron density map of the human PABPC1-oligo(A) complex identifies eight adenylate residues extending through a trough lined by the β-sheets of the RNPs (Figure 3b) [17]. Specificity for recognition of poly(A) is primarily mediated via van der Waals contacts, hydrogen bonds, and stacking interactions with conserved residues within the RNP motifs [17].

Cytoplasmic PABPs

The overall structure of the cytoplasmic PABPs is highly conserved and consists of four RRMs connected to a carboxy-terminal helical domain by an unstructured linker region rich in proline and methionine residues [12,18]. Phylogenetic analysis suggests that the four RRMs arose from successive duplications before the divergence of yeast and mammals [19]. The first two RRMs make up one functional unit and the latter two make up a second. This conclusion is derived partly from the observation that residues participating in RNA recognition within RRM1 are most similar to those in RRM3, while those of RRM2 are most like those of RRM4 [17]. Although each RRM is capable of binding RNA, they are not functionally equivalent, as they have differing affinities for poly(A) [15].

The carboxy-terminal helical domain is highly conserved. In humans it is composed of five helices (Figure 3c), while the yeast protein has only four, lacking an ortholog of the first helix [20,21]. The carboxy-terminal domain is not required for RNA recognition, is dispensable for cell viability in yeast [13,15], and is missing from PABPC5 proteins [5]. This domain is shared with HECT domain proteins in the hyperplastic disc (HYD) family of ubiquitin-protein ligases [22], but there is no evidence that PABPs play any role in protein degradation. The carboxy-terminal domain is, however, the site of interaction with factors regulating polyadenylation, deadenylation, translation initiation, and translation termination (see below).

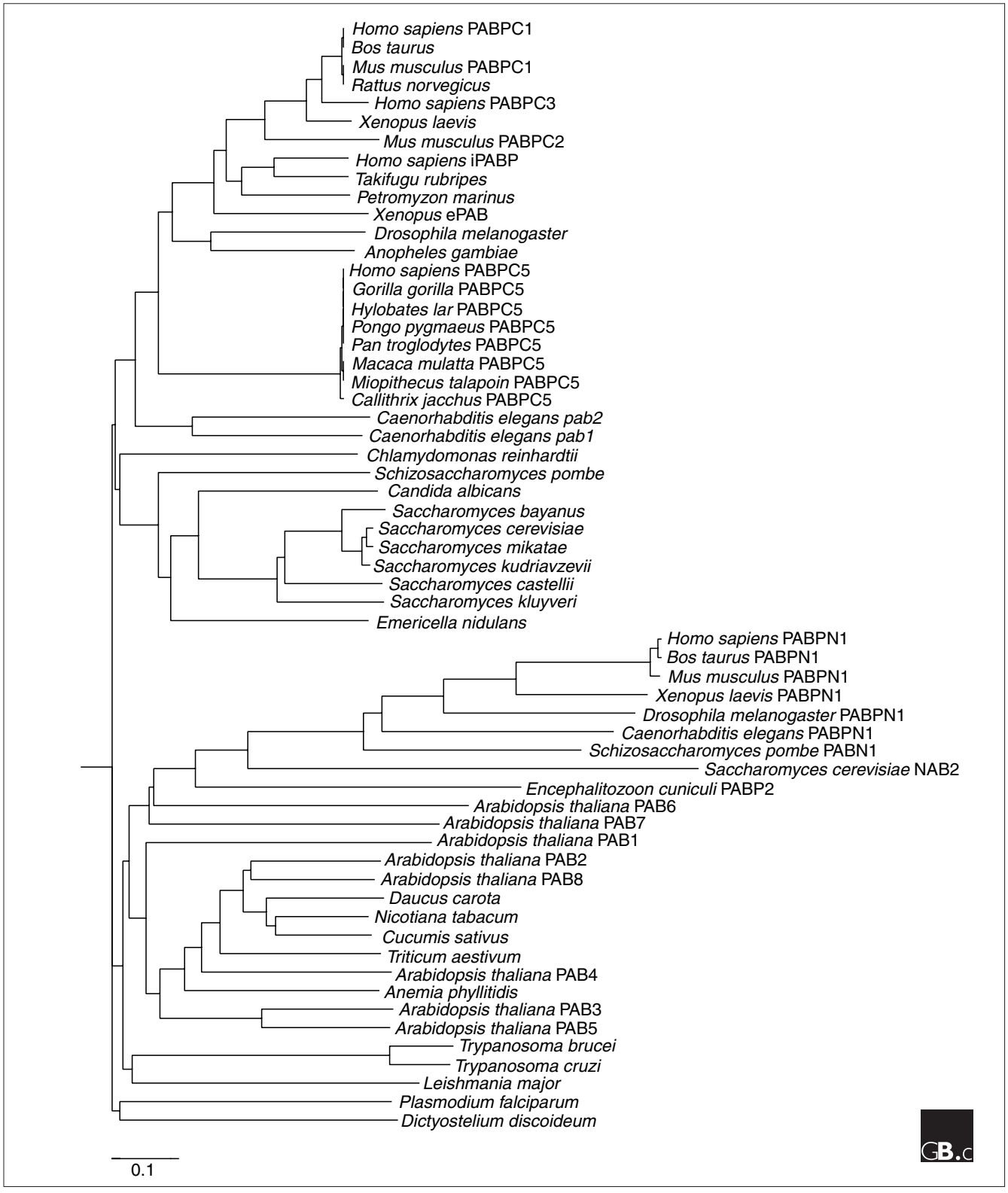


Figure 1
Predicted evolutionary relationships of PABPs. Full-length PABP sequences were compiled from various databases (see Additional data files) and aligned using the CLUSTALW program at the European Bioinformatics Institute [122]. The tree was constructed using the neighbor-joining method [123] and drawn using Phylodendron [124]. The scale bar represents 0.1 substitutions. In the instances where no PABP name is given, only a single PABP protein has been identified in that organism.

Table 2

Chromosomal location of human PABP genes

Gene name	Chromosomal location
PABPC1	8q22.2-q23
PABPC3	13q12-q13
iPABP	1p32-36
PABPC5	Xq21.3
Pseudogene 1	4
Pseudogene 2	14
Pseudogene 3	6, 12, 21, or X
Pseudogene 4 (formerly *PABP4*)	15
PABPN1	14q11.2-q13

Information is derived from [10,11,13]. The map position of Pseudogene 3 is uncertain.

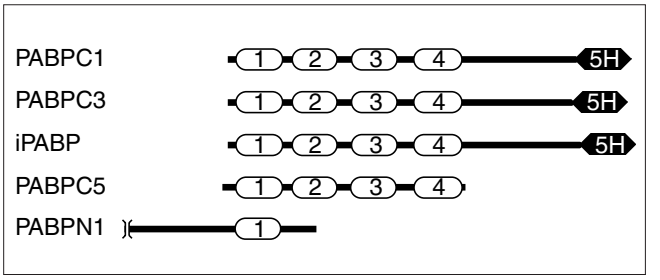


Figure 2
The domains of human PABPs. PABPC1, PABPC3, iPABP, PABPC5 and PABPN1 are shown, aligned on their first RNA-recognition motifs (RRMs). White capsules represent individual RRMs; black hexagons (5H) represent the five conserved helices at the carboxyl terminus. Inverted brackets indicate the site of expansion of a run of alanines in PABN1 that leads to the synthesis of PABPN1 with 12-17 alanines and results in the autosomal recessive disease oculopharyngeal muscular dystrophy (OMPD) [24,25]. PABPN1 accumulates in OMPD patients and forms intranuclear inclusions that appear to sequester mRNAs and associated factors and promote cell death [25].

Nuclear PABPs

The structure of the nuclear PABPs is not as well understood as that of the cytoplasmic PABPs, largely because crystal and NMR structures have yet to be determined, but it is known that they typically have an acidic amino terminus followed by a single RRM and an arginine-rich carboxy-terminal domain. Recognition of poly(A) requires both the RRM and the arginine-rich domain [23]. A run of alanines in PABN1 is expanded in the recessive disease oculopharyngeal muscular dystrophy (see Figure 2) [24,25].

In yeast, the nuclear PABP is essential for viability and is encoded by the *NAB2* gene [26]. Unlike other poly(A)-binding proteins, Nab2p uses an Arg-Gly-Gly (RGG) domain for binding. This protein also contains a Cys-Cys-Cys-His zinc-binding motif, similar to one in RNA polymerase subunits, and a glutamine-rich region that contains a variable number of Gln-Gln-Gln-Pro segments, the number of which is strain-dependent.

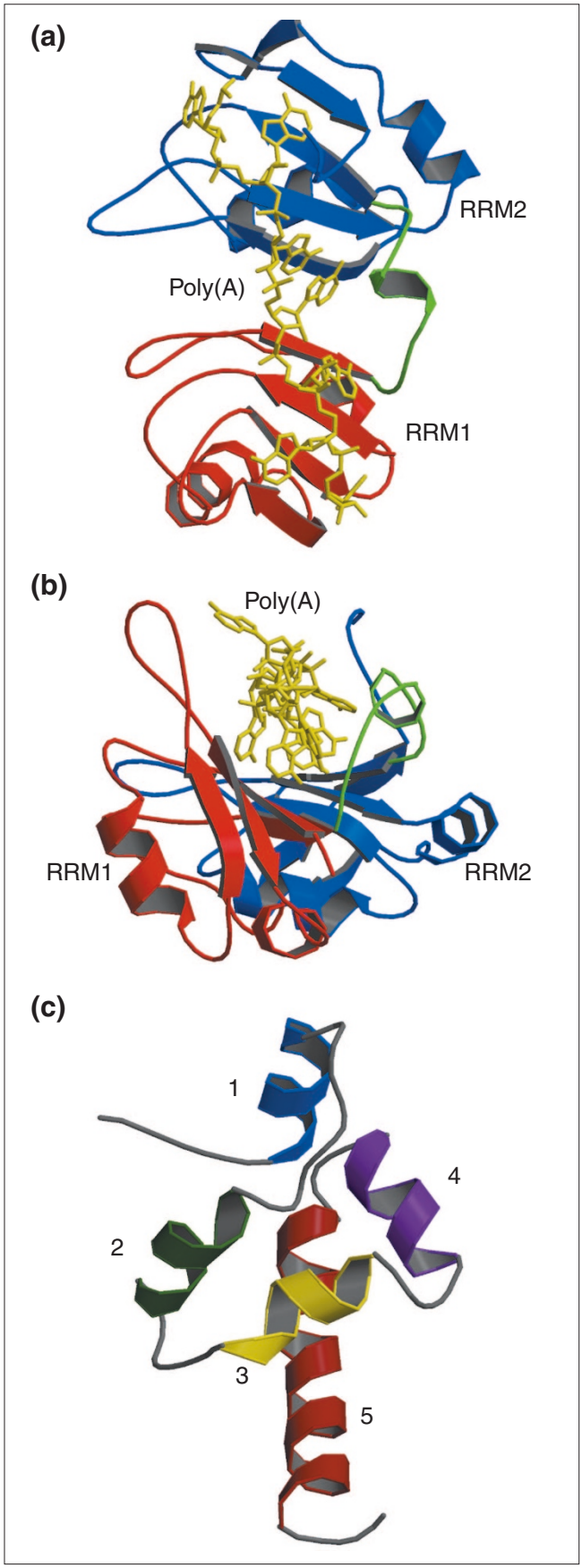
Localization and function

PABPs have crucial roles in the pathways of gene expression. They bind the poly(A) tails of newly synthesized or mature mRNAs and appear to act as *cis*-acting effectors of specific steps in the polyadenylation, export, translation, and turnover of the transcripts to which they are bound. Lacking any evident catalytic activity, PABPs provide a scaffold for the binding of factors that mediate these steps and also apparently act as antagonists to the binding of factors that enable the terminal steps of mRNA degradation.

Polyadenylation

Messenger RNAs synthesized in the nucleus generally contain a 3′ poly(A) tail; the rare exceptions to this rule are principally the transcripts of replication-dependent histone genes. Newly synthesized poly(A) tails of different mRNAs are relatively homogeneous in length and approximately 200-250 residues in mammals and 70-90 residues in yeast [27]. These poly(A) tracts are not encoded within genes but are added to nascent pre-mRNAs in a two-step processing reaction that involves site-specific cleavage and subsequent polyadenylation of the upstream cleavage product [23,28-30]. Throughout eukaryotes, pre-mRNA cleavage and polyadenylation take place in a large complex (500-1,000 kDa) that includes poly(A) polymerase (PAP) and many additional factors. In general, the factors regulating PAP stimulate both the specificity and processivity of an otherwise marginally active and indiscriminate enzyme. In so doing, they not only regulate the process of polyadenylation but also determine the ultimate size of the poly(A) tail.

In mammalian cells, PABPN1 binds nascent tracts of 11-14 adenylate residues [31] and, along with cleavage and polyadenylation specificity factor (CPSF), stimulates PAP to switch from distributive synthesis (dropping off after synthesis of a few nucleotides) to processive (continuous, high-speed) synthesis [32,33]. PABPN1 monomers continue to bind available, nascent adenylates until the full-length poly(A) tail has been synthesized and the polymerase then reverts back to its distributive mode [34]. This sequential binding is accompanied by the formation of linear filaments and 21 nm spherical particles: the latter are thought to serve as 'molecular rulers' that dictate the final length of the poly(A) tail [34]. In this model, the particle is postulated to encompass a stable polyadenylation complex and to tolerate PABPN1-poly(A) oligomers until the tail reaches 200-300 nucleotides. Beyond that point, increased poly(A) length is believed to be compromised by disruption of critical interactions between PAP and CPSF [34].



PABPs also play a role in the polyadenylation of yeast pre-mRNAs. Recent studies indicate that Nab2p is the most likely candidate for the yeast equivalent of PABPN1 function, at least for a subset of mRNAs. Mutations in *NAB2* promote hyperpolyadenylation of mRNA that cannot be reversed by overexpression of Pab1p [35]. The failure to detect this activity of Nab2p in earlier studies may be attributable to inhibitory interactions between Nab2p and its nuclear import receptor Kap104p, and/or to the preponderance of Pab1p in whole-cell extracts used for *in vitro* polyadenylation and the consequent obstruction of Nab2p activity by Pab1p bound to nascent poly(A) [35]. Interestingly, mutations in the yeast gene encoding cytoplasmic PABP, *PAB1,* cause a significant increase in mRNA poly(A) tail lengths *in vivo* and *in vitro* [36-38], and this effect, too, is partly attributable to a switch of PAP (Pap1p) between processive and distributive activities. Unlike the process in mammalian cells, the yeast switch appears to be directly regulated by Fip1p and Yth1p, two factors unrelated to nuclear or cytoplasmic PABPs, and only indirectly regulated by Pab1p [39-41]. Pab1p interactions underlying this indirect effect may include its binding to the nascent mRNA [28] or a direct interaction with the RNA-processing factor Rna15p [37].

Evidence for a direct role for Pab1p in yeast poly(A) length control comes from experiments analyzing the Pab1p-mediated regulation of poly(A) nuclease (PAN). This exonuclease, comprising the Pan2p and Pan3p proteins, appears to trim up to 20 residues from excessively long newly synthesized poly(A) tails in an mRNA-specific manner [42-44]. Pan2p, the subunit with apparent exonuclease activity, is positively and negatively regulated by interactions with Pan3p and Pbp1p, respectively; both of the latter interact with Pab1p (D.M. and A.J., unpublished observations; [43-45]).

Nuclear export

A second role for PABPs in the nuclear maturation of mRNA can be inferred from experiments in which impaired 3′ processing interferes with export of mRNAs to the cytoplasm. In both mammalian cells and yeast, mRNAs are generally retained in the nucleus when they lack a functional polyadenylation signal or when polyadenylation is inhibited by the absence or inactivity of specific catalytic factors [46-49]. Since the failure to polyadenylate an mRNA would deprive it of

Figure 3
Structures of the domains of human PABPC1. **(a)** Crystal structure of RRMs 1 and 2 in association with poly(A) [17]. The central two β strands of each RRM include two highly conserved sequence motifs, octomeric RNP1 ((K/R)-G-(F/Y)-(G/A)-F-V-X-(F/Y), where X is any amino acid) and hexameric RNP2 ((L/I)-(F/Y)-(V/I)-(G/K)-(N/G)-(L/M)), which is repeated six times. **(b)** The RNA-binding trough that is present when RRM1 and RRM2 of human associate with poly(A). **(c)** NMR structure of the five carboxy-terminal helices [21]. Figures were generated by MOLSCRIPT 2.0 using data from Protein data bank (PDB) files (a,b) 1CVJ and (c) 1G9L [125,126].

bound PABPs, nuclear retention of mRNA could be attributable to an essential role for PABPs in mRNA export.

As noted above, PABPs coat the nascent poly(A) tail and play a role in determining its ultimate length. How, then, might this poly(A)-PABP complex facilitate the exit of mRNAs and their associated proteins (mRNPs) from the nucleus? Consistent with the propensity of PABPs to form interactions critical to specific functions, yeast Pab1p has been shown to interact with specific nucleoporins [50] and the nuclear export signal export receptor, Xpo1p [49], and Nab2p has been shown to interact with Gfd1p, a nuclear-pore-associated protein [51]. The presence of bound Pab1p or Nab2p could serve as a determinant of an mRNP's export competence, in a manner analogous to the function of the RNA export factor Yra1p [52]. This view is consistent with the observed nucleocytoplasmic shuttling of yeast and mammalian PABPs [53-56] and with the inhibitory effects on mRNA export caused by interactions between the influenza virus NS1A protein and PABPN1 [57].

The notion of a direct role for PABPs in mRNA export may, however, be too simplistic. It does not accommodate examples of mRNAs that enter the cytoplasm without conventional 3′ processing [58,59], viable mutants devoid of PABP [36], or functional interactions between the 3′ processing apparatus and the factors that promote mRNA export [49,60]. The latter reflect a quality control mechanism that leads to retention of an mRNA in the nucleus (often at its transcription site) in the event of processing problems [49,61,62]. This apparent checkpoint illustrates the interdependence of many steps in gene expression and the manner in which such regulatory mechanisms can make indirect effects appear to be direct.

Translation initiation

After an mRNA enters the cytoplasm, the association of PABP with its poly(A) tail promotes 5′-3′ interactions that stimulate initiation of its translation [27,63]. Formation of this 'closed loop' [27] was shown by Sachs and colleagues [64-66] to promote the recruitment of 40S ribosomal subunits and to be dependent, at a minimum, on interactions between initiation factor eIF4G and PABP and concurrent interactions between eIF4G and the cap-binding protein eIF4E (Figure 4). The existence of a translational regulatory network involving PABP, eIF4G, and eIF4E is consistent with the impaired-translation phenotypes of yeast strains lacking functional Pab1p [36] and provides a mechanistic basis for the synergistic effects on translation known to occur when mRNAs are both capped and polyadenylated [65,67,68]. The combined cooperative interactions enhance the affinity of eIF4E for the 5′ cap of the mRNA by lowering its dissociation rate [69-72], stimulate the RNA-binding activity of PABP [73], and increase the ATPase and RNA helicase activities of eIF4A, eIF4B, and eIF(iso)4F [74]. The combination of these effects also provides an effective means for the protein synthesis apparatus to ensure preferential translation of mRNAs containing both a cap and a poly(A) tail [74] and may create an opportunity for ribosomes to recycle from the 3′ to the 5′ end of the same mRNA [27,75].

Studies in yeast and mammalian cells have shown that the Pab1p-eIF4G interaction requires RRM1 and RRM2 of Pab1p (the same RRMs required for poly(A) recognition) and an amino-terminal domain of eIF4G [65,76-78]. Several additional experiments have indicated, however, that the network of 5′-3′ interactions regulating translation initiation goes well beyond the communication of a single domain in PABP with another in eIF4G. This was initially suggested by the existence of viable yeast *pab1* mutants in which the Pab1p-eIF4G interaction could not occur [65] and others that had defects in poly(A)-dependent translation but no defects in eIF4G binding [76]. The potential complexity of PABP's translation-promoting interactions is illustrated by interactions of PABPs in wheat germ with the initiation factor eIF4B [73] and in mammals with the PABP-interacting proteins Paip1 and Paip2 [79-82]. Paip1 is homologous to the central segment of mammalian eIF4G and binds with high affinity and 1:1 stoichiometry to two sites in PABP, one in RRMs 1 and 2 and the other in the carboxy-terminal domain [79,80]. The region of eIF4G to which Paip1 is homologous encompasses one of two binding sites for the RNA helicase eIF4A. Not surprisingly, Paip1 also interacts with eIF4A, and is capable of stimulating the translation of a reporter mRNA when overexpressed in cultured cells [79]. Paip2, a low-molecular-weight acidic protein, binds PABP at two sites, one in RRMs 2 and 3 and one in the carboxyl terminus [81,82]. Binding of Paip2 to the RRM2-3 region competes effectively for binding of Paip1 to PABP, reduces PABP binding to poly(A), and inhibits the translation of polyadenylated mRNA [81,82].

Tethered-function assays in yeast and *Xenopus* that exploit PABP fusions to the bacteriophage MS2 coat protein also underscore the intricate nature of PABP's stimulatory effects on translation [83]. PABP tethered at specific MS2 coat binding sites stimulates translation of a reporter mRNA *in cis*, but not *in trans*, and can do so without its poly(A)-binding activity and in the absence of a poly(A) tail [83]. With the exception of the yeast requirement that Pab1p be bound to poly(A) in order to interact with eIF4G [64], this implies that, at least with respect to translational stimulation, poly(A) simply provides a binding site for PABP. The failure of yeast Pab1p to function in the absence of bound poly(A) may reflect the selective inability of yeast eIF4G to stabilize the packing of poly(A)-associated RRMs 1 and 2 in a manner comparable to that achieved by the eIF4Gs of other species [84]. Tethered function assays also reveal that RRMs 1 and 2, or RRMs 3 and 4, of *Xenopus* PABP are as capable of translational stimulation as the full-length protein, despite the fact that RRMs 3 and 4 lack the ability to interact with eIF4G or Paip1 [83]. Like the Pab1p-eIF4G interaction mutants in yeast [65,76], the novel PABP interactors in mammals and plants [73,79-82], and the

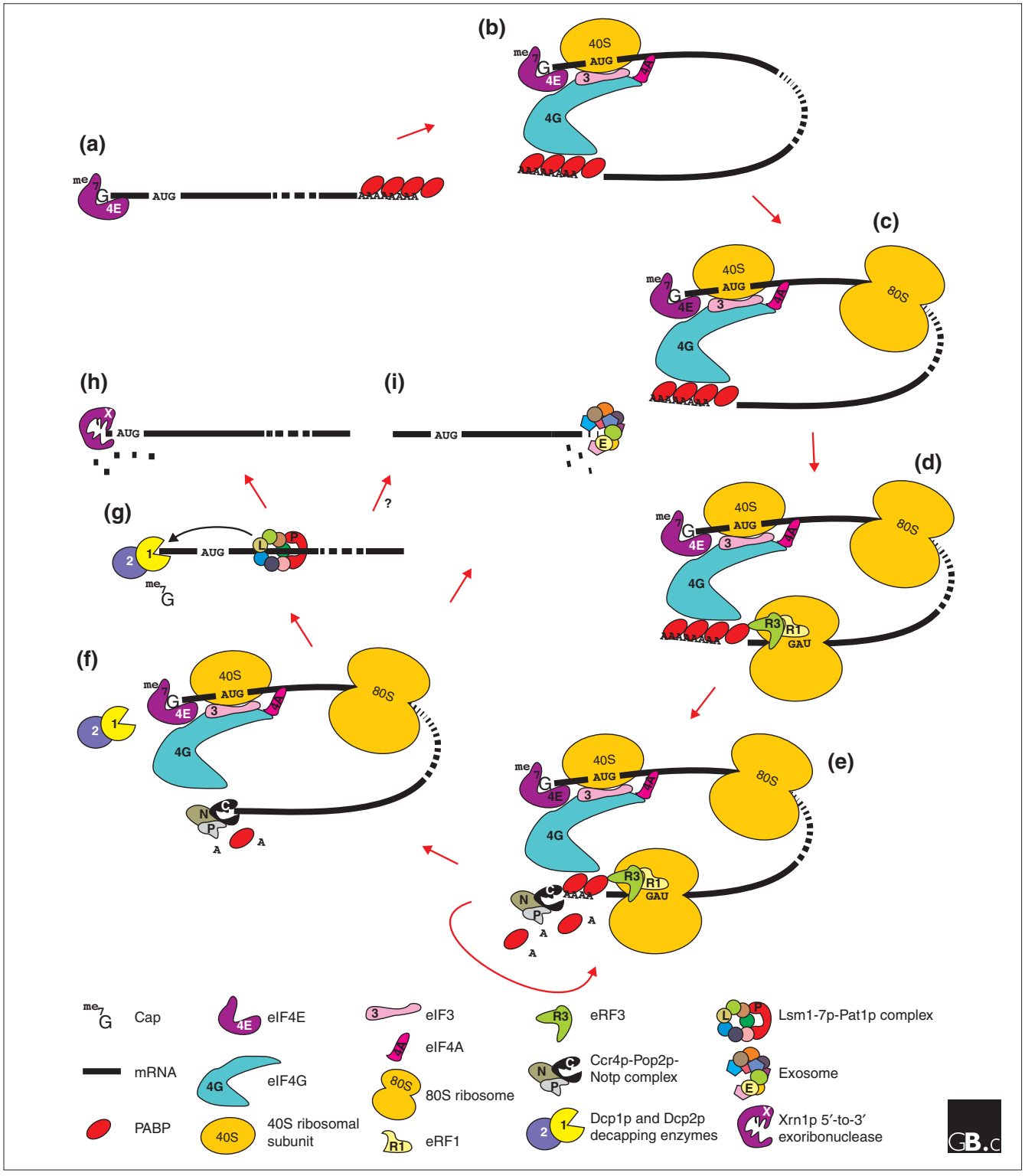


Figure 4
Roles of PABP in mRNA translation and stability. This model depicts different stages of a cytoplasmic mRNA 'life cycle', in which distinct roles can be ascribed to PABP. **(a)** Association of PABP with the mRNA poly(A) tail. **(b)** Interaction of PABP with elongation initiation factor eIF4G to promote formation of the 'closed loop', thus **(c)** initiating translation and antagonizing decapping. **(d)** Interaction of PABP with the termination factor eRF3 and recycling of the ribosome from the 5′ to the 3′ end of the same mRNA. **(e)** Poly(A) shortening by the Ccr4p-Pop2p-Notp deadenylase complex. **(f)** Loss of the poly(A) tail and PABP, facilitating **(g)** dissociation of the proteins of the mRNP, binding of the Lsm1-7p-Pat1p complex, and decapping by the decapping proteins Dcp1p and Dcp2p, and subsequent **(h)** 5′-to-3′ degradation of the mRNA by the exonuclease Xrn1p or **(i)** 3′-to-5′ degradation by the exosome.

unique domain requirements for *trans*-activation of translation by Pab1p [78], this observation implies that interaction with eIF4G is not likely to be the only mechanism by which PABP stimulates translation. One alternative model for PABP function, supported by genetic analyses in yeast [85] and the biochemical properties of poly(A)-deficient mRNAs *in vitro* [67], suggests that PABP is also a regulator of the joining of the 60S subunit to the 40S preinitiation complex. The studies in yeast indicate that PABP controls 60S joining by regulating the activities of two RNA helicases, Ski2p and Slh1p [85].

Additional insight into the translational networks affected by the presence of PABP is derived from studies of the tactics that viruses and cells use to modulate PABP structure and/or activity. For example, rotaviruses reroute translation for their own purposes by synthesizing a protein, NSP3, which serves as a PABP analog. NSP3 binds to specific 3′ sequences on viral mRNAs and effectively circularizes those transcripts, and mimics PABP, by also binding to eIF4G [86]. Enteroviruses, on the other hand, choose to eliminate the activity of PABP, rather than replace it. As part of a general assault on host cap-dependent translation, these viruses express two proteases, 2A and 3C, that not only remove the PABP-interacting domain of eIF4G but also cleave PABP into several fragments [87,88]. PABP interactions and activity, at least in plants, are also altered by changes in its phosphorylation status [89] and may be affected by arginine methylation within the domain separating RRM4 from the carboxy-terminal helices [90].

In addition to their global effects on translation initiation, PABPs can also selectively affect the translation of individual mRNAs. PABPs can bind oligoadenylate tracts in the 5′ UTRs of their own mRNAs, thereby repressing their own translation (and possibly their stability [17]) [9,91]. This autoregulation can be mimicked both *in vitro* and *in vivo*, can be abolished by deletion of the adenylate-rich region and can be conferred on other mRNAs by insertion of the adenylate-rich tract within their 5′ UTRs. In each case, the presence of PABP is required to mediate the observed effects. The inhibition of translation has been ascribed to an inability of the 60S ribosomal subunit to join the pre-initiation complex [92]. PABP can also facilitate the binding of translational repressors specific for other mRNAs, such as that encoding the iron-oxidizing protein ceruloplasmin [93], and can activate the translation of a large number of mRNAs whose polyadenylation is developmentally controlled [94], as well as functioning as an mRNA-specific activator. Cytoplasmic PABP in *Chlamydomonas reinhardtii*, normally a 69 kDa polypeptide, is imported into chloroplasts where it is processed to a 47 kDa form that binds the 5′ UTR of the *psbA* mRNA and activates its translation [95]. The latter role of PABP is particularly intriguing in light of the generally prokaryotic nature of chloroplast translation systems.

Translation termination

The eukaryotic translation termination factor eRF1, which is responsible for catalyzing polypeptide hydrolysis in response to recognition of any of the three nonsense codons by the ribosome, appears to be activated by the GTPase eRF3 [96]. The amino-terminal region of eRF3 does not participate in this interaction with eRF1, but does interact directly with the carboxy-terminal domain of cytoplasmic PABPs [21,97,98]. The eRF3-PABP interaction appears to enhance the efficiency of termination in cells with mutated or aggregated eRF3 [98] and to promote ribosome recycling for multiple rounds of translation on the same mRNA [99]. It also seems to minimize the multimerization of PABP monomers on poly(A), possibly expediting access of poly(A) shortening enzymes to their substrate and linking translational termination to normal mRNA decay [97]. Additional insights into the role of PABPs in translation termination come from analyses of instances in which termination occurs abnormally, such as at premature nonsense codons. In this case, termination is thought to be aberrant because of the creation of a 'faux' UTR, an untranslated region lacking at least one of the factors required for efficient polypeptide hydrolysis and ribosome release that are normally positioned 3′ to a termination codon by interaction with poly(A)-associated PABP [100].

Decay of mRNA

The process of mRNA decay can be initiated by three distinct events: endonucleolytic cleavage, removal of the 5′ cap, and poly(A) shortening [101]. In yeast, in which the process of mRNA decay has been extensively analyzed, most wild-type mRNAs decay by a mechanism in which the initial nucleolytic event is the shortening of the poly(A) tail to an oligo(A) length of 10-15 nucleotides. After poly(A) shortening, transcripts are decapped by the Dcp1p-Dcp2p complex. Decapped and deadenylated mRNAs are then digested exonucleolytically by the 5′-to-3′ exoribonuclease, Xrn1p, and/or the 3′-to-5′ multi-subunit exosome [102] (Figure 4).

All three decay-initiating events eliminate the closed-loop state of the mRNP by removing or separating the binding sites for the respective 5′ and 3′ interacting proteins [27], and these events also render the remaining mRNA fragments substrates for further degradation. At a minimum, then, mRNA decay generally occurs concurrently with the conversion of an mRNP from a translatable to an untranslatable (or poorly translatable) form [27,101], that is, in parallel with the termination of PABP's role in the enhancement of translation initiation.

Although the onset of mRNA decapping does coincide with the loss of PABP's binding site, and efficient translation initiation does, indeed, antagonize mRNA decay [103,104], PABP's role in the maintenance of mRNA stability is more complicated than that of a mere translation enhancer. Several observations suggest that loss of PABP's binding site, and presumptive disruption of the closed loop state, may not

always trigger immediate degradation of the remainder of the mRNA. These observations include, first, that poly(A) shortening or removal is the rate-determining event in the decay of some mRNAs whereas for others, it may be an obligate event in their degradation but not the rate-determining step [105,106]; second, that yeast *pab1* mutations that unlink mRNA decapping from poly(A) shortening do not necessarily accelerate the rate of mRNA decay [107,108]; and third, that the domains of tethered Pab1p that provide yeast mRNA stabilization and translation functions are different [83,109].

Additional roles for PABP in the regulation of mRNA stability range from being an antagonist or promoter of poly(A) shortening to a facilitator of the binding of additional factors that promote or retard rapid mRNA decay. *In vitro*, excess poly(A) is an effective competitor of PABP binding to mRNA [63,110-112]. Such competition accelerates the rate of poly(A) shortening, indicating that the presence of PABP on the poly(A) tail provides a protective effect [110-112]. This effect is, in part, attributable to physical hindrance of the deadenylase, because poly(A) tails are often shortened in discrete lengths equivalent in size to a PABP 'footprint' [113]. It is also known, however, that the principal yeast deadenylase, the Ccr4p-Pop2p-Notp complex (Figure 4), and a major mammalian deadenylase, PARN, are both inhibited in the presence of PABP [114]. In contrast, the yeast Pan2p-Pan3p deadenylase, an enzyme responsible for the initial trimming of the poly(A) tail (see above), requires Pab1p for its activity [42,44].

Much like its role in translational initiation, PABP also influences mRNA decay by interacting with key regulatory proteins, either influencing their activity or being influenced by them. Two proteins that bind the 3′ UTR of the α-globin mRNA and enhance its stability, αCP1 and αCP2, interact with human PABP [113]. PABP appears to stimulate the ability of the αCP proteins to bind to their target sequence in the 3′ UTR, thereby precluding access of an endonuclease (ErEN) to its recognition site [115]. Interestingly, the binding of PABP to the poly(A) tail is also enhanced by the αCP proteins, implying that α-globin mRNA stabilization is mediated by multiple interdependent events [115]. Stability of the mRNA encoding the transcription factor c-Fos is regulated by sequence elements in its 3′ UTR and coding region [116]. The coding region stability element, also known as the major protein-coding-region determinant (mCRD), interacts with a complex of RNA-binding proteins that includes PABP, Paip1, hnRNPD, NSAP1, and Unr [117]. Translation through the mCRD destabilizes *c-fos* mRNA by a mechanism that is thought to disrupt interactions with this complex and, in turn, promote poly(A) shortening [117]. As noted above, PABP also interacts with the termination factor eRF3 [21,97,98], a consequence of which is a decrease in the number of PABP multimers associated with the poly(A) tail. This observation links translation termination to poly(A) shortening and suggests one mechanism for orchestrating a standardized 'clock' that limits the lifetime of a poly(A) tail and, in turn, the mRNA to which it is appended [97].

Additional roles for PABP in mRNA decay are illustrated by events that occur after the poly(A) tail has been removed. As shown in Figure 4, mRNA deadenylation is accompanied by an mRNP rearrangement that allows binding of a decapping activator complex containing the proteins Lsm1p-Lsm7p and Pat1p [118]. This complex appears to promote interaction of the mRNP with the Dcp1p-Dcp2p decapping complex, thereby creating a substrate for terminal 5′-to-3′, and/or 3′-to-5′ exonucleolytic degradation [119] (Figure 4). Recent studies indicate that all steps subsequent to association of the Lsm1-7p-Pat1p complex occur at a limited number of subcellular sites called P bodies [119]. In principle, therefore, both the terminal steps of mRNA decay (from decapping onwards), and their localization to a specific subcellular site, are prevented from occurring by the presence of bound Pab1p. Pab1p may simply maintain the mRNP in its translation-favorable mode, but the formal possibility that it directly inhibits mRNA association of the Lsm1-7p-Pat1p complex has not been excluded. In the latter hypothesis, PABP's exit from the mRNP would complete a cycle in which its initial association with mRNA assists in mRNP formation, then leads to efficient mRNP utilization, and culminates in destruction of the mRNA.

Frontiers

Considering the number of functions associated with the PABPs, and their simultaneous interactions with both RNA and other proteins, the number of questions for which we have no answers far exceeds the number of those for which we do. Does the presence or absence of PABP determine an mRNP's competence for export, or does it play a more active role? How is nuclear PABP exchanged for cytoplasmic PABP and where does that exchange take place? How does interaction with PABP actually affect eIF4G and eRF3, and *vice versa*; in other words, do these proteins influence each other's conformations and interactions with other factors? Does autoregulatory PABP simply 'block' the 5′ UTR or does it promote interactions with other factors that are the ultimate regulators? Why do plants have so many PABP genes? Have they separated PABP's functions into distinct polypeptides?

A key question is which of PABP's many functions are essential. Of the functions enumerated in this review, most appear to be dispensable. For example, PABP mutants lacking the ability to interact with factors governing polyadenylation, translation initiation, and translation termination are all viable. Cross-species complementation experiments assessing the essential nature of PABP demonstrate that *Arabidopsis* Pab3p can restore PABP's role in mRNA biogenesis but fails to complement defects in mRNA decay and translation initiation [120]. What does appear to be required is the ability

of PABP to recognize RNA. The possibility remains that the essential nature of PABP lies not with a single function but with a combination of functions. That, of course, raises the final question: have all of PABP's functions been enumerated? That seems unlikely. A hint of PABP's untapped versatility is apparent from its role in the replication of zucchini yellow mosaic potyvirus, a plant virus whose RNA-dependent RNA polymerase appears to exploit PABP for viral replication [121]. Who knows - maybe PABP will find its way into splicing and transcription, completing its act as the one-man band of gene expression.

Additional data files

A list of the currently known PABP genes with accession numbers and links to their entries in the nucleotide and protein sequence databases (Additional data file 1) and the sequences of these proteins in FASTA format (Additional data file 2) are available with the online version of this article.

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