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Differential organ-specific expression of three poly(A)-binding-protein genes from *Arabidopsis thaliana*

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ABSTRACT **Poly(A)-binding protein (PABP) is considered an essential component of a eukaryotic cell; deletion of the PABP-coding gene in yeast leads to a lethal phenotype. PABP is implicated in numerous aspects of posttranscriptional regulation, including mRNA turnover and translational initiation. A nested set of degenerate PCR primers designed from regions conserved among yeast, *Xenopus*, and human PABP sequences was used to amplify genomic DNA fragments from *Arabidopsis thaliana*. Hybridization screening of genomic and cDNA libraries with a genomic PCR probe led to the isolation of three diverse *Arabidopsis* genes encoding PABPs, *PAB1*, *PAB3*, and *PAB5*. All three sequences contain the expected four RNA-recognition motifs. Sequence diversity between these genes equals or exceeds the diversity among animal and fungal sequences. One of the genes, *PAB5*, and its cDNA were completely sequenced. Its open reading frame encodes a 73.2-kDa protein containing a number of amino acid motifs characteristic of PABPs from different species. Moreover, *in vitro* synthesized PAB5 protein bound to poly(A)-Sepharose with high specificity. All three genes isolated showed organ-specific patterns of expression. *PAB5* and *PAB3* RNAs were detected only in floral organs, with the highest level of expression in immature flowers. *PAB1* RNA was observed predominantly in roots, was less abundant in immature flowers, and was not detected in any other organ examined (stems, leaves, mature flowers, siliques). This suggests a potentially unique role for PABPs in organ-specific posttranscriptional regulation in plants.**

Poly(A)-binding protein (PABP) is a ubiquitous component of eukaryotic cells, where it is found both in the nucleus and cytoplasm. It is bound to poly(A) tails of cytoplasmic mRNA and heterogeneous nuclear RNA, although in some situations there may exist a large pool of uncomplexed protein (1). In yeast it appears to be essential for viability (2). Although it is evident that its function is dependent on its interaction with poly(A), the exact role of PABP is not completely understood. PABP stimulates translation *in vitro* (3, 4) and this effect was observed only in systems with high reinitiation rates (as discussed in ref. 5). These data agree well with the fact that temperature-sensitive yeast mutants for PABP have decreased levels of polysomes and increased levels of monoribosomes after a shift to restrictive temperature (6). Moreover, extragenic suppressors of this mutation affect biogenesis of 60S ribosomal subunits, perhaps allowing the cell to bypass the requirement for PABP in translational initiation (7).

Although initial data demonstrated that PABP could protect polyadenylylated messages from rapid degradation *in vitro* (8), the mechanism(s) of PABP action in RNA turnover now appear to be far more complex. Conditional PABP mutants in yeast have abnormally elongated poly(A) tails when a functional PABP is not expressed (6), and PABP is required for activity of poly(A)-specific nuclease (PAN) *in vitro* (9, 10). Thus PABP may promote and/or regulate this early step in mRNA degradation rather than impede it. Furthermore, PAN, like PABP, is required for translation initiation. It has been suggested that an interaction between PABP and PAN may be important for both poly(A) shortening and translation initiation, suggesting that these two reactions are somehow coupled (10).

PABPs belong to a larger family of RNA-binding proteins characterized by the presence of one or several divergent 80- to 90-amino acid repeats variously called RNA-binding domains (11) or RNA-recognition motifs (RRMs) (12). Two short conserved amino acid motifs, called RNP1 (or RNP-CS) and RNP2, are found in each RRM. RNP1 and RNP2 contain basic and aromatic residues which appear to contact single-stranded RNA and unpaired ribonucleotides in higher-order structures, whereas sequence specificity may be imposed by nonconserved residues both within and outside of the RRM (12). However, both overall structure and particular sequence variations within common motifs make PABPs a unique subclass of the RRM superfamily of proteins. All known PABPs have four RRMs which constitute the N-terminal two-thirds of the protein. The C-terminal region is not as conserved across all PABPs. Also, each of the four RRM domains is more closely related to the homologous domain in other distantly related PABPs than to other domains in the same protein or to the RRMs in other RNA-binding proteins. Thus, a number of uniquely conserved PABP-specific sequences are found in PABPs that are not found in other RNA-binding proteins and that can be used to distinguish these proteins.

Information on PABPs in plants is limited. The *in vitro* translational activity of extracts prepared from dry pea embryo axes is stimulated by added PABP (13). Recently, a 70-kDa PABP from pea has been purified and partially characterized (14). The stimulatory effect of polyadenylylation on the translation of a β-glucuronidase message in electroporated plant protoplasts has been reported (15), which also suggests the involvement of PABP in translation in plants. Moreover, the poly(A) tail has been shown to act synergistically with the cap structure to enhance expression of an enzyme encoded by a reporter gene (16).

Here we report that *Arabidopsis thaliana* has at least three genes encoding PABPs† and that these genes are expressed in an organ-specific manner.

MATERIALS AND METHODS

Plant Material and Libraries. *A. thaliana* cv. Columbia was used throughout this study. Plants were grown under standard greenhouse conditions. For RNA isolation, tissues were collected, frozen in liquid nitrogen, and stored at −70°C.

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Abbreviations: PABP, poly(A)-binding protein; RT–PCR, reverse transcription–polymerase chain reaction; RACE–PCR, rapid amplification of cDNA ends by polymerase chain reaction; RRM, RNA-recognition motif.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M97657).

Immature flowers are defined as unopened and mature flowers as opened and actively pollinating. *Arabidopsis* genomic and immature floral cDNA libraries were provided by J. Mulligan and R. Davis (Stanford University) and E. Meyerowitz (California Institute of Technology), respectively.

PCR with Degenerate Oligonucleotide Primers. Degenerate PCR primers designed to correspond to amino acid motifs conserved in PABPs from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Xenopus*, and humans (17) are shown in Fig. 1. Thirty-five cycles of PCR (1 min at 94°C, 1 min at 48°C, 1 min at 72°C) were performed with 0.5 μg of *Arabidopsis* genomic DNA as a template and external primers 118Sa plus 369N or 118Sb plus 369N. The 760-bp product was either used directly as a hybridization probe or reamplified in the second round of PCR using internal oligonucleotide primers 175S plus 298Na or 175S plus 298Nb under the same conditions to give a 400-bp PCR product.

Rapid Amplification of cDNA Ends (RACE)–PCR, Reverse Transcription (RT)-PCR, and Primer Extension Analysis. Published protocols were used for 3′-RACE–PCR (18) and 5′-RACE–PCR (19). RT–PCR with gene-specific primers and primer extension with *PAB5* oligonucleotide GATGCAACCGCCGCAGCCATAGCGATAAGA were performed essentially as described (20). In RT–PCR experiments, successful cDNA synthesis was monitored and approximately normalized between organ samples by incorporation of small amounts of radioactive dATP during reverse transcription. *Arabidopsis* actin-specific primers, CTAAGCTCTCAAGATCAAAGGCTTA and ACTAAAACGCAAAACGAAAGCGGTT, for the constitutively expressed *ACT2* RNA, were used as a positive control on each organ sample. The sense and antisense pairs of oligonucleotides TGATTCGAGTGTCGAAGA/TGCAAGAGAGAACTTTGC, TGCACTGAAGCAGCGGCTTC/CGTGAAGTGGTTCGTGCCTT, and 38S/620N (Fig. 1) were used to amplify *PAB1*, *PAB3*, and *PAB5* cDNA sequences, respectively.

Name	From	To	Sequence
			start
38S	304	325	tatacc *atG* GCT TTG CAA ACC CAT CCA ACC
			NcoI A L Q T H P N
118Sa	714	733	ATG TGG TCN CAR MGN GAY CC
118Sb	714	733	ATG TGG AGY CAR MGN GAY CC
			M W S Q R D P
175S	885	901	gctctaga GGN TTY GTN CAY TTY GA
			XbaI G F V H F D
			GGN CGN GCN CAR AAR AAgaattcc
298Na	1270	1254	CCN GCN CGN GTY TTY TT cttaagg - 5′
298Nb	1270	1254	CCN TCY CGN GTY TTY TT cttaagg - 5′
			G R A Q K K EcoRI
			GGN TTY GGN TTY GTN TGY TT
369N	1581	1562	CCN AAR CCN AAR CAN ACR AA - 5′
			G F G F V C F
			stop
			AG ATG GAT CAA GCT GAG ATT *Tag* tcgacca
620N	2733	2713	TC TAC CTA GTT CGA CTC TAA *Atc* agctggt -5′
			E M D Q A E I Sal I

FIG. 1. Oligonucleotides used to isolate PABP-encoding sequences. The sequences of sense oligodeoxynucleotides are written in 5′ to 3′ direction and antisense, in 3′ to 5′ direction (5′-end indicated) with the complementary sense strand shown above. Amino acid translations are shown below the sequences of oligonucleotides. Numbers in their names denote the position of the first (closest to N terminus) codon covered by the oligonucleotide, S or N stands for sense and antisense orientation, respectively. The 5′-end additions containing restriction enzyme sites are in lowercase. Start and stop codons used for creating the pab5-1.8 expression construct are italicized. All nucleotide coordinates (From, To) of oligonucleotides are given relative to the first transcription start point of *PAB5* (i.e., that closest to the TATA box).

pab5-1.8 Expression Construct. A 1.8-kb fragment of *PAB5* cDNA comprising almost all the protein-coding region was PCR-amplified directly from cDNA synthesized from floral poly(A)$^+$ RNA by using primers 38S and 620N (Fig. 1). An ATG codon (in the context of a translation initiation consensus sequence) and a stop codon, as well as *Nco* I and *Sal* I sites, were incorporated into the oligonucleotide primers. The resulting PCR product was cloned into pET-15b expression vector (Novagen, Madison, WI), and the structure of the entire construct (pab5-1.8) was verified by sequencing.

Poly(A)-Binding Assay. Assays were performed essentially as described (17) except that binding reactions were performed in the presence of a 10- or 100-fold excess of polyribonucleotide competitors. After four washes with binding buffer, bound material was eluted from poly(A)-Sepharose by boiling in SDS sample buffer and resolved by SDS-PAGE. The gel was dried and exposed to x-ray film, and the autoradiogram was scanned on a Molecular Dynamics scanning densitometer, model 300A.

Northern Blot Analysis. *Arabidopsis* total and poly(A)$^+$ RNAs were isolated as described (20). For Northern analysis, total RNA was electrophoresed in a formaldehyde/agarose gel, transferred to a GeneScreen*Plus* membrane (DuPont) and hybridized with probe at 10^6 cpm/ml.

DNA Sequence Analysis. Sequencing by the dideoxy chain-termination method (21) was performed with the Sequenase kit (United States Biochemical). The Genetics Computer Group (Madison, WI) or IntelliGenetics software provided by the University of Georgia Biological Sequence Computation Facility was used for sequence analysis. Multiple sequence alignments and gene trees were constructed by using the PILEUP program of the GCG package, according to the methods of Higgins and Sharp (22).

RESULTS

***Arabidopsis* Contains at Least Three Genes Encoding PABPs.** A nested set of degenerate primers corresponding to conserved amino acid motifs found in yeast, human, and *Xenopus* PABPs was designed (17) (Fig. 1 and *Materials and Methods*) and used in PCR amplification of *Arabidopsis* genomic DNA. Major products of 760 bp and 400 bp were obtained in the first and second rounds of PCR, respectively, which comigrated with PCR products from a yeast genomic DNA control. The PCR products were used as probes in the screening of an *Arabidopsis* immature floral cDNA library. The PCR products and initial clones were used in probing of a genomic library and reprobing of the cDNA library. Three genomic clones (*PAB1*, *PAB3*, *PAB5*) and two partial cDNAs lacking 5′ and 3′ end sequences (*PAB3* and *PAB5*) were obtained. *PAB5* was subjected to extensive characterization (below). When DNA fragments encoding *PAB1*, *PAB3*, and *PAB5* were hybridized at moderately high stringency to Southern blots of genomic DNA, each revealed a single distinct genomic DNA band with most restriction enzymes (data not shown). Use of either the *PAB5* partial cDNA or the 760-bp PCR product as a probe gave ≈20 signals per *Arabidopsis* genome equivalent calculated according to ref. 23. The first three characterized, *PAB1*, *PAB3*, and *PAB5*, corresponded to one of the weakest and two of the strongest signals, respectively. These data suggest that *Arabidopsis* may contain a large number of PABP-related sequences. However, the possibility that other RRM-containing sequences might hybridize to our probes cannot be ruled out.

The amino acid translations of a portion of the *A. thaliana* PABP sequences, the corresponding portions of PABP genes from other species, and several other known or potential RNA-binding proteins containing the RRM and showing the greatest sequence similarity (11, 24) were compared. The results are summarized in the gene tree presented in Fig. 2. *PAB1*, *PAB3*, and *PAB5* are more closely allied to known

PABPs than to other RRM-containing proteins. Further analysis of *PAB5* (below) and *PAB1* and *PAB3* sequences (data not shown), beyond the portion compared in Fig. 2, demonstrated that they all encode typical PABPs with four N-terminal RRMs. It is clear that the three *Arabidopsis* genes are very diverse; in fact, human and *Xenopus* sequences are more closely related than the two most similar *Arabidopsis* sequences, *PAB5* and *PAB3*. *PAB1* is quite diverged from *PAB3* and *PAB5*.

Structural Features of *PAB5*. The sequence of the PAB5 genomic clone was obtained from a set of nested deletions. This sequence information allowed us to employ a PCR strategy in order to obtain complete *PAB5* cDNA sequence. First, oligonucleotide primers 38S and 620N derived from genomic sequence were used in PCR amplification of cDNA reverse-transcribed from poly(A)$^+$ RNA of immature flower buds. An 1800-bp PCR product covering almost all of the protein-coding region was subcloned (pab5-1.8, Fig. 3) and sequenced. The 3′ end of *PAB5* cDNA was isolated by the 3′-RACE–PCR technique. The sequence of the 5′ end and location of transcription start points were obtained by a combination of 5′-RACE–PCR, RT–PCR, and primer extension analysis.

The resulting sequence information allowed the physical structure of the gene to be deduced (Fig. 3). *PAB5* consists of seven exons and six A+T-rich (≥70% A+T) introns ranging in size from 94 to 169 bp. All exon/intron junctions comply with the "GU . . . AG" rule. The first intron splits an alanine codon (Ala^{98}), whereas the other introns occur between codons. Sequencing of 3′-RACE products revealed four poly(A)-addition sites (positions 3014, 3015, 3086, and 3131; pA_{A-D}, Fig. 3).

The open reading frame of 668 amino acids starts at an ATG codon in exon 1 and ends at a TGA stop codon in exon 7. The calculated molecular mass of the protein is 73.2 kDa, which is close to values for PABPs from other species, including plants (14). The N-terminal two-thirds of the protein contains the expected four repeated RRMs (I–IV) each containing two conserved sequences (RNP-1 and RNP-2). Each RRM shows significantly higher similarity to the corresponding domain in the PABPs from other species than to any other domain within the same protein, in agreement with previously reported sequence analyses (11). There is an alanine cluster in the nonconserved N-terminal portion of the PAB5 protein (A_n) similar to that found in the C-terminal region of other PABP proteins (2, 26–28, 34) and a glutamine cluster in the C-terminal region (Q_n). The C-terminal third of the protein is not highly conserved except for a short, 20-amino acid motif (17).

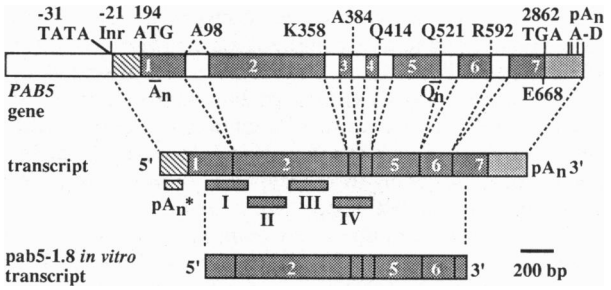

FIG. 3. Physical map of the *PAB5* gene (*Top*), its cDNA (*Middle*), and the pab5-1.8 expression construct (*Bottom*). Gray and white boxes represent exons and introns, respectively. The striped portion of exon 1 corresponds to the 5′ untranslated leader and the light gray portion of exon 7 to the 3′ untranslated region. Indicated are the TATA box, initiator (Inr), start and stop codons, four poly(A)-addition sites (pA_nA-D), and the alanine-rich (A_n) and glutamine-rich (Q_n) regions. Codon positions are shown for intron/exon junctions and the C-terminal amino acid. pA_n* corresponds to the adenine-rich portion of the 5′ untranslated leader. Bars I–IV also denote positions of RRMs encoded by the transcript and by a portion of *PAB5* expressed *in vitro* from plasmid pab5-1.8.

Two transcription start points identified by primer extension analysis lie 29 and 30 nt downstream from the last T in the sequence TATAAAT (TATA box) and 14 and 15 nt downstream from the sequence CTCATTCT (initiator element, Inr). Inr is known to direct transcription initiation at a specific position within itself in both TATA-less and TATA-containing genes. The TATA-binding factor TFIID must be positioned strictly 30 nt away from Inr in order to activate transcription specifically from Inr (35). Thus, the significance, if any, of Inr in regulation of *PAB5* transcription is unclear.

A relatively long A-rich sequence (pA_n*, Fig. 2) found in the 5′ untranslated leader of *PAB5* is similar to the sequence present in 5′ untranslated leaders of yeast (25), human (28), and *Xenopus* (36) PABP mRNAs. The yeast PABP can bind to this sequence *in vitro* with an affinity comparable to binding to poly(A) (25), but the implied autoregulatory role could not be confirmed *in vivo* (2).

PAB5 Protein Binds Poly(A) *in Vitro*. To confirm that *PAB5* indeed encodes a functional PABP with binding preference for poly(A) over other homopolymers, an *in vitro* binding experiment was performed. The 1.8-kb cDNA fragment in the construct pab5-1.8 (Fig. 3) contains the first four RRMs and most of the C-terminal domain under control of a T7 phage promoter. This construct was transcribed and translated *in vitro* in the presence of [^{35}S]methionine. The binding of the ≈65-kDa translation product to a poly(A)-Sepharose column

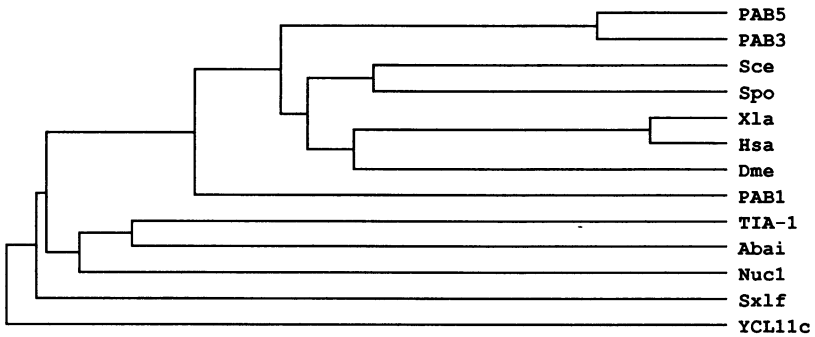

FIG. 2. Gene tree constructed from partial amino acid sequence comparison of *A. thaliana* PABPs (encoded by *PAB1*, *PAB3*, and *PAB5*); PABPs from *S. cerevisiae PAB1* (see ref. 25), *Sch. pombe* [Spo (17)], *Drosophila melanogaster* [Dme (26)], *X. laevis* [Xla (27)], and *Homo sapiens* [Hsa (28)]; and some of the most closely related RRM-containing proteins, including the sex-lethal factor (Sxlf) from *Drosophila* (29), nucleolin (Nuc1) from hamster (30), the abscisic acid-inducible (Abai) protein from maize (31), the human apoptosis factor TIA-1 (32), and the product of a gene from yeast, YCL11c (33). The region containing most of RRM-II and RRM-III from the PABPs were compared with the most homologous regions of these other proteins. The approximate scale of the tree can be taken from the *PAB5* vs. *PAB1*, *PAB5* vs. *PAB3*, and Hsa vs. Xla comparisons (48%, 81%, and 90% amino acid identity, respectively).

was assayed in the presence or absence of ribonucleotide competitors. As shown in Fig. 4, a 10-fold excess of poly(A) as a competitor was sufficient to reduce binding by a factor of at least 4–5, and the effect was more pronounced (factor of about 20) with a 100-fold excess of poly(A). Binding was not affected significantly by poly(G) and poly(C). This further demonstrates that the *PAB5* gene encodes a functional PABP in *Arabidopsis*.

***PAB1*, *PAB3*, and *PAB5* Are Expressed in an Organ-Specific Manner.** The 1.8-kb *PAB5* cDNA probe was used to examine steady-state levels of *PAB5* mRNA in different organs of *Arabidopsis* by Northern hybridization. Two major transcripts of ≈1.3 kb and ≈2.5 kb (indicated by dot and arrowhead, respectively, in Fig. 5*A*) were detected. The shorter transcript was observed in all organs except roots, whereas the 2.5-kb band was present only in immature flowers. The size of the longer transcript agreed well with that of *PAB5* cDNA sequence. Moreover, the ratio of hybridization signal from the 2.5-kb band to that from the 1.3-kb band increased significantly after high-stringency washes (data not shown), suggesting that the 1.3-kb band represented a transcript from a different PABP gene. To further examine the expression of *PAB5*, RT–PCR with *PAB5*-specific oligonucleotide primers was performed on mRNA from different organs of *Arabidopsis*. RT–PCR (results of a representative experiment are shown in Fig. 5*C*) confirmed that *PAB5* RNA was expressed primarily in immature flowers, but it also was detected at lower levels in mature flowers and siliques. In this and subsequent RT–PCR experiments (below), similar results were obtained if the reverse transcriptase step was primed with oligo(dT) (Fig. 5*C*) or random hexamers. Amplifications of actin cDNA gave bands of equal intensity from all organs examined (data not shown). Both the Northern analyses and the RT–PCR assays were repeated several times with essentially the same results on independent RNA preparations from each organ or stage.

The expression of the *PAB3* gene was assayed similarly (Fig. 5 *B* and *D*). Only one RNA band, of ≈2.4 kb, was detected on Northern blots in immature flowers and not in any other organ examined (Fig. 5*B*). RT–PCR using *PAB3*-specific primers produced a strong band from RNA of immature flowers but not other organs. This confirmed the organ-specific expression of *PAB3* RNA (Fig. 5*D*). The faint amplification product obtained from mature-flower RNA may have resulted from some immature flowers contaminating the mature-flower sample or from continued low-level expression of *PAB3* RNA.

Attempts to detect *PAB1* transcript by Northern hybridization proved unsuccessful due to an apparent low abundance of this message. However, RT–PCR assay (Fig. 5*E*) using *PAB1*-specific primers consistently demonstrated that *PAB1* RNA was present in roots and, to a lesser extent, in immature flowers, but not in stems, leaves, mature flowers, or siliques. Thus, *PAB1*, *PAB3*, and *PAB5* were all expressed in an organ-specific manner.

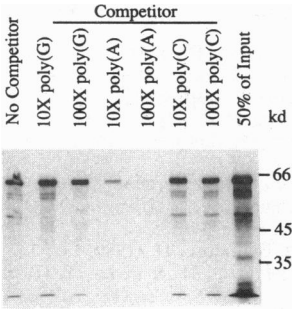

FIG. 4. Poly(A)-binding assay for PAB5. ^{35}S-labeled *in vitro* translation products were incubated with poly(A)-Sepharose in the absence (left lane) or presence of polyribonucleotide competitors: 10- and 100-fold excess poly(G); 10- and 100-fold excess poly(A); 10- and 100-fold excess poly(C). Translation products in the lane at right correspond to 50% of the *in vitro* synthesized protein used in binding reactions. Poly(A)-Sepharose-bound material was washed, eluted by boiling with SDS, resolved by SDS/9% PAGE, and autoradiographed.

DISCUSSION

The results suggest that *A. thaliana* has a diverse family of PABP genes expressed in an organ-specific manner. Hybridization of a genomic library with a PCR probe generated from genomic DNA gave ≈20 signals per genome equivalent. At this point there is no formal proof that all the recombinant phages detected indeed encoded PABPs. However, one of the primers employed to generate the PCR probe corresponded to a unique and conserved (PABP-specific) junction between RRM-I and RRM-II (17), and thus the population of amplified fragments should contain primarily PABP sequences and not those of other RNA-binding proteins. Furthermore, independent experiments using *PAB5* partial cDNA gave essentially the same number of signals per genome equivalent. Finally, all three plaques that were purified encoded PABPs. The great diversity among the three PABP gene family members characterized, *PAB1*, *PAB3*, and *PAB5*, suggests that there may be more PABP genes than we were able to detect by hybridization with the PCR-generated probe or by using any one member of the gene family as a probe. Pierre Hilson and Patrick Masson (personal communication; University of Wisconsin, Madison) have isolated PABP-encoding sequences from *A. thaliana*, some of which are distinct from the three characterized here. Taking the diversity and a potential requirement for the organ-specific expression of *PAB* genes into account, and the fact that *Arabidopsis* has one of the smallest genomes among higher plants, it is likely that all higher plants contain complex multigene families encoding PABPs.

Northern analysis and RT–PCR data allowed us to conclude that the expression of *PAB1*, *PAB3*, and *PAB5* is organ-specific. *PAB1* is expressed predominantly in roots and at much lower levels in immature flowers, whereas *PAB3* and *PAB5* are expressed predominantly in immature flowers. There have been reports of genes expressed predominantly in roots, and in most cases they are involved in development of the root or well-established root-specific functions, such as plant–microbe interactions (37). It is not at all obvious what the root-specific role of PAB1 might be, although it has been reported that DNA sequences located 3′ to the protein-coding region are required for correct spatial expression of the AX92 gene in the cortex of the embryonic axes and the root apex in oilseed rape (38). Given that 3′ untranslated regions appear to modulate the activity of the poly(A)–nuclease–PABP complex (9), it is possible that PAB1 is involved in similar mechanisms controlling expression of genes specific for root tissues.

Flowers develop as a complex of four organs each composed of several cell and tissue types. Organ-specific gene expression has been observed for genes in stamen, pistil, and petals, and tissue-specific expression has been detected in tapetal cells and pollen (37). In several cases individual members of a multigene family, such as the chalcone synthase, phenylalanine ammonia-lyase, and agamous genes (37, 39, 40) are expressed only in particular floral organs. However, each of these sequences has obvious flower-specific tasks (i.e., pigment biosynthesis or floral transcription). It is not clear why *PAB5* and *PAB3* expression should be restricted to flowers.

The expression of *PAB5* and *PAB3* in immature flowers implies a role in organ and/or stage-specific posttranscriptional processes. One example of such processes may be temporal translational inactivation of certain classes of

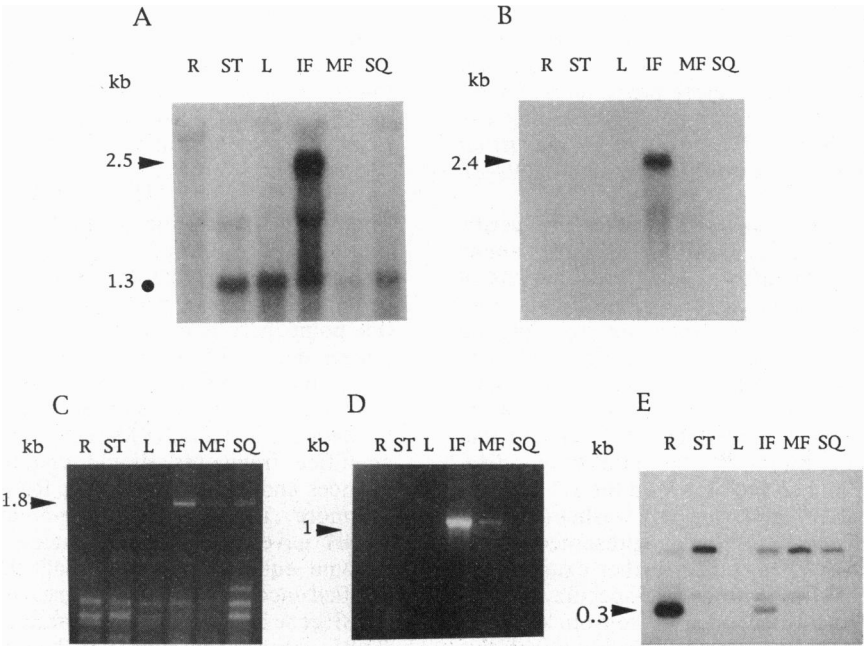

FIG. 5. Northern and RT–PCR analyses of *PAB* genes. (*A* and *B*) Northern blots. Samples (10 μg) of total RNA from roots (R), stems (ST), leaves (L), immature (IF) and mature (MF) flowers, and siliques (SQ) were hybridized with probe specific for *PAB5* (*A*) or *PAB3* (*B*). Equal loading of samples and RNA integrity were verified by ethidium bromide staining and the presence of equivalent amounts of 18S and 26S rRNAs in all samples. (*C–E*) RT–PCR. PCR was performed on cDNA templates reverse-transcribed from total RNAs from indicated organs by using *PAB5* (*C*), *PAB3* (*D*), or *PAB1* (*E*)-specific oligonucleotide primers. Gene-specific products are indicated by arrowheads. In the case of *PAB1* (*E*), a Southern blot of the respective agarose gel probed with the *PAB1*-specific probe is shown. The higher molecular weight band in the *PAB1* experiment (*E*) results from amplification of the traces of genomic DNA present in these RNA samples.

mRNA during oocyte development in animals, which is mediated by a reversible deadenylylation. It has been suggested that such processes can be mediated by special isoform(s) of PABP (41). Given that the development of gametes occurs in immature flowers, it is quite possible that PAB5 and PAB3 have a similarly unique function in the posttranscriptional regulation of gene expression in early flower development.

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