The Plant Journal (2000) **22**(3), 199–210

Conserved expression of *Arabidopsis thaliana* poly (A) binding protein 2 (PAB2) in distinct vegetative and reproductive tissues

Ravishankar Palanivelu[1,†], Dmitry A. Belostotsky[2] and Richard B. Meagher[1,*]
[1]*Department of Genetics, Life Sciences Building, University of Georgia, Athens, GA 30602, USA, and*
[2]*Department of Biology, State University of New York, Albany, NY 10222, USA*

Received 22 October 1999; revised 15 February 2000; accepted 15 February 2000.
*For correspondence (fax +706 542 1387; e-mail meagher@arches.uga.edu).
[†]Present address: Department of Molecular Genetics and Cellular Biology, University of Chicago, Chicago, IL 60637, USA.

Summary

The poly(A) tails of eukaryotic mRNAs are complexed with poly(A) binding protein (PABP). The poly(A)–PABP complex is central to the efficient translation initiation and control of poly (A) tail length and is required in some pathways of mRNA decay. A large gene family encodes PABPs in *Arabidopsis thaliana*. In striking contrast to the floral and root specific expression of three previously reported *Arabidopsis* PABPs, we demonstrate that RNA and protein for one highly diverse member of this family, *PAB2,* are expressed in roots, stems, leaves, flowers, pollen and siliques of *Arabidopsis*. However, cell-type specific analysis of a *PAB2* reporter gene fusion revealed that *PAB2* is spatially and temporally regulated in each organ. For example, strong expression was detected only in the stele and meristem region of roots and a dramatic decrease in expression was observed upon fertilization of ovules. Furthermore, the *PAB2-reporter* construct gave a nearly identical expression pattern in transgenic tobacco, demonstrating that *PAB2* expression is under strong selective constraint. The *PAB2*-reporter was also strongly expressed in the transmittal tissues of both *Arabidopsis* and tobacco, raising the possibility of its involvement in the pollination-dependent poly(A) tail shortening of transmittal tissue specific mRNAs previously reported in tobacco (Wang *et al.*, 1996, *Plant J.* 9, 715–727). In view of its potential role in poly(A) tail shortening, we demonstrated the strong and distinct presence of PAB2 protein in transmittal tissues of *Arabidopsis*. The evolutionary and functional implications of the expression pattern of *PAB2* and its possible functional roles in post-transcriptional regulation in transmittal tissues are discussed.

Introduction

Arabidopsis poly(A) binding proteins (PABP) are encoded by a very diverse gene family that may contain up to 20 different *PABP* genes (Belostotsky and Meagher, 1993; Belostotsky and Meagher, 1996; Hilson *et al.*, 1993). Those few family members that have been characterized are differentially expressed at the organ and tissue level. In wheat, alterations in the expression of PABP genes were also observed during seed development and after heat shock (Gallie, 1998). The functional significance of the differential expression of PABP gene family members and the mechanistic roles plant PABPs play in post-transcriptional regulation are only recently being determined.

The RNA polymerase II transcripts of nearly all eukaryotes are polyadenylated at their 3′ ends in the nucleus and then exported to cytoplasm. There is evidence for poly(A) tails playing nuclear roles (reviewed in Baker, 1997); however, they are not well defined. In contrast, the cytoplasmic roles of poly(A) have been intensively studied and are implicated in the regulation of mRNA stability and translation initiation (reviewed in Baker, 1997). Among the proteins that bind to poly(A), PABP is the best characterized. It is likely that PABP mediates most, and perhaps all, of the functions of poly(A). Thus, it is proposed that the poly(A)/PABP ribonucleoprotein complex is the biological functional unit (Baker, 1993). PABP is a member of a superfamily of RNA binding proteins and contains four distinct RNA binding domains (RRM) (Kenan *et al.*, 1991) that vary in their specificity for polypyrimidine RNA sequences and RNA polynucleotides (Burd *et al.*, 1991; Deardorff and Sachs, 1997; Kuhn and Pieler, 1996).

Functional roles of yeast PAB1 in several post-transcriptional processes have been well documented. Yeast PAB1 plays a critical role in translation initiation (Sachs *et al.*, 1997; Tarun and Sachs, 1995), mRNA stabilization (Coller *et al.*, 1998; Tarun and Parker, 1997), poly (A) tail formation (Minvielle-Sebastia *et al.*, 1997) and poly (A) tail length maturation for newly synthesized mRNAs (Brown and Sachs, 1998). Compared to the data on yeast PABP, little is known about the functions of PABPs of higher plants. PABP mediates a functional link between the 5′ end cap and 3′ end poly (A) through its interaction with translation initiation factors eIF4G and eIF4B in wheat as reviewed by Gallie (1998). In one study, reporter gene translation was stimulated when purified pea PABP was added to *in vitro* translation extracts made from dry pea embryo axes (Sieliwanowicz, 1987).

Yeast has been used directly to help dissect the molecular functions of plant PABPs. *Arabidopsis PAB5* (Belostotsky and Meagher, 1996) and *PAB2* (Palanivelu *et al.*, 2000) and wheat *PAB1* (Le *et al.*, 1997) have all been shown to restore viability to yeast *pab1* mutant strains. Molecular characterization of PABP defective yeast strains complemented with PAB5 and PAB2 revealed that while the translation initiation and poly (A) tail shortening functions of yeast PAB1 are conserved in both PAB2 and PAB5, only PAB2 is capable of restoring the linkage between deadenylation and decapping during mRNA degradation (Belostotsky and Meagher, 1996; Palanivelu *et al.*, 2000).

These data on the molecular functions of *Arabdiopsis* PAB2 in yeast strongly suggest that it plays similar roles in plant poly (A) metabolism. To investigate the functional roles of PAB2 in *Arabidopsis*, we characterized its expression pattern in detail. Through Northern and Western analysis we demonstrated that *PAB2* is expressed in different organs of *Arabidopsis* in both vegetative and reproductive tissues. Analysis of cell-type specific expression revealed that *PAB2* is under a dynamic spatial and temporal regulation within several vegetative and reproductive tissues. In addition, this pattern of expression is highly conserved in tobacco, demonstrating its importance in dicots, and perhaps all angiosperms. These results provide evidence that the expression pattern of *PAB2* is strikingly different from that of other characterized *Arabidopsis* PABP genes.

Results

PAB2 is expressed in all organs of Arabidopsis

Preliminary characterization of *Arabidopsis PAB2* revealed that the *PAB2* gene is expressed in roots and shoot tissues (Hilson *et al.*, 1993). Because shoot tissues are comprised of several organs, we wanted to further characterize the expression of *PAB2*. We first examined the steady-state levels of *PAB2* mRNA in different organs of *Arabidopsis* through Northern hybridization. The complete coding region of *PAB2,* as mapped in Figure 1(a), was used to probe a Northern blot under high stringency conditions. A 2.5 kb transcript was detected in all organs of *Arabidopsis,* as indicated in Figure 2(a). The size of this transcript was the same as previously reported (Hilson *et al.*, 1993). The intensity of hybridizing bands showed that *PAB2* mRNA was most abundant in stems, followed in descending order by flowers, leaves, roots, siliques and pollen. Hybridization of the same blot with an 18S rRNA specific probe confirmed equal loading of RNA in each lane (Figure 2b). In addition, Northern results were corroborated by RT–PCR results obtained using *PAB2*-specific primers in the

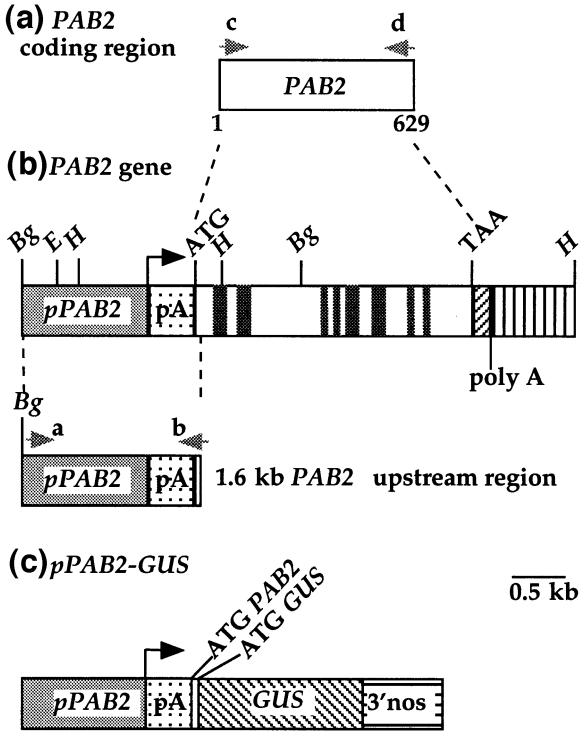

Figure 1. Gene and cDNA structure of *PAB2* and its translational fusion construct with *GUS* reporter gene.
(a) Diagram of a *PAB2* complete coding region amplified by RT–PCR and used as a probe in Northern analysis.
(b) A *PAB2* genomic DNA structure adapted from Hilson *et al.* (1993). The light grey spotted region represents the *PAB2* promoter region (*pPAB2*) used in this study. pA indicates the 5′ untranslated region including the nine adenine rich stretches. The positions of the transcription initiation site (rightward facing arrow), translational initiation site (ATG), stop codon (TAA) and polyadenylation sites (poly A) are indicated. The right-slashed region and vertical lines represent *PAB2* 3′UTR and downstream sequence up to *Hin*dIII restriction site, respectively. White and dark grey boxes represent exon and intron sequences, respectively. The indicated restriction sites are H-*Hin*dIII, Bg-*Bgl*II, E-*Eco*RI.
(c) Diagram of the p*PAB2-GUS* construct used in experiments to analyze the *PAB2* expression pattern in *Arabidopsis* and tobacco.
a, b, c and d represent the primers used (see Experimental procedures).

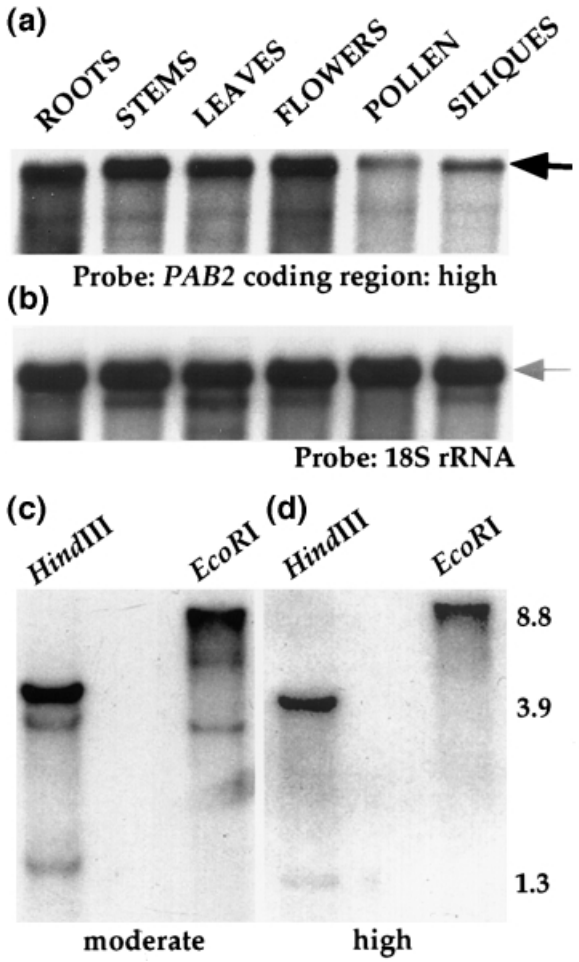

Figure 2. Northern analysis of *PAB2* transcript levels.
(a) Five μg of total RNA from indicated tissues were used to perform Northern blotting, using the *PAB2* coding region as the probe. A *PAB2* transcript of the expected size (~2.5 kb, black arrow) was detected in all the organs of *Arabidopsis*. The filter was probed under high stringency conditions.
(b) An equal loading of total RNA was confirmed by stripping the blot and re-probing with an 18S rRNA specific oligonucleotide. Equal amounts of 18S rRNA (grey arrow) were detected in every lane. The approximate sizes of RNAs were determined relative to standards examined on the stained gel (data not shown).
(c,d) Southern gel blot analysis to confirm gene specificity of the probe used in Northern analysis. The autoradiograms of duplicate blots probed under moderate- (c) or high-stringency conditions (d) are shown. The positions of the DNA molecular weight markers (in kb) are indicated on the right side of (d).

highly divergent 3′ end of the *PAB2* gene (data not shown). Based on these observations, we conclude that *PAB2* is expressed in all organs of *Arabidopsis.*

Because of the size and complexity of the PABP gene family and to exclude the possibility that the probe used in the Northern analysis could have detected other PABP transcript(s), we tested the specificity of the probe in Southern blots either under the same conditions used in Northern blotting (40% formamide, high stringency, Figure 2d) or with moderate stringency conditions (30% formamide, Figure 2c). Under high stringency conditions, the size and restriction profile of the bands detected by the probe were as predicted from the restriction map of the *PAB2* genomic structure (Figure 1b). In addition, the pattern was consistent with the pattern reported earlier for *PAB2* (Hilson *et al.*, 1993). Under moderate stringency conditions, however, the same probe detected a few additional weak bands. These Southern blot results strongly suggested that the probe specifically detected *PAB2* transcripts under the high stringency conditions used in Northern blotting.

PAB2 protein is expressed in all organs of Arabidopsis

To correlate PAB2 protein expression with mRNA expression patterns, we raised polyclonal antibodies against PAB2, using a multiple antigenic peptide (MAP) peptide (*PAB2*-CT) derived from sequences in the C-terminal region of PAB2 protein. Sequences in this region displayed considerable divergence amongst known plant PABP sequences and PABP sequences from other organisms, as shown in the alignment in Figure 3(a). When total *Arabidopsis* plant extracts were probed with anti-PAB2 antibody, it detected an approximately 69 kDa band, which is the same size as the estimated size of PAB2 protein (Figure 3c, lane 1). When similar blots were probed with pre-immune serum these bands were not detected (data not shown). The specificity of the antibody was determined by testing it against three different *in vitro* translated PAB proteins. Successful translation and equal loading of each PAB protein – PAB2 (~69 kDa, Figure 3b, black arrow), PAB3 (D. Belostotsky, unpublished results), and PAB5 (~72 kDa, Figure 3b, grey arrow) – was confirmed with translations performed in the presence of ^{35}S-methionine. Duplicate translation reactions were carried out with cold methionine and then used to perform Western blots probed with anti-*PAB2* antibody. Although the antibody was able to detect the ~69 kDa PAB2 band (black arrow, Figure 3c, lanes 1 and 2), it was unable to detect the ~72 kDa PAB3 and PAB5 bands (grey arrow indicating where the bands were expected, Figure 3c, lanes 3 and 4). Western blot results were further confirmed by demonstrating the ability of the PAB2-CT antibody to specifically immunoprecipitate ^{35}S-methionine labeled *in vitro* translated PAB2 protein but not PAB3 and PAB5 proteins (data not shown). These data demonstrated that anti-PAB2 antibody distinguished PAB2 from PAB3 and PAB5 proteins.

Total protein extracts from roots, stems, leaves, flowers, pollen and siliques were isolated and used to perform Western blot analyses using the PAB2-CT antibody. As can be seen in Figure 3(d), a strong band of the expected size of ~69 kDa was observed in all organs of *Arabidopsis*. The PAB2 protein expression pattern is thus similar to the

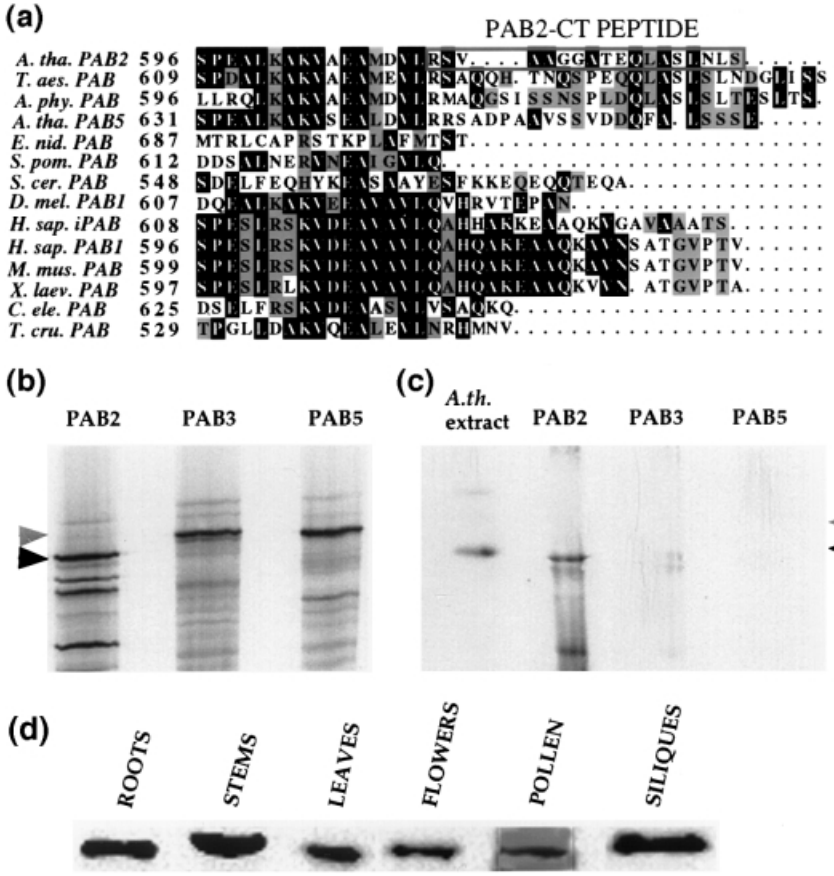

Figure 3. Characterization of PAB2 protein expression in *Arabidopsis*.
(a) A highly divergent region of PAB2 (indicated by grey box) used to design a MAP peptide to raise polyclonal antibody (PAB2-CT). Codon numbers are given to the right of each gene name.
(b) Autoradiogram of a protein gel showing *in vitro* translation of ^{35}S methionine labeled; PAB2 (black arrow, ~69 kDa, lane 1), PAB3 (grey arrow, ~72 kDa, lane 2), and PAB5 (grey arrow, ~72 kDa, lane 3) proteins.
(c) PAB2-CT does not detect the PAB*3* and PAB5 proteins. The Western blot analysis of *in vitro* translated PAB2, PAB3 and PAB5 with PAB2 CT antibody is shown.
(d) PAB2 protein is present in different organs of *Arabidopsis*. Total protein extracts obtained from indicated organs and tissues of *Arabidopsis* were used to prepare a Western blot, probed with *PAB2*-CT antibody. The size of the expected band is indicated (black arrow, ~69 kDa).

PAB2 mRNA expression pattern. Loading of equal amounts of protein from each organ was confirmed by quantification of coomassie blue stained duplicate gels (data not shown).

Temporal and spatial regulation of pPAB2-GUS in Arabidopsis

The tissue- and cell-type-specific expression of *PAB2* at the whole plant level were visualized using a translational fusion between 1.6 kb of *PAB2* 5′ sequence and the β-glucuronidase (*GUS*) reporter gene (*pPAB2-GUS* construct, Figure 1c). *pPAB2-GUS* contained 1.2 kb of *PAB2* sequence upstream of the transcription start site, the 5′ UTR sequence of *PAB2,* and the first seven codons of *PAB2* coding sequence fused in translational frame with *GUS* coding region (Figure 1c and Experimental procedures). The 5′ UTR of *PAB2* was included in the construct because the leader sequence contains distinct poly(A) stretches, within 200 bp upstream of ATG start codon (Hilson *et al.*, 1993), that have been implicated in autoregulation (Sachs *et al.*, 1987; Wu and Bag, 1998) and growth-dependent regulation (Berger *et al.*, 1992; Hornstein *et al.*, 1999) of PABP expression. The first seven codons of *PAB2* were also included because previous studies with other genes suggested that they might contain important regulatory sequences (Cleveland, 1988). *pPAB2-GUS* was used to generate transgenic *Arabidopsis* plants (see Experimental procedures). Six independent, kanamycin resistant transformants were selfed to produce T2 and T3 generation plants that were assayed for *GUS* activity. At different stages of the life cycle of each transformed line, expression was examined in at least three to four plants. The expression patterns controlled by the *PAB2* 5′ region in all six of these lines are summarized in Table 1 and shown in detail in Figure 4. While quantitative variations among the lines in some particular tissues were occasionally observed, the fundamental pattern of expression was the same in all lines.

Seedlings

When 5- to 7-day-old seedlings were analyzed, strong expression of *pPAB2-GUS* was observed in root tips and root meristem (Figure 4a). In addition, strong expression was observed in the mesophyll cells, veins and hydathodes of cotyledons (Figure 4a). Strong expression was also observed in emerging true leaves (Figure 4b).

Table 1. Conserved patterns of *pPAB2-GUS* expression in transgenic *Arabidopsis* and tobacco

Plant species	Root tip	Stele of roots	Lateral root initials	Transition zone	Cotyledons	Emerging new leaves
Arabidopsis	6/6[a]	6/6	6/6	6/6	6/6	6/6
Tobacco	5/5	5/5	5/5	5/5	5/5	5/5
Plant species	Leaf veins	Mature leaf tips	Tapetum tissues	Pollen	Ovules	Transmittal
Arabidopsis	6/6	6/6	6/6	6/6	6/6	6/6
Tobacco	5/5	5/5	5/5	5/5	5/5	5/5

[a]Numbers indicate independent transgenic lines demonstrating the indicated expression pattern versus the number assayed.

Juvenile plants

Cell-type specific expression in the roots of 2-week-old seedlings was similar to that observed in 1-week-old seedlings including intense staining in root tips, and root meristem and spatially restricted expression in the stele region of roots in the elongation (Figure 4e) and maturation zones (Figure 4d). In addition, strong expression was also observed in lateral root primordia (Figure 4d). In old cotyledons, expression was restricted to the veins and hydathodes. Emerging leaves showed very intense staining but the older leaves showed relatively less staining (Figure 4c).

Flowers

Striking temporal and spatial regulation of *pPAB2-GUS* expression was found in flowers. Expression was observed in inflorescence and floral stalks (data not shown). *pPAB2-GUS* showed weak staining from the tetrad stage up to the binucleate stage of developing microspores (Figure 4h–j). However, *GUS* staining intensified during the trinucleate stage of mature pollen grain (Figure 4k,l), and the staining in pollen continued until pollen germination on the stigma. Strong expression was seen in the tapetum of developing anthers, which decreased after stage 12 (compare anthers in Figure 4h and g) probably due to the degeneration of the tapetum (Bowman, 1994).

pPAB2-GUS expression was seen in transmittal tracts throughout pistil development. The staining continues to be observed until the transmittal tissue degenerates after pollination (Figure 4f,g,m,n). Staining was also seen in nectaries throughout flower development (Figure 4g,m). Expression of *pPAB2-GUS* was not detected in the very earliest stages of ovule development (stage 10 flower, Bowman, 1994, Figure 4f). Expression could be seen in ovules in stage 12 flowers (Figure 4g) through fertilization (Figure 4m). Expression in ovules decreases dramatically after fertilization (Figure 4o) and expression completely ceases by the globular stage of embryo development (Figure 4p). The funiculi of fertilized ovules is the only pistil expression of *pPAB2-GUS* found after fertilization (Figure 4p). These results clearly demonstrate a dynamic spatial and temporal regulation of *PAB2* expression in flowers.

Temporal and spatial regulation of Arabidopsis pPAB2-GUS is conserved in tobacco

Evolutionary conservation of expression patterns of genes among distant species is one measure of their functional significance (Meagher, 1995; Mitsialis and Kafatos, 1985). To analyze the extent to which PAB2 expression was conserved, we analyzed the expression pattern of *Arabidopsis pPAB2-GUS* in tobacco, which has not had a common ancestor with *Arabidopsis* for more than 100 million years (Cronquist, 1981; Taylor and Taylor, 1993). For this analysis, we generated transgenic tobacco plants (see Experimental procedures) with the *Arabidopsis pPAB2-GUS* reporter construct (Figure 1c). Three plants from each of five independent transgenic tobacco lines were analyzed for GUS activity. The typical expression pattern as observed in these five lines is presented in Figure 5.

1-week-old seedlings

Strong expression was observed in the root tip, root meristem and elongation zone. In cotyledons, the mesophyll cells, veins and vein endings showed *GUS* staining (Figure 5a).

3-week-old seedlings

Expression in the roots was similar to that observed in 1-week-old seedlings, i.e. strong staining in the root tip and meristem (Figure 5d). In the mature region of the root, expression was restricted to the stele, with little or no staining in the epidermis and cortex (Figure 5c). The lateral root primordia also showed intense staining (Figure 5c) whilst in old cotyledons, expression was restricted to the

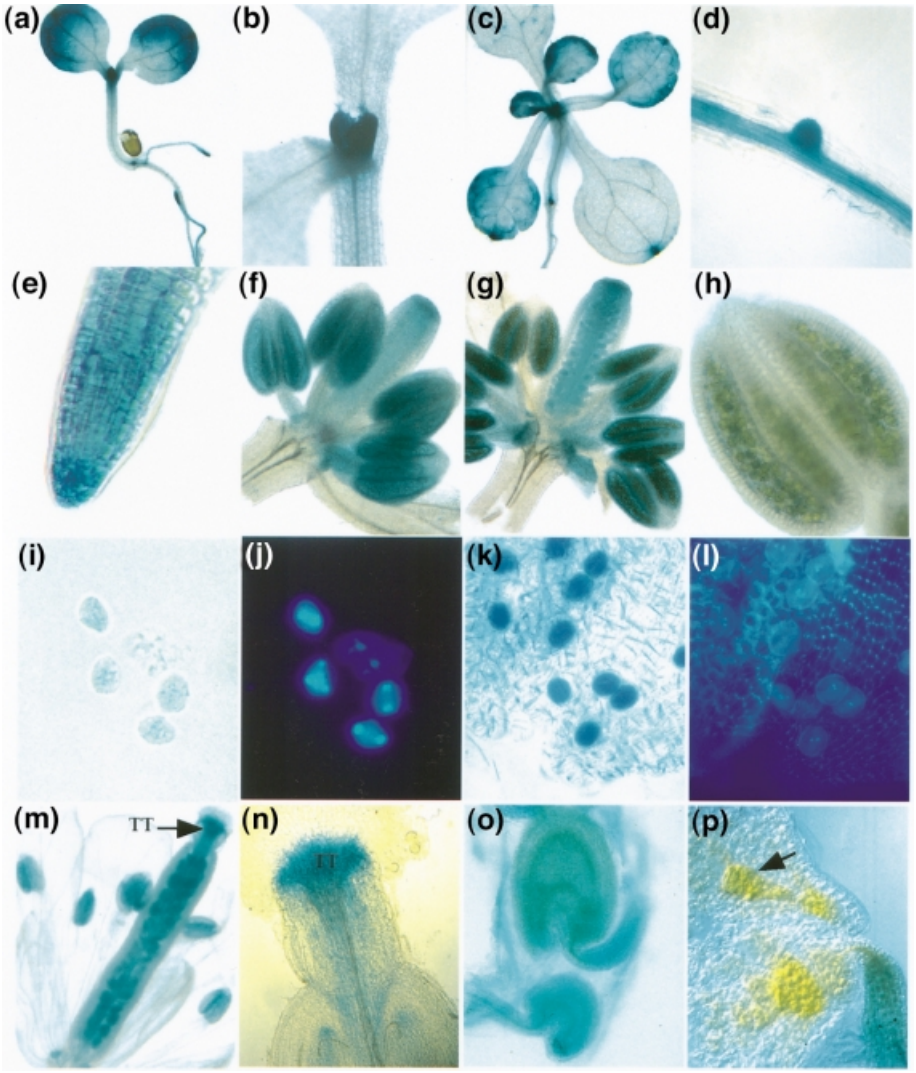

Figure 4. Tissue-specific expression of *pPAB2-GUS* in different organs of transgenic *Arabidopsis*.
(a) *GUS* staining in shoot and root tissues of a 6-day-old seedling. (b) Expression in emerging true leaves of a 6-day-old seedling. (c) *GUS* expression in shoot tissues of a 2-week-old seedling. (d) *GUS* expression in a lateral root primordia and the central stele region of a root of a 2-week-old seedling. (e) Expression in the root tip of a 2-week-old seedling. (f) *GUS* expression in male and female cell types of a stage 10 flower and a stage 12 flower (g). (h) Weak expression in developing binucleate microspores within anther locules. (i,j) Bright field (i) and UV epifluroscence (j) images of DAPI-stained microspore mother cells. (k,l) Bright field and UV epifluroscence images, respectively, of mature trinucleate pollen. (m) Expression in transmittal tissues (TT), ovules and pollen grains of an opened flower. (n) Expression in the transmittal tissues (TT) of a pollinated style. (o) Expression is found in an unfertilized ovule but diminished in a fertilized ovule. (p) Expression ceases completely by the globular embryo stage (black arrow). The yellow colour of the embryos is probably due to a non-specific reaction with ferrocyanide in the staining solution.

veins (Figure 5b). While the emerging leaves showed intense staining, relatively less staining was observed in mature leaves (Figure 5b).

Flowers

During microspore development, weak staining was observed at the tetrad stage (Figure 5g) up to the uninucleate stage of developing microspores (not shown). However, mature binucleate pollen grains, which are equivalent to the trinucleate pollen of *Arabidopsis* (McCormick, 1993), showed strong expression (Figure 5h). During anther development, expression was found only in the inner tapetum (Goldberg *et al.*, 1993) and not in the outer tapetum (Figure 5e). Expression was restricted only to pollen grains in mature anthers (Figure 5f,h). Strong expression was found in transmittal tissues throughout flower development (Figure 5j,k) until the transmittal tissues degenerated after pollination. No *GUS* staining was observed during very early stages of ovule development (not shown). In later stages of ovule development, *GUS* staining was observed (Figure 5l). These

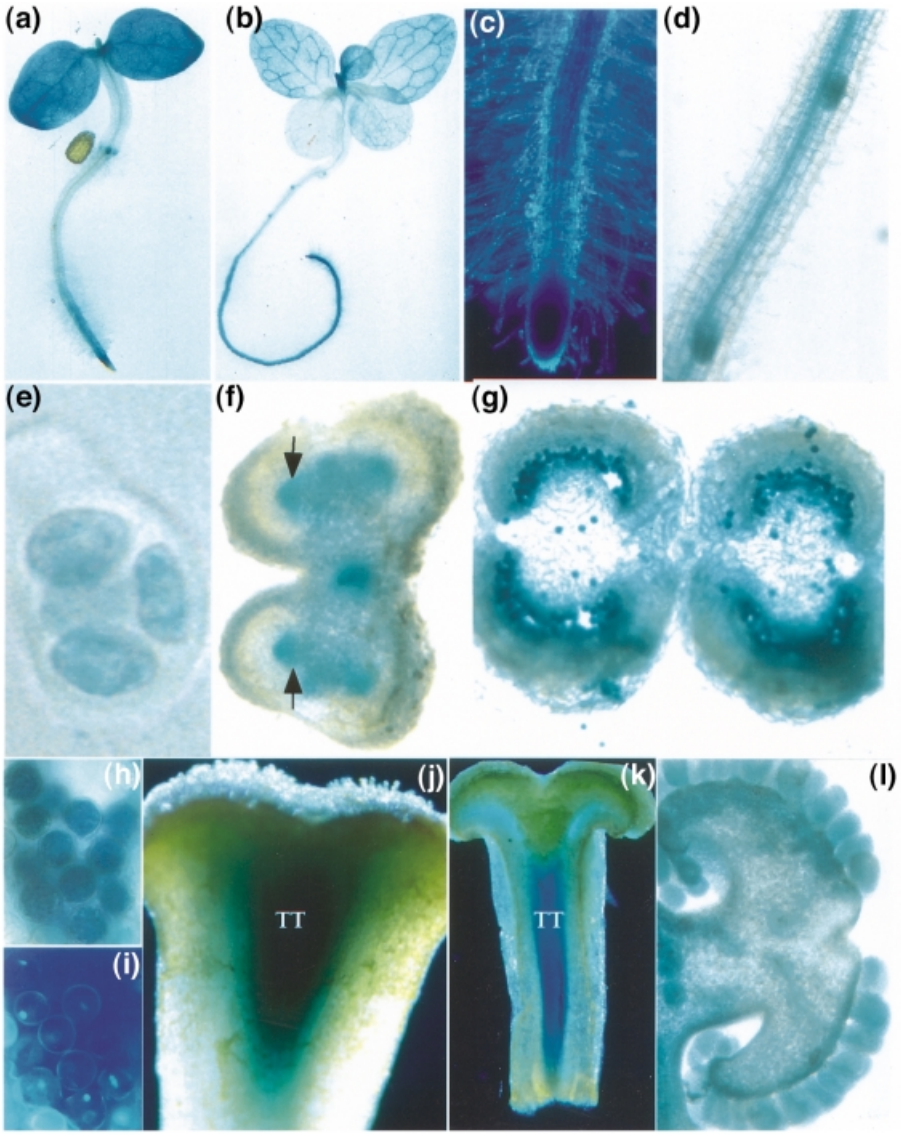

Figure 5. Conserved tissue specific expression of *Arabidopsis pPAB2-GUS* in different organs of transgenic tobacco.
(a) *GUS* expression in a 1-week-old tobacco seedling. (b) *GUS* staining in the root and shoot tissues of 3-week-old seedling. (c) *GUS* staining in the root tip (dark field image). (d) Staining in stele and lateral root primordia of 3-week-old roots. (e) Bright field image showing staining in a single tetrad derived from microspore mother cell. (f) Cross-section of an immature anther showing *GUS* staining in the inner tapetum (black arrows). (g) Cross-section of a mature anther showing expression only in the pollen grains. (h,i) Bright field and UV epifluroscence images, respectively, of a mature pollen grain within an anther lobe. (j,k) *GUS* expression in immature and mature transmittal tissues (TT), respectively. (l) Cross-section of a developed ovary revealing expression in ovules.

results demonstrate the remarkable conservation of expression patterns between the two plant species and supports the argument that selective constraint is acting to preserve most aspects of *PAB2* regulation.

Immunolocalisation of PAB2 protein in transmittal tissues of Arabidopsis

In yeast, reporter mRNAs undergo the removal of the poly(A) tails before they are subjected to degradation. Yeast PAB1 is involved in the removal of poly(A) tails (Caponigro and Parker, 1995). Recently, a few tobacco transmittal tissue-specific (TTS) mRNAs were shown to undergo poly(A) tail shortening prior to their degradation (Wang *et al.*, 1996). In this context, our data which demonstrate a strong expression of *pPAB2-GUS* in transmittal tissues of *Arabidopsis* and tobacco present a possible involvement of PAB2 protein in these post-transcriptional processes. To lend support for potential roles of PAB2 in post-transcriptional processes, we used PAB2-CT antibody to immunolocalize PAB2 protein in *Arabidopsis* transmittal tissues. As seen in Figure 6(b),

when PAB2-CT antibody was used to immunostain a section of *Arabidopsis* pistil, it specifically bound to the transmittal tissues of mature flowers (stage 20). No staining (silver grains) was seen in the adjacent papillar cells of stigma or cortical cells of stylar tissue. In addition, no staining in any tissue of the pistil was observed when similar sections were probed with pre-immune serum (Figure 6a) or secondary antibody alone (data not shown). PAB2 protein was also detected in the transmittal tissues of younger (stage 12) developing flowers (data not shown). The immunolocalization of PAB2 protein in transmittal tissues is in excellent agreement with the transcriptional expression of the *pPAB2/GUS* reporter fusion in this tissue.

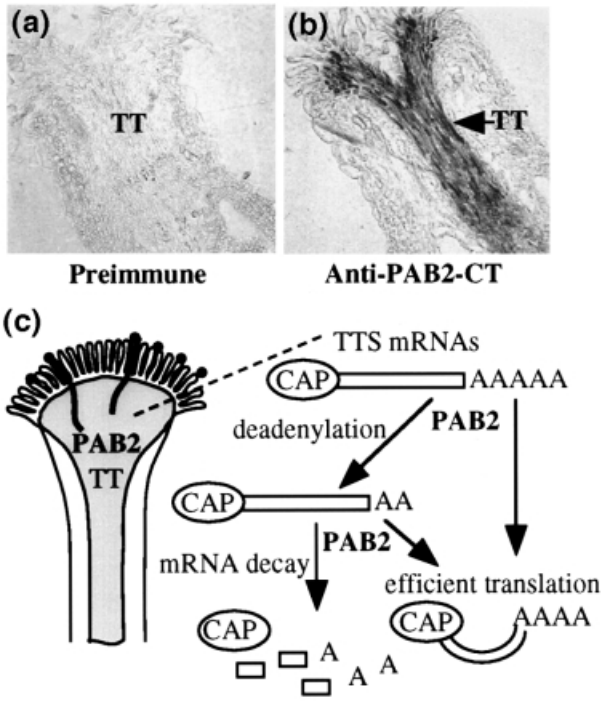

Figure 6. Immunolocalization of PAB2 protein in the transmittal tissues of *Arabidopsis* and a model of PAB2 function.
(a,b) Sections of pistils incubated with pre-immune serum (a) or *PAB2*-CT antibody (b). Note the intense staining in the transmittal tissue (TT) in (b). Staining was performed with a silver-enhanced gold-labeled secondary antibody.
(c) A model depicting the proposed roles of PAB2 in the post-transcriptional regulation of mRNAs in transmitttal tissues of *Arabidopsis*. PAB2 might mediate the deadenylation and subsequent degradation of transmittal tissue specific mRNAs. In addition, it could also mediate the efficient translation initiation of non-deadenylated mRNAs.

Discussion

Distinct expression pattern of PAB2

Our data demonstrate that *PAB2* is expressed in leaves, stems, flowers, pollen and siliques of shoot tissues. Within each organ *PAB2* is under distinct spatial and temporal regulation. Parallel evidence for distinct expression of *PAB2* comes from the analysis of the *PAB2* mRNA and PAB2 protein and the regulation of a reporter gene by 5′ flanking sequences. The *GUS* staining data correlated well with the levels of *PAB2* mRNA and protein in most of the cell types except mature pollen. In all of the lines we examined, very intense *GUS* staining was observed in pollen. However, Northern and Western analysis reveal that mRNA and protein levels were the lowest in pollen when compared to other organs. One possible explanation for these observations is that *PAB2* mRNA and protein may be subjected to distinct post-transcriptional control in mature pollen. Cell-type specific studies also yielded information on the temporal and spatial regulation of *PAB2*. Throughout plant development, high levels of *GUS* staining were observed in cell types involving active cell division (for example, the meristem of root tip and shoots, lateral primordia of roots, Figure 4). This observation is consistent with the report that PABP mRNA was one of the significantly up-regulated transcripts found in a rapidly dividing pancreatic cancer cell line compared to a normal cell line (Zhang *et al.*, 1997).

With the demonstration of *PAB2* expression in immature flowers, it is interesting to note that all four PABPs characterized so far are expressed in immature flowers (Belostotsky and Meagher, 1993). *PAB2* expression overlaps with the expression of *PAB1* in roots (Belostotsky and Meagher, 1993) and with *PAB5* expression in flowers (Belostotsky and Meagher, 1996). Specifically, *PAB2* and *PAB5* are both expressed in anther tapetal tissues. While both displayed weak expression during the early stages of pollen development, their expression in mature pollen was higher. Although there are overlaps in *PAB2* and *PAB5* expression patterns, they are essentially distinct. In mature pollen, *PAB2* is expressed only until pollen germination, whereas *PAB5* expression continues beyond pollen germination until fertilization. Whereas *PAB2* expression is found in transmittal tissues and nectaries, *PAB5* expression is not found in these cell types. In ovule development, *PAB2* is expressed throughout ovule development, but *PAB5* is expressed only in mature ovules. During early embryo development, *PAB5* expression ceases by the heart stage, but *PAB2* expression ceases even by the globular stage. Taken together, these data demonstrate the complex regulation of expression of the PABP gene family in *Arabidopsis*.

The functional significance of the same cell type expressing multiple and diverse PAB proteins may mean that different PABPs must be recruited for different molecular functions. This is consistent with the fact that PAB1 in yeast performs multiple functions (Caponigro and Parker, 1995; Sachs *et al.*, 1997) and that functional

differences have been found between PAB2 and PAB5 in restoring linkage between deadenylation and decapping in yeast (Belostotsky and Meagher, 1996; Palanivelu *et al*. 2000). Alternatively, different PAB proteins might be needed for the same function on different transcripts. Substrate mRNA specificity of each PABP in such instances could be influenced either by its interacting proteins or by the interaction of PABP with specific sequences within a substrate mRNA. The latter possibility is supported by *in vitro* studies that demonstrated PABP's ability to bind sequences other than poly(A) (Burd *et al*., 1991; Deardorff and Sachs, 1997; Gorlach *et al*., 1994) and an *in vivo* study that found PABP as a message-specific translation factor, with a non-poly(A) sequence as its binding site in the 5'UTR of *psbA* mRNA of *Chlamydomonas reinhardii* chloroplasts (Yohn *et al*., 1998). Cell-type specific expression of PAB proteins may also reflect the distinct occurrence of post-transcriptional processes in which PABPs are involved. The developmental specific occurrence of deadenylation dependent degradation pathway in *Chlamydomonas reinhardii* (Gera and Baker, 1998) lends credence to such a possibility.

Evolutionary conservation of PAB2 expression

Conservation of a gene's expression pattern over long, evolutionarily significant time periods is a measure of its importance to the organism. To determine the degree of conservation of the PAB2 regulation, we analyzed the expression of *PAB2* in a distant plant species, tobacco. Our analysis demonstrated that the spatial and temporal expression of *pPAB2-GUS* is remarkably well conserved between *Arabidopsis* and tobacco. The *Arabidopsis PAB2* 5′ flanking sequence contains *cis* elements that must interact with *trans*-acting factors in various tobacco tissues to display the same expression patterns as observed in *Arabidopsis*. Such an extensive conservation of the regulatory information implies that the observed *PAB2* expression is under strong selective constraint in both organisms. Based on the observed differential expression of four *Arabidopsis* PABP genes and that our results show conservation of expression for at least one of those genes, it can be suggested that differential regulation of PABP expression is important and could be one of the reasons why such a large gene family is maintained by the small genome of *Arabidopsis*.

Potential function for PAB2 in transmittal tissues of Arabidopsis

The major mRNA degradation pathway in yeast begins with poly(A) tail removal followed by decapping and then degradation of the body of the message (reviewed in (Caponigro and Parker, 1996). In the yeast *pab1* mutant, it was observed that reporter mRNAs could undergo decapping even though their poly(A) tails remained intact. This observation suggested that the lack of PAB1 uncoupled deadenylation from the degradation of mRNA (Caponigro and Parker, 1995). Although in higher plants there is no direct evidence for the existence of deadenylation-dependent degradation pathway, results from two studies suggest that such a pathway might exist: a deadenylation-dependent degradation pathway has been suggested to partially account for the pattern of Oat *PHYA* mRNA degradation products (Higgs and Colbert, 1994) and three transmittal tissue mRNAs (TTS; β-1, 3, glucanase; and MG-15) have been shown to undergo specific poly(A) tail shortening in the transmittal tissues of tobacco upon pollination (Wang *et al*., 1996). Additionally, two of these mRNA levels declined after pollination (Wang *et al*., 1996). Since the mRNAs that undergo poly(A) tail shortening are involved in several fundamental aspects of pollination, homologues of these mRNAs probably exist in other self-pollinating dicotyledonous plants like *Arabidopsis*. With the demonstrated ability of PABP to mediate poly(A) tail shortening function, expression of one or more poly(A) binding proteins can be expected in transmittal tissues of self-pollinating plants.

In this context, the following observations suggest that poly(A) tail shortening of transmittal tissue-specific mRNAs may be mediated by PAB2 as modeled in Figure 6(c): (i) p*PAB2-GUS* analysis revealed strong *PAB2* expression in transmittal tissues of *Arabidopsis* ((Figure 4 and Table 1) and conservation of this pattern in tobacco (Figure 5 and Table 1). (ii) *PAB2* is expressed in transmittal tissues of *Arabidopsis* and tobacco during and after pollination (Figures 4n and 5k), the same timepoint at which poly(A) tail shortening of certain mRNAs were observed in tobacco (Wang *et al*., 1996). In addition, poly(A) tail shortening of these mRNAs can be induced by ethylene even in immature transmittal tissues, much prior to pollination (Wang *et al*., 1996). In agreement with this, we have found *pPAB2-GUS* expression in immature transmittal tissues in both *Arabidopsis* and tobacco (Figures 4g,m and 5) and PAB2 protein expression in immature transmitted tissues of *Arabidopsis* (data not shown). (iii) Using the PAB2-CT antibody, we have demonstrated the distinct presence of PAB2 protein in *Arabidopsis* transmittal tissues (Figure 6a,b). (iv) *PAB2* has been shown to function in the poly(A) tail removal that precedes degradation of reporter mRNAs in a yeast *pab1* deletion strain rescued with *PAB2* (Palanivelu *et al*., 2000).

Given the demonstrated ability of PAB2 to participate in translation initiation (Palanivelu *et al*., 2000), it is also possible that PAB2 could be involved in the efficient translation initiation of the non-deadenylated mRNAs in the transmittal tissues (Figure 6c). Thus, PAB2 could be essential to the post-transcriptional regulation of mRNAs

involved in pre- and post-pollination events in transmittal tissues. This view is consistent with our detection of PAB2 protein in transmittal tissue of early stage 12 through to stage 20 flowers. Future work will use *Arabidopsis* as a model system to explore the functions mediated by PAB2 in the transmittal tissues. The functional correlation between PAB2 and transmittal tissue-specific mRNAs also brings into question the role of PAB2 in other cell types where it is expressed. Characterization of *PAB2* mutants and mRNA substrates of PAB2 in the different cell types should greatly aid in the understanding of PAB2 functions in these *Arabidopsis* cell types.

Experimental procedures

RNA methods

Different organs and tissues of *Arabidopsis* were collected into liquid nitrogen and stored at –70°C before use. After isolating total RNA as described in Huang *et al.* (1997), 5 μg of total RNA from each tissue were fractionated on a 1% agarose/formaldehyde gel (Sambrook *et al.*, 1989) and blotted to a nylon membrane (0.45 μm, Biotrans +, ICN) for 20 h. The filters were pre-hybridized overnight in 1× RNA hybridization mixture containing 40% formamide at 48°C. Because there are no full-length clones for the *PAB2* coding region (Figure 1a), we PCR amplified the region from the pCDNAII flower library (Invitrogen) using *PAB2*–1S and *PAB2*–624N primers (c and d, respectively, in Figure 1b and Table 2). Using previously published *PAB2* sequences (Hilson *et al.*, 1993), the PCR product was confirmed for its sequence. Subsequently, it was used to probe the Northern blot. 3×10^6 cpm ml^{-1} of the probe labeled by the random primer method of Feinberg and Vogelstein (1983) was added to the blot and hybridized for 34 h under the high stringency conditions described for pre-hybridization. Filters were washed once for 15 min at 48°C in 2× SSC/0.2% SDS and twice for 15 min at 48°C in 1× SSC/0.2% SDS. After a brief drying period, the filters were exposed to X-ray film (Kodak) with one intensifying screen at –70°C for 1–3 days. Equal loading of the RNA samples was confirmed by rRNA hybridization as follows: the Northern blots were stripped and rehybridized with a 26 nt 18S rRNA-specific oligonucleotide (Tanzer and Meagher, 1994). The hybridization was performed at 48°C for 20 h with approximately 1×10^7 cpm of the 5′ end labeled probe at approximately 1×10^6 cpm pmol^{-1} oligonucleotide. Washes were conducted three times for 15 min at 48°C in 2× SSC/0.5% SDS. After a brief drying period, the filters were exposed to X-ray film for 20 min with one intensifying screen.

Southern blotting

Genomic DNA was isolated from *Arabidopsis* (RLD ecotype) plants using a modified cetyltrimethylammonium bromide (CTAB) DNA extraction procedure as described by Murray and Thompson (1980). The DNA was restricted with the indicated restriction enzymes (Figure 2c,d) and resolved on a 0.8% agarose gel at 50 V for 10 h followed by blotting to a nylon membrane (Biotrans plus, ICN) according to a rapid transfer procedure described by Reed and Mann (1985). Duplicate blots were probed with the *PAB2* coding region either with 30% (moderate-stringency) or 40% formamide (high-stringency) using the same pre-hybridization, hybridization and washing conditions as described under RNA methods. The blots were exposed to X-ray film with one intensifying screen for 2–4 days.

Table 2. Oligonucleotides used in this study

PAB2–1S	5′CAGCGTCGACCCATGGCGCAGGTTCAACTTCAG
PAB2–624N	5′GTGCGTCGACCTCGAGTTAAGAGAGGTTCAAGGAAGC
PPAB2-P1S	5′GATCTATTTGGCCGATGTGTGATTGTAGAC
PAB2–1N	5′CTGCGGATCCCTGAAGTTGAACCTCGCCCAT

GUS fusion constructs and plant transformations

The 1.6 kb of *PAB2* 5′ region including codons for the first seven amino acids of *PAB2* was amplified by PCR from a 3.0 kb pPAB1 genomic clone (Hilson *et al.*, 1993) using *PAB2-P1S* and *PAB2*–1N primers (a and b, respectively, in Figure 1b and Table 2) with synthetic *Sal*I and *Bam*HI sites. The PCR product was then cloned into pBluescript SK + to yield p*PAB2*-pBS. From this clone, the *Sal*I and *Bam*HI digested fragment was subcloned into pBI101.1 (Clontech), which contains the *GUS* coding region. This ligation rendered *PAB2* sequences in frame with the *GUS* coding region of pBI101.1 to yield *pPAB2-GUS* (Figure 1c). The construct was transformed into the LBA4404 *Agrobacterium* strain and then used for generating transgenic *Arabidopsis* plants (cv RLD) essentially as described by An *et al.* (1996). Kanamycin resistant primary transformants were self-fertilized to yield T2 and T3 generations. Detailed *GUS* histochemical analysis was performed with kanamycin resistant T2 and T3 plants. LBA4404 *Agrobacterium* containing the same construct was also used to generate transgenic tobacco plants according to the leaf disc transformation procedure (Horsch *et al.*, 1988).

Assay for GUS activity

Different tissues of *Arabidopsis* and tobacco were subjected to *GUS* histochemical analysis essentially as described in An *et al.* (1996), omitting the acetone fixation step when staining tobacco tissues to prevent the excessive browning of tissue samples.

Computational analysis

PAB protein sequences of different organisms (Figure 3a) were obtained from GenBank, and protein sequence alignment was created using the Pileup program of GCG software (Devereaux *et al.*, 1984). Subsequently, boxshading was carried out using the software available on the worldwide web (http://www.isrec.isb-sib.ch/software/BOX_form.html). Results reported in each color plate were scanned into a computer and edited with Adobe PhotoShop and Clarisdraw software.

Western blot analysis

For Western blot analysis, plant tissue samples were harvested and ground in liquid nitrogen to yield fine powder. Further grinding of the powder was undertaken in the presence of 2× Lammelli buffer (0.5 M Tris, pH 6.8, 10% SDS, 33% glycerol, 0.1% β-mercaptoethanol and bromophenol blue). The whole extract was boiled for 5 min and spun down at 13 000 ***g*** for 5 min. The supernatant was aliquoted and stored at –20°C. Approximately 15 μg of total protein extracts were separated on a 10%

polyacrylamide gel and transferred to Immobilon membrane (Amersham) in a semi-dry transfer unit (Hoefer semiphor, Pharmacia) using transfer solution (4 mM Tris, 3 mM glycine, and 20% methanol). Membranes were blocked for 30 min in an incubation solution (5% non-fat dry milk and 10% normal goat serum) in TBS-T (20 mM Tris, 136 mM NaCl, and 0.1% Tween, pH 7.5). The membranes were then incubated with protein A (Bio-Rad) affinity purified *PAB2*-CT polyclonal antibody at a dilution of 1 : 2000 (~90 μg of antibody/40 ml incubation solution) for 1 h at room temperature. After washing three times at 5 min intervals with TBS-T, the membranes were probed (1/2 h at room temperature) with anti-rabbit IgG conjugated to Horseradish peroxidase secondary antibody (Amersham) at a dilution of 1 : 5000. Membranes were washed again three times at 5 min intervals with 1XTBS -T. Protein bands were detected using an ECL protein detection kit (Amersham).

Full-length clones of *PAB2*, *PAB3* (D. Belostotsky *et al*., unpublished results) and *PAB5* cDNAs under the control of the T7 promoter in a pBluescript vector were *in vitro* transcribed and translated in the presence of ^{35}S-labeled methionine (Figure 4b) using the rabbit reticulocyte based TnT system (Promega) following the manufacturer's instructions. The proteins were then separated on a 10% polyacrylamide gel, fixed in 10% methanol + 10% glacial acetic acid for 15 min and dried for 1 h. The gel was then exposed to a phospho-imager (Molecular Dynamics) screen and quantified using Image quant software (Molecular Dynamics) to determine the relative amounts of each reaction needed to obtain an equal amount of full-length PAB proteins (Figure 4b). *In vitro* translation reactions (as above) were performed in the presence of cold methionine and relative amounts of each reaction that would yield the same amount of each of the PAB proteins were used to perform Western blot analysis (Figure 4c) as described for *Arabidopsis* extracts.

Immunogold localization

Arabidopsis cv RLD flowers were harvested from plants grown in growth chambers at 24°C, under 12 h day/night cycles and used to perform immunogold silver staining essentially as described by Kandasamy *et al*. (1989). Ten micron sections of flowers were probed with PAB2-CT primary antibody (1 : 50 dilution) for 1 h at room temperature, in a moist chamber followed by incubation at room temperature for 1 h with 1 : 60 diluted gold-labeled secondary antibody (Amersham). Silver enhancement and mounting was carried out as described by McLean *et al*. (1990) using an Intense silver enhancement kit (Amersham).

Acknowledgements

We thank Drs Patrick Masson and Pierre Hilson (University of Wisconsin, Madison, WI, USA) for their generous gift of *PAB2* clones. We acknowledge Dr M.K. Kandasamy for his help with immunolocalisation. We thank Ms Gay Gragson and members of the R.B.M. lab for their comments on the manuscript. This project was supported by Department of Energy grant no. DEFG05–95ER20177 to R.B.M. and USDA NRICGP grant no. 96–35301–3146 to D.A.B. This research serves as a partial fulfilment for a PhD degree in Genetics for R.P.

References

An, Y.-Q., Huang, S., McDowell, J.M., McKinney, E.C. and Meagher, R.B. (1996) Conserved expression of the *Arabidopsis ACT1* and *ACT3* actin subclass in organ primordia and mature pollen. *Plant Cell,* **8**, 15–30.

Baker, E.J. (1993) Control of Poly (A) length. In *Control of Messenger RNA Stability*. New York: Academic Press, pp. 367–415.

Baker, E.J. (1997) mRNA Polyadenylation: Functional implications. In *mRNA Metabolism and Post-Transcriptional Gene Regualtion*. New York: Wiley-Liss, pp. 85–105.

Belostotsky, D. and Meagher, R.B. (1993) Differential organ-specific expression of three poly (A) binding protein genes from *Arabidopsis*. *Proc. Natl Acad. Sci. USA,* **90**, 6686–6690.

Belostotsky, D.A. and Meagher, R.B. (1996) A pollen-, ovule-, and early embryo-specific poly (A) binding protein from *Arabidopsis* complements essential functions in yeast. *Plant Cell.* **8**, 1261–1275.

Berger, L.C., Bag, J. and Sells, B.H. (1992) Translation of poly (A) -binding protein mRNA is regulated by growth conditions. *Biochem. Cell Biol.* **70**, 770–778.

Bowman, J. (1994) *Arabidopsis: an Atlas of Morphology and Development.* New York: Springer-Verlag.

Brown, C.E. and Sachs, A.B. (1998) Poly (A) tail length control in saccharomyces cerevisiae occurs by message-specific deadenylation [In Process Citation]. *Mol Cell Biol.* **18**, 6548–6559.

Burd, C.G., Matunis, E.L. and Dreyfuss, G. (1991) The multiple RNA-binding domains of the mRNA poly (A) -binding protein have different RNA-binding activities. *Mol. Cell Biol.* **11**, 3419–3424.

Caponigro, G. and Parker, R. (1995) Multiple functions for the poly (A) -binding protein in mRNA decapping and deadenylation in yeast. *Genes and Dev.* **9**, 2421–2432.

Caponigro, G. and Parker, R. (1996) Mechanisms and control of mRNA turnover in *Saccharomyces cerevisiae*. *Microbiol. Rev.* **60**, 233–249.

Cleveland, D.W. (1988) Autoregulated instability of tubulin mRNAs: a novel eukaryotic regulatory mechanism. *TIBS,* **13**, 339–343.

Coller, J.M., Gray, N.K. and Wickens, M.P. (1998) mRNA stabilization by poly (A) binding protein is independent of poly (A) and requires translation. *Genes Dev.* **12**, 3226–3235.

Cronquist, A. (1981) *An Integrated System of Classification of Flowering Plants*. New York: Columbia University Press.

Deardorff, J.A. and Sachs, A.B. (1997) Differential effects of aromatic and charged residue substitutions in the RNA binding domains of the yeast poly (A) -binding protein. *J. Mol Biol.* **269**, 67–81.

Devereaux, J., Haeberli, P. and Smithies, O. (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucl. Acids Res.* **12**, 387–395.

Feinberg, A.P. and Vogelstein, B. (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**, 6–13 and Addendum.

Gallie, D.R. (1998) A tale of two termini: a functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation. *Gene,* **216**, 1–11.

Gera, J.F. and Baker, E.J. (1998) Deadenylation-dependent and -independent decay pathways for alpha1- tubulin mRNA in Chlamydomonas reinhardtii. *Mol. Cell Biol.* **18**, 1498–1505.

Goldberg, R.B., Beals, T.P. and Sander, P.M. (1993) Anther development: Basic principles and practical applications. *Plant Cell,* **5**, 1217–1229.

Gorlach, M., Burd, C.G. and Dreyfuss, G. (1994) The mRNA poly (A) biding protien: Localization, abundance, and RNA-binding specificity. *Exp. Cell. Res.* **211**, 400–407.

Higgs, D.C. and Colbert, J.T. (1994) Oat phytochrome A mRNA

degradation appears to occur via two distinct pathways. *Plant Cell.* **6**, 1007–1019.

Hilson, P., Carroll, K.L. and Masson. P.H. (1993) Molecular characterization of *PAB2*, a member of the multigene family coding for poly (A) binding proteins in *Arabidopsis thaliana*. *Plant Physiol.* **103**, 525–533.

Hornstein, E., Git, A., Braunstein, I., Avni, D. and Meyuhas, O. (1999) The expression of poly (A) binding protein gene is translationally regulated in a growth dependent fashiion through a 5′-terminal oligopyrimidine tract motif. *J. Biol. Chem.* **274**, 1708–1714.

Horsch, R.B., Fry, J., Hoffmann, N., Neidermeyer, S., Rogers, S.G. and Fraley, R.T. (1988) Leaf disc transformation. *Plant Molecular Biology Manual* (Hingman, M.A., Gelvin, S.B. and Schilperoot, R.A., eds). **A5**, Kluwer: 1–9.

Huang, S., An, Y.-Q., McDowell, J.M., McKinney, E.C. and Meagher, R.B. (1997) The *Arabidopsis ACT11* actin gene is strongly expressed in tissues of the emerging inflorescence, pollen and developing ovules. *Plant Mol. Biol.* **33**, 125–139.

Kandasamy, M.K., Paolillo, D.J., Faraday, C.D., Nasrallah, J.B. and Nasrallah, M.E. (1989) The S-locus specific glycoproteins of *Brassica* accumulate in the cell wall of developing stigma papillae. *Dev. Biol.* **134**, 462–472.

Kenan, D.J., Query, C.C. and Keene, J.D. (1991) RNA recognition: towards identifying determinants of specificity. *TIBS,* **16**, 214–220.

Kuhn, U. and Pieler, T. (1996) Xenopus poly (A) binding protein: functional domains in RNA binding and protein–protein interaction. *J. Mol. Biol.* **256**, 20–30.

Le, H., Chang, S.C., Tanguay, R.L. and Gallie, D.R. (1997) The wheat poly (A) -binding protein functionally complements pab1 in yeast. *Eur. J. Biochem.* **243**, 350–357.

McCormick, S. (1993) Male gametophyte development. *Plant Cell.* **5**, 1265–1275.

McLean, B.G., Eubanks, S. and Meagher, R.B. (1990) Tissue specific expression of divergent actins in soybean root. *Plant Cell.* **2**, 335–344.

Meagher, R.B. (1995) The impact of historical contingency on gene phylogeny: Plant actin diversity. In *Evolutionary Biology, 28* (Hecht, M.K., MacIntyre, R.J. and Clegg, M.T., eds). New York: Plenum Press, pp. 195–215.

Minvielle-Sebastia, L., Preker, P.J., Wiederkehr, T., Strahm, Y. and Keller, W. (1997) The major yeast poly (A) -binding protein is associated with cleavage factor IA and functions in premessenger RNA 3′-end formation. *Proc. Natl Acad. Sci. USA,* **94**, 7897–7902.

Mitsialis, S.A. and Kafatos, F.C. (1985) Regulatory elements controlling chorion gene epxression are conserved baetween flies and moths. *Nature,* **317**, 453–456.

Murray, M.G. and Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res.* **8**, 4321–4325.

Palanivelu, R., Belostotsky, D.A. and Meagher, R.B. (2000) *Arabidopsis thaliana* poly (A) binding protein 2 (PAB2), functions in yeast translational and mRNA decay processes. *Plant J.* **22**, 187–198.

Reed, K.C. and Mann, D.A. (1985) Rapid transfer of DNA from agarose gels to nylon membrane. *Nucl. Acids Res.* **13**, 7207–7221.

Sachs, A.B., Davis, R.W. and Kornberg, R.D. (1987) A single domain of yeast poly (A) -binding protein is necessary and sufficient for RNA binding and cell viability. *Mol. Cell. Biol.* **7**, 3268–3276.

Sachs, A.B., Sarnow, P. and Hentze, M.W. (1997) Starting at the beginning, middle, and end: translation initiation in eukaryotes. *Cell,* **89**, 831–838.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Sieliwanowicz, B. (1987) The influence of poly (A) -binding proteins on translation of poly (A) $^{+}$ RNA in a cell-free system from embryo axes of dry pea seeds. *Biochim. Biophys. Acta,* **908**, 54–59.

Tanzer, M.M. and Meagher, R.B. (1994) Faithful degradation of soybean *rbcS* mRNA *in vitro*. *Mol. Cell. Biol.* **14**, 2640–2650.

Tarun, S. and Parker, R. (1997) Mechanisms of mRNA turnover in eukaryotic cells. In *mRNA Metabolism and Post Transcriptional Gene Regulation* (Harford, J.B. and Morris, D.R., eds). New York: Wiley-Liss.

Tarun, S.Z. and Sachs, A.B. (1995) A common function for mRNA 5′ and 3′ ends in translational initiation in yeast. *Genes Dev.* **9**, 2997–3007.

Taylor, T.N. and Taylor, E.L. (1993) *The Biology and Evolution of Fossil Plants.* Englewood Cliffs: Prentice Hall.

Wang, H., Wu, H.M. and Cheung, A.Y. (1996) Pollination induces mRNA poly (A) tail-shortening and cell deterioration in flower transmitting tissue. *Plant J.* **9**, 715–727.

Wu, J. and Bag, J. (1998) Negative control of the poly (A) -binding protein mRNA translation is mediated by the adenine-rich region of its 5′-untranslated region. *J. Biol. Chem.* **273**, 34535–34542.

Yohn, C.B., Cohen, A., Danon, A. and Mayfield, S.P. (1998) A poly (A) binding protein functions in the chloroplast as a message-specific translation factor. *Proc. Natl Acad. Sci. USA,* **95**, 2238–2243.

Zhang, L., Zhou, W., Velculescu, V.E., Kern, S.E., Hruban, R.H., Hamilton, S.R., Vogelstein, B. and Kinzler, K.W. (1997) Gene expression profiles in normal and cancer cells. *Science,* **276**, 1268–1272.