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A Pollen-, Ovule-, and Early Embryo-Specific Poly(A) Binding Protein from Arabidopsis Complements Essential Functions in Yeast

Dmitry A. Belostotsky[1] and Richard B. Meagher[2]

Department of Genetics, University of Georgia, Athens, Georgia 30602-7223

Poly(A) tails of eukaryotic mRNAs serve as targets for regulatory proteins affecting mRNA stability and translation. Differential mRNA polyadenylation and deadenylation during gametogenesis and early development are now widely recognized as mechanisms of translational regulation in animals, but they have not been observed in plants. Here, we report that the expression of the *PAB5* gene encoding one of the poly(A) binding proteins (PABPs) in Arabidopsis is restricted to pollen and ovule development and early embryogenesis. Furthermore, *PAB5* is capable of rescuing a PABP-deficient yeast strain by partially restoring both poly(A) shortening and translational initiation functions of PABP. However, *PAB5* did not restore the linkage of deadenylation and decapping, thus demonstrating that this function of PABP is not essential for viability. Also, like endogenous PABP, *PAB5* expressed in yeast demonstrated genetic interaction with a recently characterized yeast protein SIS1, which is also involved in translational initiation. We propose that *PAB5* encodes a post-transcriptional regulatory factor acting through molecular mechanisms similar to those reported for yeast PABP. This factor may have evolved further to post-transcriptionally regulate plant sexual reproduction and early development.

INTRODUCTION

The functions of poly(A) tails post-transcriptionally added to 3′ ends of most eukaryotic mRNAs have been puzzling ever since their discovery in the early 1970s. Hypotheses that have gained experimental support are related to the role of poly(A) tails in translation and decay of mRNA (Sachs, 1990) and more recently to the associations of the mRNA with the cytoskeleton (Taneja et al., 1992). Poly(A) binding proteins (PABPs) are found in association with poly(A) tails in most if not all eukaryotic cell types and are likely to mediate the effects of poly(A) tails on gene expression. Genes encoding PABPs have been cloned from a number of organisms ranging from yeast to humans. Sequence analyses revealed that PABPs constitute a distinct subclass within a superfamily of RNA binding proteins containing a so-called RNA recognition motif. Overall structures of PABPs from various eukaryotes appear to be highly conserved. The direct analysis of PABP functions in vivo has been performed only in yeast, and the results are consistent with a major role of PABPs in translation and mRNA decay (Sachs and Davis, 1989). However, our mechanistic understanding of these functions of PABP is still very limited, and it is not clear whether PABPs in higher eukaryotes act similarly (Ross, 1995).

Several observations suggest that PABP is required for efficient translation. First, shifting the temperature-sensitive (*ts*) yeast mutant *pab1-F364L* to a restrictive temperature leads to a translational initiation defect manifested as a reduction in the relative amount of polysomes and a concomitant accumulation of 80S monoribosomes that have terminated translation and apparently are unable to reinitiate (Sachs and Davis, 1989). Furthermore, extragenic suppressors of the *pab1-F364L* mutation have been isolated and shown to affect the structure and/or amount of the 60S ribosomal subunit (Sachs and Davis, 1990). In addition, at least some of the suppressor mutations that permit the deletion of the *PABP* gene also suppress certain defects in another essential gene, *SIS1*, recently shown to be involved in translation initiation as well (Zhong and Arndt, 1993). Finally, translation in vitro is inhibited by exogenously added poly(A). Translation can be restored by the addition of purified PABP (Grossi de Sa et al., 1988), which is consistent with the view that PABP must be bound in *cis* for efficient translation. Also, much evidence demonstrates that polyadenylated mRNAs are translated preferentially when compared with their nonadenylated counterparts, at least when the translational apparatus is limiting (Munroe and Jacobson, 1990b)—an effect that must be PABP dependent. Translational control during the maturation of transcriptionally inactive animal oocytes represents a particularly clear example of the causative relationship between the mRNA polyadenylation status and its translation and has been reviewed recently (Wickens, 1992; also see Discussion).

[1] Current address: Department of Biological Sciences, State University of New York, Albany, NY 12222.

[2] To whom correspondence should be addressed.

PABP also regulates deadenylation—the first and rate-limiting step of the major mRNA decay pathway in eukaryotes (Decker and Parker, 1994). However, there are two seemingly conflicting sets of data about the precise role of PABP in this process. In yeast, shifting the *pab1-F364L ts* mutant to a restrictive temperature produces longer poly(A) tails; the same effect is seen when expression of the *PABP* gene is abolished by shutting off its promoter (Sachs and Davis, 1989). Consistent with the presence of longer poly(A) tails in the absence of PABP activity, poly(A) nuclease (PAN), which is an enzyme degrading poly(A) tails, has been partially purified from yeast and shown to be absolutely PABP dependent, whereas PABP serves only as an activating factor and by itself has no exonucleolytic activity (Lowell et al., 1992). However, in a mammalian polysome-based in vitro mRNA decay system, PABP appears to protect rather than destabilize poly(A) tails (Bernstein et al., 1989). This conclusion is based on the observation that the addition of synthetic poly(A) to the decay system resulted in increased decay rates of polyadenylated mRNA substrate, and this destabilizing effect could be abolished by adding either purified human or recombinant yeast PABP. It is possible that the in vitro decay system did not contain an active homolog of PAN, and under these circumstances, PABP might indeed have a protective effect.

Although potentially there are other ways to reconcile these observations, purified yeast PAN has not been assayed in the mammalian mRNA decay system, and attempts to complement the yeast *Pab1* mutant with mammalian (or any other) PABP homolog have not been reported. Therefore, the general mechanism of PABP action in mRNA decay remains unclear. The mechanism of PABP action in translational initiation must also be clarified. Based on genetic data, a common function suggested for PABP is to bring together mRNA 5′ and 3′ ends to aid in translational reinitiation and 60S joining (Munroe and Jacobson, 1990a), whereas other data suggest that it activates 40S joining (Tarun and Sachs, 1995). Interacting protein partners of PABP have not been identified biochemically or genetically (i.e., all of the suppressors of PABP mutations analyzed so far in yeast are bypass suppressors).

We and others previously cloned several diverse genes encoding PABPs in Arabidopsis and provided initial evidence that the individual *PABP* genes are expressed in an organ-specific manner (Belostotsky and Meagher, 1993; Hilson et al., 1993). In this study, we demonstrate that the expression of one Arabidopsis *PABP* gene, *PAB5*, is restricted to tissues and developmental stages associated with sexual reproduction and early embryogenesis. We also demonstrate by functional complementation that despite the remarkably specific *PAB5* expression pattern and the very high sequence divergence between *PAB5* and the yeast *PAB1* gene (i.e., only 44% amino acid identity), basic functions (activation of poly[A] shortening, translational initiation, and genetic interaction with *SIS1*) are conserved between Arabidopsis PAB5 and the yeast PABP. Implications of these results are discussed with regard to the possible role of *PAB5* in the regulation of stability and translational activity of specific mRNAs during plant sexual reproduction and early development.

RESULTS

PAB5 Functionally Complements the Yeast *PAB1* Gene

PAB5 is one of numerous genes encoding PABPs in Arabidopsis, and it is expressed only in flowers. The PAB5 protein contains all of the structural features common to other PABPs, as diagrammed in Figure 1A, and binds poly(A) with high specificity (Belostotsky and Meagher, 1993). We sought to confirm further the functionality of the Arabidopsis *PAB5* gene by attempting to complement the yeast *pab1* mutant with *PAB5* cDNA. This would also provide us with a convenient heterologous system in which various aspects of PAB5 activity could be tested.

To this end, a *PAB5* cDNA was placed under control of the yeast β-galactosidase (*GAL1*) promoter (Figure 1B; detailed descriptions of all plasmid constructs are given in Methods) on a yeast–*Escherichia coli* shuttle vector containing *TRP1* as a selectable marker. This plasmid, pYPAB5, along with several control constructs, was transformed into the strain YAS319, which has its only functional copy of the yeast *PAB1* gene on

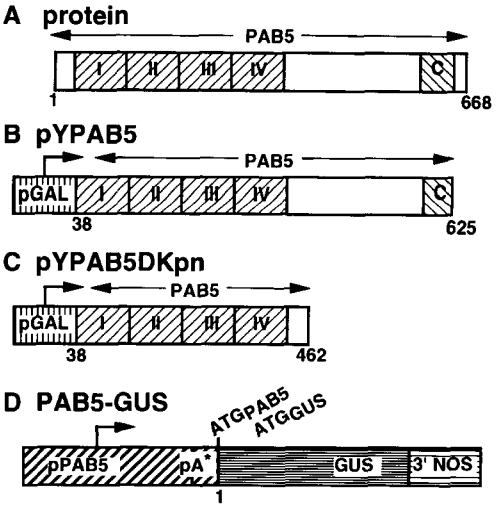

Figure 1. Diagrams of *PAB5* Constructs.

(A) Wild-type PAB5 protein of 668 amino acids. RNA recognition motifs are marked I through IV (light diagonal lines), and the conserved C-terminal region is indicated with a C.

(B) *GAL* promoter (vertical lines) fusion to the *PAB5* cDNA present on plasmid pYPAB5 used in yeast complementation experiments. The first and last amino acids of the PAB5 protein encoded by the construct are shown below the diagram.

(C) *GAL* promoter fusion to the *PAB5* cDNA present on plasmid pYPAB5DKpn (see **[B]**).

(D) *PAB5–GUS: PAB5* promoter (dark diagonal lines) fusion to the *GUS* reporter gene (horizontal lines) contained in pPAB5–GUS used to create transgenic Arabidopsis plants. Shown are the *PAB5* leader sequence containing poly(A) stretch (pA*) as well as the *PAB5* and *GUS* start codons (ATG_{PAB5} and ATG_{GUS}). 3′ NOS, nopaline synthase polyadenylation signals.

Table 1. Genotypes of Yeast Strains Used in This Study

Strain	Genotype	Plasmid[a]	Source
YAS319	*MATa, ade2, his3, leu2, trp1, ura3, pab1::HIS3*	pAS77 (*PAB1, URA3, CEN4*)	Isogenic to YAS100 (Sachs and Davis, 1989) but Gal$^+$; from A. Sachs (University of California, Berkeley)
RSY763	*MATa, ade2-1, his3-11, his15, leu2-3, leu112, trp1-1, ura3-1, sec61::HIS3*	pGAL-SEC61 (*pGAL-SEC61, URA3, CEN4*)	Gift from S. Sanders and R. Schekman (University of California, Berkeley)
YDB1	As YAS319	pYPAB5 (*pGAL-PAB5, TRP1, CEN4*)	This work
YDB2	As YAS319	pYPAB5DKpn (*pGAL-PAB5DKpn, TRP1, CEN4*)	This work
YDB11-7	As YAS319	pYPAB5$^{11\text{-}7ts}$ (*pGAL-PAB5$^{11\text{-}7ts}$, TRP1, CEN4*)	This work
yRP840	*MATa, leu2-3, leu112, ura3-52, his4, trp1-1, cup1::(pGAL-MFA2/pG, pGAL-PGK1/pG, LEU2)*	None	Caponigro and Parker (1995)
yRP881	*MATa, leu2-3, leu112, ura3-52, his4, trp1-1, cup1::(pGAL-MFA2/pG, pGAL-PGK1/pG, LEU2), spb2::URA3, pab1::URA3*	None	Caponigro and Parker (1995)
yRP881$^+$ PAB5	As YRP881	pYPAB5 (*pGAL-PAB5, TRP1, CEN4*)	This work
YDB1$^+$ MFA2/pG	As YDB1	pRP590	This work

[a] *PAB1* and *PAB5* refer to yeast and Arabidopsis *PABP* genes, respectively.

plasmid pAS77 containing the *URA3* selectable marker (yeast strains used in this study are described in Table 1). The transformants were selected on minimal galactose media lacking Trp and Ura, for which this strain is auxotrophic. The *URA3* plasmid can be removed by using the plasmid shuffle technique in which these transformants are simply plated on galactose media (with Ura and without Trp) containing suicide substrate 5-fluoroorotic acid (Sikorski and Boeke, 1991). We reasoned that the *URA3* plasmid, pAS77, carrying the yeast *PABP* gene, could be lost from the Trp$^+$ transformants only if the *PAB5* construct on the *TRP1* plasmid could functionally substitute for the essential yeast *PABP* gene. These two strains and additional controls were streaked on a galactose/5-fluoroorotic acid plate for comparison, as shown in Figure 2A, to illustrate the extent to which PAB5 complements PABP deficiency in yeast. As expected, no growth was observed when the empty *TRP1* vector was used (Figure 2A, pAS135). Strain F364 with plasmid pAS85 expressing a highly truncated yeast *PAB1* gene (Figure 2A) grew very slowly; the strain with the full-length yeast gene on plasmid pAS137 (Figure 2A) grew as well as the wild type; and strain YDB1 with plasmid pYPAB5 expressing the Arabidopsis *PAB5* cDNA (Figure 2A) supported an intermediate level of growth. The results of this experiment demonstrated that *PAB5* at least partially complemented an essential function(s) of PABP in yeast.

The *GAL1* promoter is highly repressed by glucose. Therefore, switching strain YDB1, carrying the *PAB5* cDNA as a sole source of PABP, from galactose to glucose medium resulted in growth arrest after a certain lag period (Figure 2B). The observed lag time most likely reflects the time required to dilute the PAB5 protein previously synthesized during growth on galactose and/or to degrade its mRNA. Transcriptional shutoff of *PAB5* expression on glucose was confirmed by RNA gel blot hybridization with a *PAB5*-specific probe. No *PAB5* RNA was detected 12 hr after the carbon source switch (data not shown).

PAB5 Partially Restores Poly(A) Shortening in Yeast

Because the requirement for poly(A) shortening can be circumvented in certain yeast genetic backgrounds (Sachs and Davis, 1989), we tested whether the Arabidopsis PAB5 protein actually activated poly(A) shortening in YDB1. YDB1 cells were grown in galactose media and then shifted to glucose to repress *PAB5* transcription. Poly(A)$^+$ RNA was isolated at the indicated times after blocking *PAB5* transcription and 3′ end labeled with radioactive pCp and RNA ligase. The non-poly(A) portions of the mRNAs were destroyed by RNase A (which cleaves 3′ to pyrimidine residues), and the remaining intact poly(A) tails were visualized on a sequencing gel, which is shown in Figure 3A (YDB1). Even at the earliest time point after blocking *PAB5* transcription (14 hr), the elongation of poly(A) tails was apparent on glucose relative to those seen on galactose. The peak of poly(A) tail lengths shifted from ~54

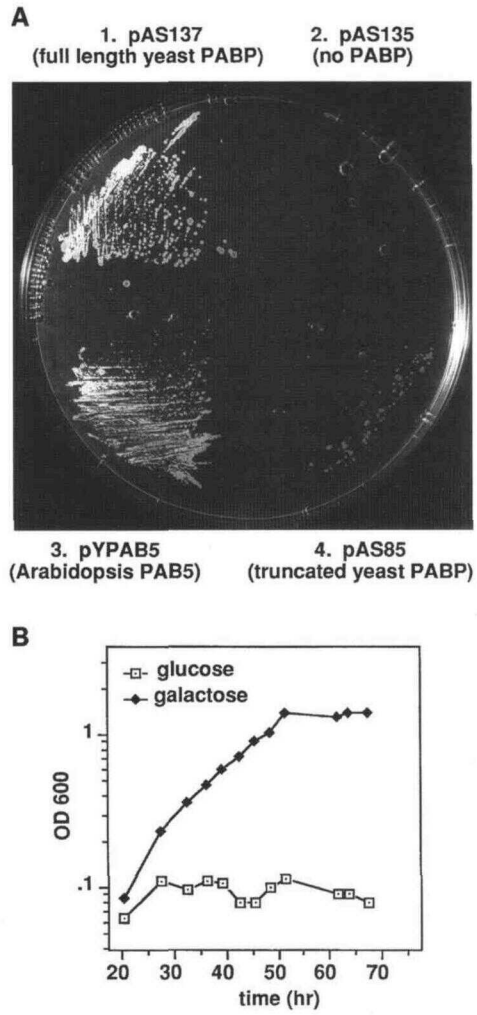

Figure 2. The Arabidopsis cDNA Encoding *PAB5* Restores Viability of the PABP-Deficient Yeast Strain.

(A) The yeast strain YAS319 carrying a disruption of the chromosomal copy of the endogenous *PABP* gene, *PAB1,* and an intact copy of that gene on a *URA/CEN* plasmid (pAS77) was transformed with the *TRP/CEN* plasmid containing the following: (1) pAS137, a vector the full length of the yeast *PABP* gene, *PAB1*, driven by the *GAL1* promoter; (2) pAS135, the vector without an insert; (3) pYPAB5, a vector expressing a nearly full-length *PAB5* cDNA from Arabidopsis under the control of the *GAL1* promoter; and (4) pAS85, a vector with a highly truncated version of the yeast *PAB1* gene driven by its own promoter. Transformants were streaked onto a galactose/5-fluoorotic acid plate to select for cells that had lost the *URA/CEN* plasmid, pAS77, carrying a wild-type copy of the yeast *PAB1* gene.

(B) Growth of the yeast strain YDB1 complemented by *PAB5* under the control of the *GAL1* promoter (Figure 1B) and lacking the yeast PABP gene is dependent on a carbon source. A culture of YDB1 was grown to late log phase in rich galactose medium (yeast extract, peptone, galactose—YPGal), washed with sterile water, and diluted into fresh rich glucose (yeast extract, peptone, dextrose—YPD) medium (dotted squares) or rich galactose medium (closed diamonds) to achieve an equal starting optical density at 20 hr.

nucleotides on galactose to 64 nucleotides on glucose (Figure 3A, striped arrows). Independent repetitions of this experiment showed very similar results. The size distribution of accumulating poly(A) tails approached their reported maximal size attained during nuclear polyadenylation (70 to 90 nucleotides; Sachs, 1990). This demonstrated that *PAB5* shutoff resulted in an inhibition of poly(A) shortening. Even during growth on galactose, when PAB5 is maximally expressed, polyadenylate tails were significantly elongated when compared with the strain having the wild-type *PAB1* gene (data not shown), indicating that PAB5 activated poly(A) tail shortening less efficiently than did yeast PABP.

To demonstrate that poly(A) elongation was not merely a nonspecific consequence of growth arrest, an identical experiment was performed with a yeast strain, RSY763 (a gift from S. Sanders and R. Schekman, University of California at Berke-

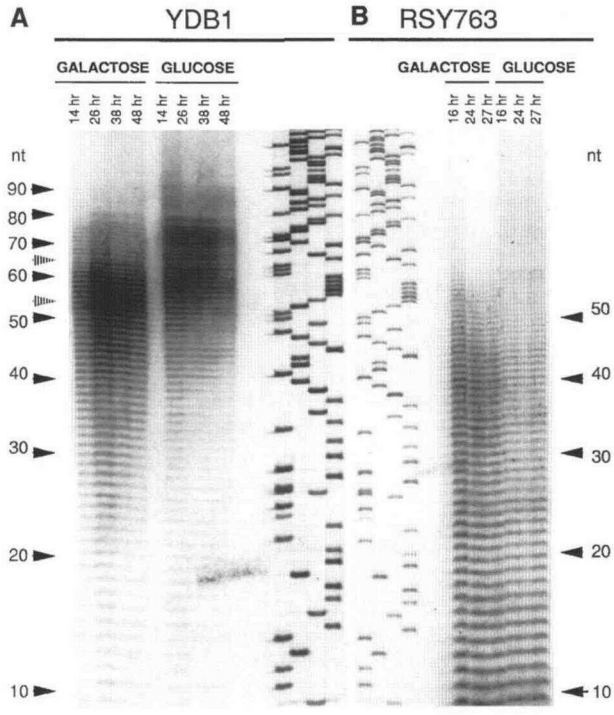

Figure 3. Poly(A) Shortening in YDB1 Requires Expression of *PAB5.*

(A) Galactose-grown or glucose-shifted cultures of YDB1 were collected at the time points indicated at top, and poly(A)$^+$ RNA was isolated by oligo(dT)–cellulose chromatography, 3′ end-labeled with ^{32}P-pCp and RNA ligase, treated with RNase A, and separated on a 12% denaturing polyacrylamide gel to visualize the poly(A) tails. Size markers are shown at left in nucleotides (nt). The peaks of poly(A) tail lengths on galactose at 54 nucleotides and on glucose at 64 nucleotides are indicated at left with striped arrowheads.

(B) The identical experiment was performed with strain RSY763, which has its only copy of the essential gene *SEC61* driven by the *GAL1* promoter. Size markers are shown at right.

ley), carrying its only copy of the essential *SEC61* gene under the control of the *GAL1* promoter. The *SEC61* gene product is an integral endoplasmic reticulum membrane protein that is involved in protein secretion, and it has no direct role in poly(A) metabolism (Deshaies and Schekman, 1987; Rapoport, 1992). As expected, cell growth arrest due to *SEC61* gene product depletion was not accompanied by poly(A) elongation (Figure 3B).

PAB5 Partially Restores Translation Initiation in Yeast

In yeast, suppressors of the *PAB1* gene deletion restore translational initiation but not poly(A) shortening (Sachs and Davis, 1989). Thus, at least in these suppressor backgrounds, the role of PABP in supporting translational initiation, rather than poly(A) shortening, appears to be essential for viability. Therefore, it seemed likely that *PAB5* performed that function in yeast as well. Indeed, the transcriptional block of *PAB5* expression via a carbon source shift resulted in progressive accumulation of 80S monosomes and, particularly at later time points, in the loss of polysomal material (data not shown). However, the control strain containing the *GAL1* promoter-driven *SEC61* gene behaved similarly in this experiment. Thus, the true translation initiation block resulting from PAB5 protein depletion could not be distinguished from the nonspecific effect due to growth arrest under these experimental conditions.

There are at least two potential problems with such carbon source shift experiments, namely, (1) the existence of a large pool of gene product presynthesized during growth on galactose, and (2) some degree of leakiness of the *GAL1* promoter on glucose. These conditions would result in a slow and very gradual depletion of the PAB5 protein after the carbon source shift and therefore might obscure any direct effect PABP may have on initiation. We reasoned that these problems could be circumvented by an abrupt functional inactivation of all PABP in the cell, which could be achieved by using *ts* alleles of *PAB5*. We have constructed a number of *ts* mutations in *PAB5* by random polymerase chain reaction (PCR) mutagenesis (see Methods). One of the resulting mutant strains, YDB11-7, was used for analysis of polysomal profiles before and after the shift to 37°C (its growth curve at 30 versus 37°C is shown in Figure 4A). After only 3 hr of incubation at 37°C, a significant increase in the relative amounts of 80S monosomes and a decrease in the amount of polysomes were observed (Figure 4B). Importantly, loss of viability occurred at a significantly later time point, between 12 and 25 hr after the shift, as evidenced by viable cell counts at different time points (Figure 4C).

In the wild-type strain, a temperature shift resulted in a much milder change in the polysome/monosome ratio. Furthermore, the polysomal profile returned to normal within 1 hr after the shift, with continued incubation at the restrictive temperature (Cigan et al., 1991). In YDB11-7, the relative amount of 80S monosomes continued to increase and the amount of polysomes continued to decrease with time until the polysomal material disappeared completely (Figure 4B, *t* of 12 hr). The results of this experiment demonstrate that *PAB5* rescues, at least to a certain degree, the translational initiation defect in PABP-deficient yeast.

Genetic Interaction of *PAB5* and *SIS1* in Yeast

Some mutational alterations in the 60S ribosomal subunit that suppresses defects in the yeast *PAB1* gene also can supress certain mutations in the essential *SIS1* gene, encoding a putative molecular chaperone required for the initiation of translation (Zhong and Arndt, 1993). Furthermore, overexpression of *SIS1* in the *pab1-F364L* background resulted in a decreased growth rate compared with an isogenic control strain that does not overexpress SIS1, thus supporting the idea of genetic interaction between *PAB1* and *SIS1*. Neither of the *ts* mutants in *GCD1* or *PRT1*, encoding subunits of the translation initiation factors eIF-2B and eIF-3, respectively, exhibited slow growth when SIS1 was overexpressed, thus demonstrating that the genetic interaction between *SIS1* and *PAB1* is specific and suggesting that the proteins they encode may act at the same distinct step (or linked steps) in the translation initiation pathway (Zhong and Arndt, 1993).

Therefore, we tested whether *PAB5* also has the potential to interact genetically with *SIS1*. Surprisingly, when YDB1 was transformed with *SIS1* on a high-copy-number plasmid, growth was enhanced rather than inhibited, particularly at 37°C. Under these conditions, higher levels of *SIS1* expression are induced (Luke et al., 1991). The overexpression of *SIS1* in YDB1 also led to a dramatic increase in the efficiency of plating on selective media at 37°C, as shown in Figure 5B (YDB1/SIS1), relative to the control transformed with the same high-copy vector without the insert (Figure 5A, YDB1/YEp13). Under these conditions, only a few colonies of much smaller size were established. Interestingly, the effect of *SIS1* overexpression was allele specific because neither stimulation nor inhibition of growth was observed in YDB2 (isogenic to YDB1 but with a truncated version of *PAB5*; Figure 1C). Notably, the original observation that *SIS1* overexpression has an inhibitory effect on growth rate (Zhong and Arndt, 1993) was made with a strain containing a more highly truncated version of yeast PABP encoded by the *pab1-F364L* allele (Sachs et al., 1987). Although it is not clear how the structural differences between these PABP proteins translate into different effects on growth rate when *SIS1* is overexpressed, the specificity and reproducibility of *PAB5–SIS1* genetic interaction strongly suggest that *PAB5* is competent to enter the same functional network in which yeast PABP and SIS1 are normally involved and which is essential for efficient translational initiation. The fact that genetic interactions of yeast *PAB1* and Arabidopsis PAB5 with *SIS1* are allele specific seems to imply that these proteins interact physically (Botstein and Maurer, 1982).

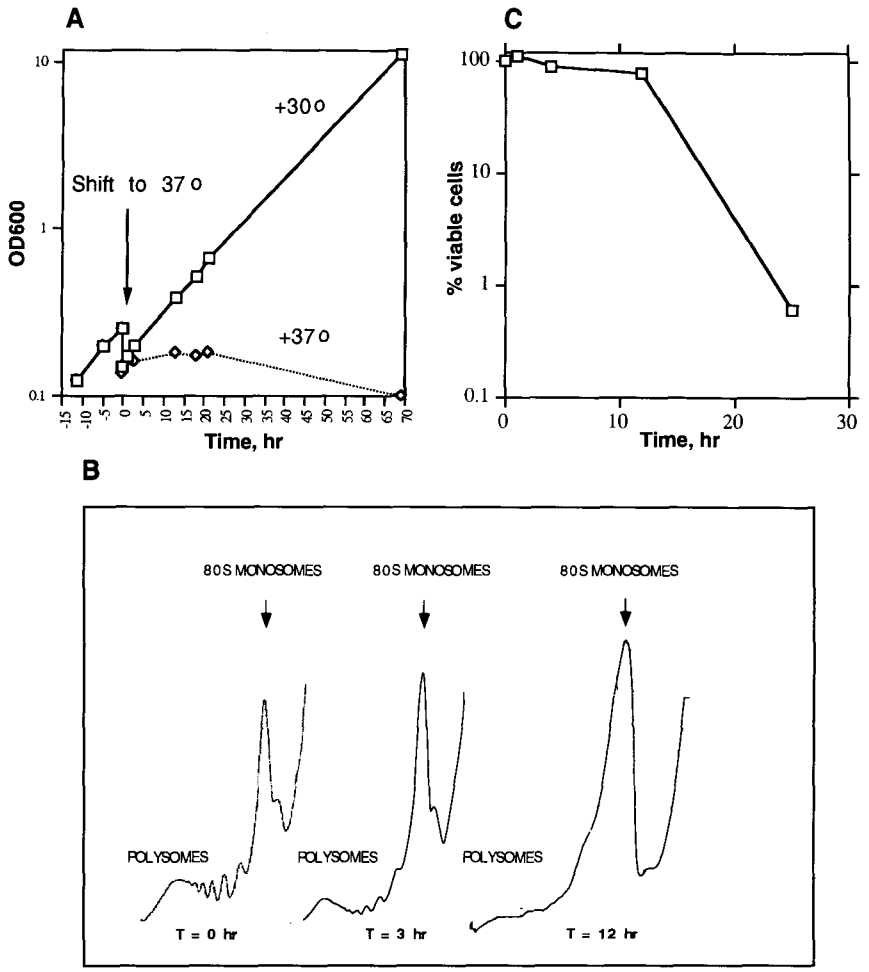

Figure 4. *PAB5* Is Essential for Translational Initiation.

(A) Shown is a growth curve of strain YDB11-7 containing a *ts* allele of *PAB5* (*PAB5*$^{11\text{-}7ts}$) driven by the *GAL1* promoter, grown at 30°C in rich galactose medium, and shifted to 37°C.

(B) Polysomal profiles were determined after sucrose gradient fractionation of YDB11-7 cell extracts prepared just before the shift (*t* of 0 hr) and 3 and 12 hr after the shift. Extracts were loaded onto 15 to 50% linear sucrose gradients, centrifuged for 2 hr at 39,000 rpm, and fractionated through a UV cell detector (Pharmacia) with simultaneous recording. Horse spleen ferritin (~65S) was used as a standard. Positions of 80S monosomes and polysomes are indicated.

(C) Shown is the relative cell viability during the course of this experiment; viability was monitored by plating a dilution series of YDB11-7 culture onto rich galactose medium.

PAB5 Does Not Restore Linkage of Deadenylation and Decapping in Yeast

Recent experiments by R. Parker and colleagues have delineated the major pathway of mRNA decay in yeast. Decay proceeds in the following order: deadenylation → decapping → 5′ to 3′ exonucleolytic decay (Figure 6A, left; Decker and Parker, 1994). Furthermore, in Pab$^-$ strains, decapping occurs directly, without prior deadenylation, suggesting that PABP is essential to such an ordered pathway (Figure 6A, right; Caponigro and Parker, 1995).

The reporter system utilized by Caponigro and Parker (1995) essentially consists of the yeast *MFA2* gene (mRNA $t_{1/2}$ of 3.5 min), modified by the insertion of the poly(G) sequence into its 3′ untranslated region. The inserted sequence forms a stable RNA secondary structure that blocks the action of 5′ to 3′ exonuclease, the primary mRNA degrading activity in yeast. The 5′ to 3′ exonuclease attacks decapped (but not cap-containing) mRNA and produces a discrete distal degradation product whose 5′ boundary is defined by the position of poly(G) insert in the reporter mRNA. Assessing the length of the poly(A) tail of this product via oligo(dT)–RNase H cleavage allowed

us to discriminate between direct and deadenylation-dependent decapping: the presence of the distal product with short poly(A) tails indicates a normal sequence of deadenylation → decapping → 5′ to 3′ exonucleolytic decay, whereas a distal product with long tails is indicative of direct decapping occurring without prior deadenylation.

These results prompted us to investigate whether Arabidopsis *PAB5* can restore the link between deadenylation and decapping in yeast by using the reporter system described above (kindly provided by G. Caponigro and R. Parker, University of Arizona, Tucson, AZ). Because yeast *PAB1* is an essential gene, these experiments were done in a Spb$^-$ background (where Spb indicates suppressor of *Pab1*). The *spb2* allele is a bypass suppressor that allows a *Pab1*$^-$ strain to grow. In agreement with the data of Caponigro and Parker (1995), we observed only short-tailed distal fragments in the wild-type strain yRP840, as shown in Figure 6B, lane 1, in which the *MFA2* transcripts went through the deadenyation-dependent decapping pathway and were then degraded by a 5′ to 3′ exonuclease up to the poly(G) tract (Figure 6A, left). Conversely, in an otherwise isogenic Pab$^-$/Spb$^-$ strain, yRP881, tails were long (Figure 6B, lane 3), because the *MFA2* transcripts went through the direct decapping pathway (Figure 6A, right). When *PAB5* was introduced into Pab$^-$/Spb$^-$ strain yRP881, poly(A) tail lengths on the distal fragment remained long (Figure 6B, lane 5), demonstrating that *PAB5* did not restore the link of deadenylation and decapping, as was observed in wild-type (Pab$^+$) yeast. Similar results were obtained when the decay pattern of the stable *PGK* mRNA ($t_{1/2}$ of 45 min), similarly modified by a poly(G) insert, was examined in these three strains (data not shown).

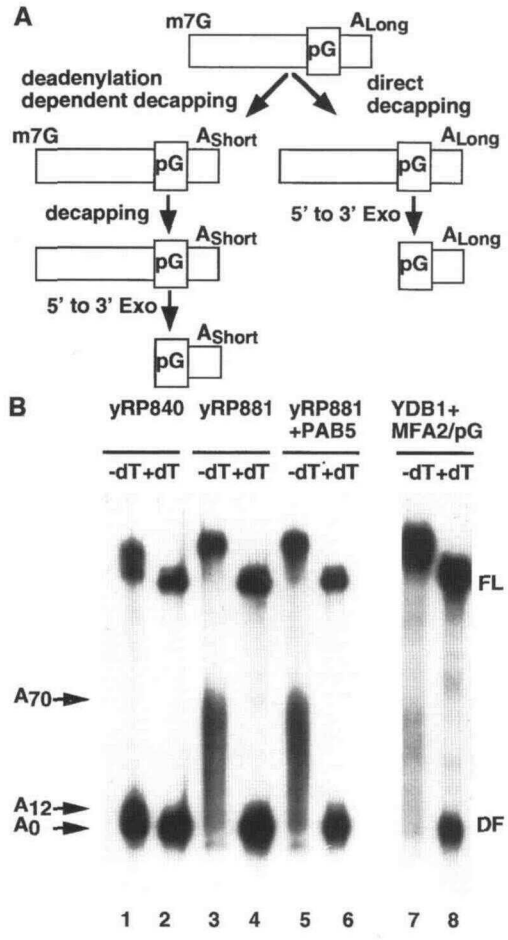

Figure 6. PAB5 Restores Viability but Not the Linkage of Deadenylation and Decapping in Yeast.

(A) The decay of most yeast mRNAs proceeds though a pathway (left) in wild-type strains in which deadenylation leads to decapping, followed by 5′ to 3′ exonucleolytic degradation, and through a pathway (right) in PABP-deficient strains in which direct decapping and decay occur independent of the poly(A) tail length. A_{Long} and A_{Short} indicate long and short poly(A) tail lengths. Exo, exonuclease. pG, a poly(G) sequence in the mRNA that slows the 5′ to 3′ exonuclease.
(B) Total RNAs from the strains shown in **(A)** were treated with RNase H in either the absence (−dT) or presence (+dT) of oligo(dT) as indicated, resolved on a polyacrylamide gel, and hybridized with the MFA2/pG–specific oligonucleotide oRP140. Upper and lower bands correspond to full-length MFA2/pG species (FL) and its distal fragment (DF, with its 5′ end delimited by poly[G] tract), respectively. Size distributions of these bands (estimated relative to pUC18-HpaII size standards and RNA samples completely deadenylated by RNase H/oligo[dT] treatment) reflect poly(A) tail length distributions of the respective RNA species. A_0, A_{12}, and A_{70} indicate the number of A residues measured on the poly(A) tail.

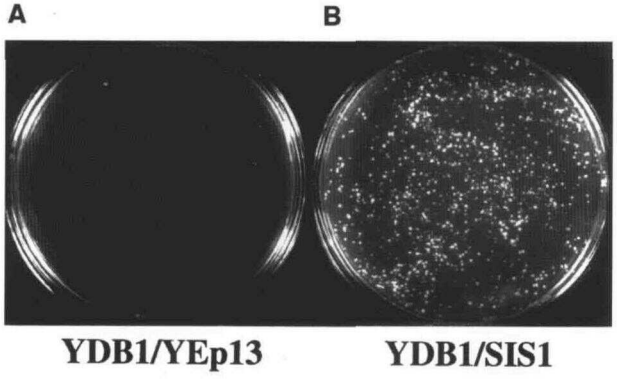

Figure 5. Overexpression of *SIS1* Enhances Plating Efficiency of the YDB1 Strain.

(A) Strain YDB1 containing the *GAL1* promoter–driven *PAB5* cDNA transformed with high-copy vector YEp13.
(B) Same strain transformed with the *SIS1* clone in YEp13. Cultures were diluted and plated on SC-minimal medium (synthetic complete glucose) lacking tryptophan and leucine and incubated at 37°C (see Methods).

Results of the above-mentioned experiment suggest that linking of deadenylation and decapping is not essential in yeast, because *PAB5* restores viability to the Pab$^-$ yeast strain (above) but cannot restore the link between deadenylation and decapping. One caveat, however, stems from the presence of the *Spb2* mutation in yRP881. Although Spb2 has no influence on mRNA decay rates and patterns by itself (Caponigro and Parker, 1995), it can bypass the requirement for PABP. Therefore, we introduced the MFA2–poly(G) reporter into the strain YDB1, which is Spb$^+$, and examined the length of poly(A) attached to the distal product. Figure 6B (lanes 7 and 8) clearly demonstrates that tails on the distal product remained long in this strain, supporting the conclusion that *PAB5* cannot restore linkage of deadenylation and decapping in yeast and therefore that the function of PABP in linking deadenylation and decapping in yeast is not essential for viability.

Chromosomal Map Position of the Arabidopsis *PAB5* Gene

Arabidopsis *Pab5*-deficient mutant(s) would be invaluable for understanding the role of *PAB5* in the plant. Experiments with complementation in yeast suggest that such mutations may result in complex pleiotropic, and possibly lethal, phenotypes in those cell types in which *PAB5* is normally expressed, thus making the design of a straightforward genetic screen difficult. However, knowledge of the *PAB5* map position and its expression pattern may help to identify Arabidopsis *Pab5* mutants among existing mutant collections. To this end, we identified the MboII restriction site polymorphism between Columbia and Landsberg *erecta* ecotypes in a PCR fragment amplified from the promoter region of the *PAB5* gene. Segregation of the cleaved and amplified polymorphic sequences (Konieczny and Ausubel, 1993) was scored in recombinant inbred lines (Lister and Dean, 1993) (see Methods for details). *PAB5* was mapped between restriction fragment length polymorphism markers g4552 and m532 on chromosome 1. *PAB5* is located at map position 106.5, ~5 centimorgans proximal to m532.

Given the association of *PAB5* expression with reproductive processes (below) as well as its ability to perform essential functions in mRNA decay and translation in yeast, it can be expected that loss-of-function mutations in *PAB5* can lead to male and/or female sterility and/or lethality at an early embryo stage. The possible identity between *PAB5* and the closely linked genes, such as those identified by embryonic lethal mutations (Franzmann et al., 1995), is currently being tested.

Construction of the Promoter-Reporter Fusion and the Production of Transgenic Arabidopsis Lines

The ability of *PAB5* to perform essential functions in yeast seemed particularly interesting because its expression in Arabidopsis was restricted to flowers and siliques, according to our RNA gel blots and reverse transcriptase–PCR analyses (Belostotsky and Meagher, 1993). To gain further insight into the details of *PAB5* expression in the plant, transgenic Arabidopsis plants were created containing *PAB5* promoter fusion to a reporter gene encoding β-glucuronidase (GUS). Approximately 2 kb of *PAB5* sequence upstream from the translational start codon was fused to the *GUS* coding sequence in the vector PBI101.2 (Jefferson et al., 1987) to make p*PAB5–GUS* (Figure 1D and Methods). The complete 5′ untranslated leader sequence was preserved in the construct to retain all potential regulation it might confer. This may be essential to reproduce accurately the expression pattern of *PAB5* because leader sequences in all *PABP* genes analyzed so far, including *PAB5*, contain a poly(A) stretch that can serve as a potential binding site for PABP itself (Sachs et al., 1986).

Eight independent transgenic Arabidopsis lines resistant to kanamycin (transformation marker) were obtained, and all expressed GUS activity. One line clearly showed ectopic expression on top of the normal *PAB5* pattern of tissue specificity, presumably due to a fortuitous enhancer trapped upon insertion in the genome. The rest of the transgenic lines expressed GUS in a very consistent pattern, with minor quantitative differences. Only the patterns of expression that were consistent for at least five of eight of the independent transgenic lines are discussed here. Our results, described in detail below, demonstrate that the *PAB5* promoter/leader fragment directed expression of the *GUS* reporter gene in the tapetum, pollen, ovules, and early embryos (below) and in no other organ or tissue, corroborating well our previous reverse transcriptase–PCR and RNA gel blot analyses (Belostotsky and Meagher, 1993).

PAB5 Is Expressed in Tapetum and in Developing and Mature Pollen

Histochemical staining for GUS demonstrated consistently high *PAB5* promoter activity in the anthers of transgenic Arabidopsis plants (Figure 7A). Staged anthers were used for more detailed study of the timing and localization of *PAB5* expression relative to the following developmental hallmarks: (1) meiosis and tetrad formation; (2) first nuclear division; (3) second nuclear division; (4) anther dehiscence; (5) pollen germination; (6) pollen tube growth; and (7) fertilization. High levels of GUS activity were already evident in the tapetum before comparable activity could be observed in developing pollen mother cells (Figure 7B). The first reliably detectable expression of *PAB5* in developing microspores was observed at the tetrad stage (stage 9 according to Smyth et al. [1990]), when young microspores are still within the callose wall (yellow in Figures 7E and 7F). At this stage, expression in the tapetum is at its maximum. Subsequently, tapetal expression diminishes, probably mirroring degeneration of the tapetum later in anther development, which begins at the stage of the callose wall breakdown and is essentially complete by the end of the first microspore mitosis (Bowman, 1994). The *PAB5* promoter remains active in developing microspores throughout pollen development (trinucleate pollen; Figures 7C and 7D), reaching its apparent maximum at dehiscence (Figure 7G, dehiscent anthers; Figure 7H, pollen from heterozygous plant

on the stigma surface). Strong staining was observed in germinating pollen, including pollen tubes growing through the style, along the funiculi (Figure 7I), and into the micropylar openings of the ovules (Figure 7J).

PAB5 Is Expressed in Ovules Undergoing Both Productive Development and Degeneration

During ovule and early embryo development, two patterns of *PAB5* expression could be distinguished. First, in developing seeds (Figures 7K and 7L), staining appeared to be concentrated in globular embryos (~36 to 60 hr after flowering) as well as at the chalazal pole of the embryo sac, which contains free-nuclear chalasal endosperm. Both embryo proper and suspensor cells expressed the p*PAB5–GUS* fusion at the globular stage. Less intense staining was observed throughout the embryo sac, probably representing *PAB5* expression in the cytoplasmic islands surrounding peripheral endosperm nuclei (cellularization of the endosperm in Arabidopsis occurs via cell wall formation, starting from the early heart stage of embryogenesis [Bowman, 1994]). Remarkably, staining has never been observed in heart-stage embryos (~66 to 84 hr after flowering; Figure 7M), thus demonstrating very tight temporal control of *PAB5* expression.

Second, in unfertilized ovules, GUS activity was observed in the degenerating embryo sac (Figures 7K and 7O). The degree of *PAB5* expression varied from a single blue spot at the micropilar end of the ovule (Figure 7N) to an intensely blue tubelike structure extending all the way from the micropilar to the chalasal end, representing the embryo sac undergoing degeneration (Figure 7O). Notably, in all cases, staining appeared more concentrated toward the micropilar region. These observations could reflect a temporal sequence of *PAB5* activation, starting in one or two cells at the micropilar pole and subsequently spreading to more proximal regions of the embryo sac. Although the identity of the cell(s) in which *PAB5* expression is first observed has not been established, their location suggests that they could be synergids and/or the egg cell.

To determine whether the expression program observed in degenerating ovules actually required pollination and fertilization or could have been programmed autonomously, flowers of transgenic plants were emasculated before their pollen reached maturity to prevent self-pollination and were stained for GUS activity 2 days later. The pattern of staining observed in this experiment was essentially identical to that shown in Figures 7K (arrows), 7N, and 7O. Therefore, the promoter of *PAB5* can be activated independent of pollination and fertilization, and thus, *PAB5* expression observed in globular embryos also could be programmed in part by maternal *PAB5* mRNA.

DISCUSSION

The data presented in this article suggest that the Arabidopsis *PAB5* gene encodes a gametogenesis and/or an early embryogenesis-specific post-transcriptional factor. This factor likely acts through a basic mechanism similar to that of yeast PABP, as demonstrated by (1) the ability of *PAB5* to restore viability of a PABP-deficient yeast strain, (2) the ability of *PAB5* to restore poly(A) shortening in yeast, (3) the ability of *PAB5* to restore translational initiation in yeast, and (4) the competence of *PAB5* to interact genetically with *SIS1* in yeast. This remarkable conservation of basic PABP functions and interactions is of particular interest because the expression of *PAB5* in Arabidopsis is highly regulated both spatially and temporally. These data, the high sequence divergence among Arabidopsis *PABP* genes, and the existence of the constitutively expressed *PABP* gene, *PAB2* (Hilson et al., 1993), may also indicate that the complexity and versatility of post-transcriptional control of gene expression during plant development may be much greater than is required during the life cycle of simple eukaryotes. Moreover, the spatial pattern and dynamics of *PAB5* expression represent an intriguing parallel to the characteristic changes in poly(A) status occurring during the maturation of animal sperm and oocytes as well as during fertilization and early development. These changes are known to direct the translational inactivation and/or recruitment of respective mRNAs.

Conserved Functions of Eukaryotic PABPs

Previously, we obtained evidence that the in vitro–synthesized PAB5 protein binds to poly(A) with high specificity (Belostotsky and Meagher, 1993). In this study, we confirm that *PAB5* is indeed a functional *PABP* gene by using even more rigorous tests. We found that Arabidopsis PAB5, which shares only 44% amino acid identity with yeast PAB1, restores viability of a PABP-deficient yeast strain. Furthermore, by engineering a conditionally expressed *PAB5* in yeast, we were able to show that *PAB5* partially restores both translational initiation and poly(A) shortening. Testing these two activities separately was essential, because it has been shown that in all studied *Spb* (suppressor of *pab1*) mutants, translational initiation is restored in the absence of PABP but poly(A) shortening is not (Sachs and Davis, 1989).

Based on recent data in yeast, it has been suggested that the absence of PABP results in several mRNA processing defects, which could by various means result in longer poly(A) tails (Caponigro and Parker, 1995). These mechanisms include (1) a reduced activity for PAN, (2) a lag before the onset of deadenylation and decay, (3) deadenylation-independent decapping and decay, and (4) the synthesis of longer poly(A) tails in the nucleus. This latter possibility has not been demonstrated experimentally and seems unlikely. Our data exclude to some extent the third mechanism because PAB5 restored viability without restoring the linkage between deadenylation and decapping. Finally, the fact that PAB5 can activate poly(A) shortening in yeast implies that it may also activate a "normal" deadenylation-mediated mRNA decay pathway in plants in a similar fashion.

It takes up to 3 hr after the temperature shift to detect noticeable changes in the polysome structure in YDB11-7 containing

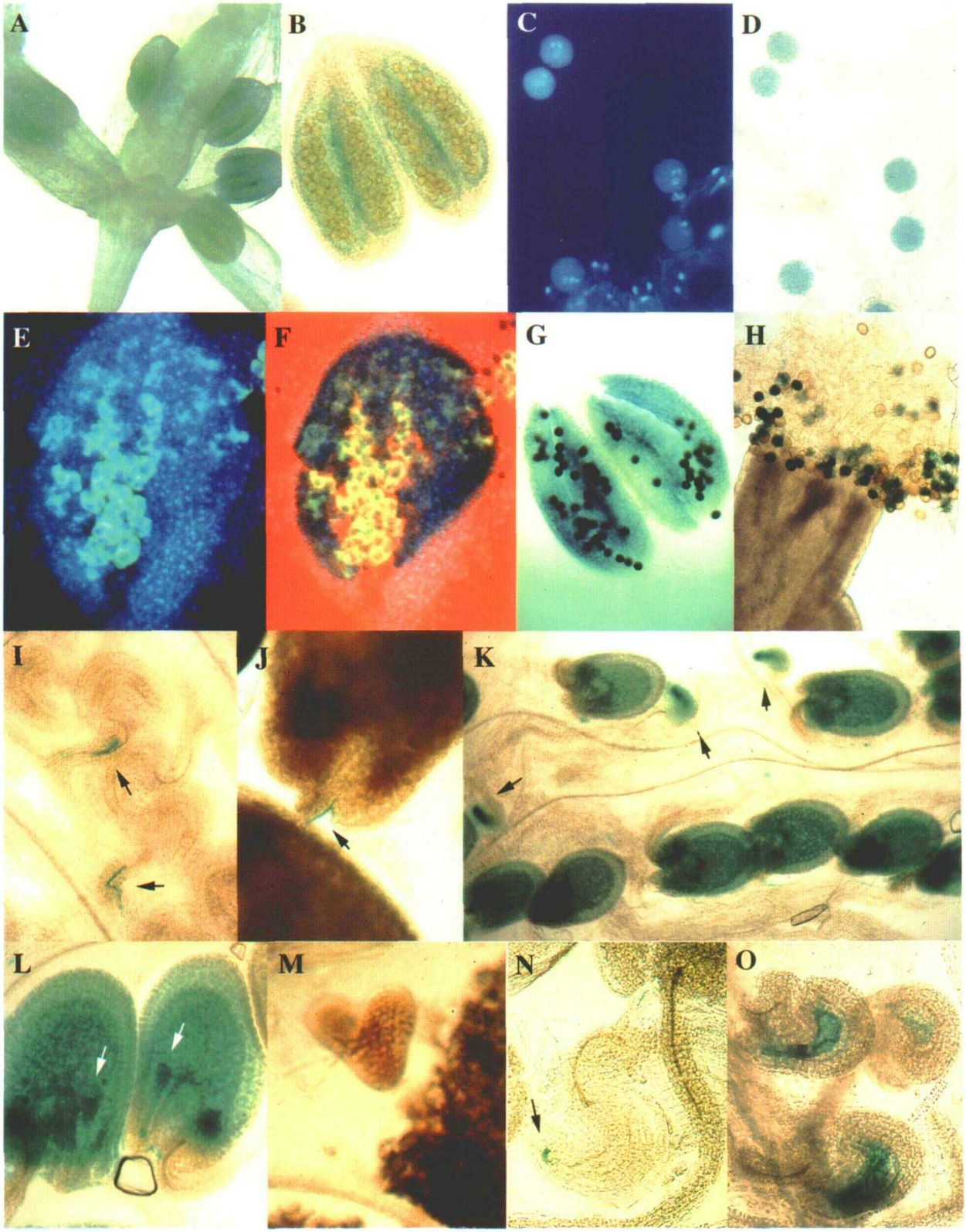

Figure 7. *PAB5* Promoter Activity Is Associated with Pollen and Embryo Development in Transgenic Arabidopsis Plants.

the *PAB5 ts* allele, *PAB5*$^{11\text{-}7ts}$, whereas an effect of similar magnitude was observed after only 15 to 30 min at restrictive temperature in strains carrying *ts* mutations in certain translation initiation factors or *SIS1* (Cigan et al., 1991; Zhong and Arndt, 1993). It is possible that the observed lag time simply reflects a turnover rate of the partially functional *ts* mutant of the PAB5 protein. However, the time lag was the same or even longer when the yeast *pab1-F364L ts* mutant was tested similarly (Sachs and Davis, 1989). Functional turnover kinetics of both PABPs need to be examined to test the possibility that the similarity in lag times observed for strains containing *PAB5*$^{11\text{-}7ts}$ and *pab1-F364L* is coincidental. Alternatively, this similarity may reflect some fundamental property of PABP-dependent translational initiation. For example, there may be a greater requirement for PABP in translational reinitiation rather than in initiation de novo, in agreement with the data of Galili et al. (1988).

We have also considered the possibility that polysomal disintegration observed upon PAB5 depletion is an indirect consequence of the loss of PABP and not a primary cause of inviability of Pab$^-$ cells (even though it occurs before loss of viability; Figures 4B and 4C). Recent experimental observations demonstrate that deadenylation in yeast is followed by decapping, the latter leading to rapid 5′ to 3′ exonucleolytic decay (Decker and Parker, 1994). Importantly, in the absence of PABP, deadenylation and decapping are uncoupled (Caponigro and Parker, 1995). These data define a plausible scenario in which direct decapping in the absence of PABP results in abnormally fast turnover of mRNA and consequently leads to insufficient levels of the protein products necessary for viability. Therefore, the essential role of PABP in the cell could be due at least partially to its ability to delay the onset of decapping until after deadenylation. This idea is further supported by the fact that the deletion of the gene *XRN1*, encoding the major 5′ to 3′ exonuclease in yeast, restores viability of Pab$^-$ strains, possibly via compensatory slowdown of the decay of directly decapped mRNAs (Caponigro and Parker, 1995).

Although such a scenario seems conceivable, a number of independent observations (reviewed in the Introduction) strongly argue for a direct stimulatory role of PABP in translation. Also, this model does not explain the mechanism of suppression of the Pab$^-$ phenotype by *Spb* mutations (all of which affect the amount of 60S subunit and therefore probably affect the function of the ribosome too). Furthermore, because XRN1 is a multifunctional protein (Interthal et al., 1995), all of its various activities must be considered to exclude possible alternative explanations of the ability of *XRN1* deletion to suppress the Pab$^-$ phenotype. It can be envisioned that the primary role of PABP is to establish and/or maintain an appropriate messenger ribonucleoprotein structure, one aspect of which is the circularization of polysomes often observed by electron microscopy (Christensen et al., 1987). PAB5 might support this structure in yeast to an extent sufficient to allow moderate levels of translational reinitiation but insufficient to link deadenylation and decapping. Finally, although PAB5 does not restore linkage of deadenylation and decapping in yeast, our data do not exclude the possibility that PAB5 may link deadenylation to decapping and translation in Arabidopsis.

Do Plants Regulate Translation of Their mRNAs via Cytoplasmic Polyadenylation/Deadenylation?

Previously, we provided initial evidence that at least three genes encoding poly(A) binding proteins in Arabidopsis are expressed in an organ-specific manner (Belostotsky and Meagher, 1993). In this study, we used transgenic Arabidopsis plants transformed with the *PAB5* promoter fusion to a reporter gene to demonstrate that *PAB5* expression is confined to the tapetum, developing pollen, ovules, and embryos. The fact that *PAB5* is expressed exclusively in association with reproductive development is of particular interest in view of the numerous examples of polyadenylation/deadenylation–dependent translational control that are operative during oocyte and/or sperm differentiation and early development in many evolutionarily diverse animal species, including a nematode (*Caenorhabiditis*

Figure 7. (continued).

(A) Histochemical staining of the whole flower reveals *GUS* promoter activity in anthers.
(B) Dissected young anther. Expression in tapetum is stronger than in developing pollen grains at this stage.
(C) and **(D)** *GUS* expression in mature trinucleate pollen viewed with UV epifluorescence and bright-field illumination, respectively.
(E) and **(F)** Triple staining for GUS in young developing pollen (with X-gluc, blue in **[E]**) and callose (yellow, with aniline blue) and in nuclei (light blue, with 4′,6-diamidino-2-phenylindole) of a single anther lobe viewed under UV epifluorescence optics or with the combination of UV and bright light, respectively. GUS activity is detectable both in the developing pollen grains (still within the tetrads) and in surrounding tapetum tissue.
(G) Mature dehiscent anther with darkly stained pollen grains.
(H) Segregation of GUS-positive and GUS-negative pollen on the surface of the stigma of a plant heterozygous for the p*PAB5-GUS* transgene.
(I) and **(J)** GUS activity in the pollen tubes (arrows) growing along the funiculi **(I)** and into the micropylar opening of the ovule **(J)**.
(K) Staining is observed in fertilized as well as unfertilized (arrows) ovules.
(L) Closeup of the developing fertilized ovules showing *PAB5* promoter expression in both the globular embryos (arrows) and surrounding tissues of the embryo sacs.
(M) *PAB5* expression ceases by the heart stage of embryos development.
(N) and **(O)** Different degrees of staining can be observed in unfertilized ovules undergoing degeneration.

elegans), a marine worm (Urechis), a surf clam (Spisula), Drosophila, Xenopus, and mouse (Jackson and Standart, 1990; Wickens, 1992). Recent data demonstrate that not only is this poly(A)-mediated translational control an evolutionarily widespread mechanism of gene regulation, but it is also essential for normal reproduction and early development in animals (Ahringer et al., 1992; Kuge and Inoue, 1992; Gebauer et al., 1994; Salles et al., 1994).

Even though the details of poly(A)-mediated translational control in the course of oocyte maturation and/or early development may differ significantly in different systems and for different mRNAs, the common trend is that cytoplasmic polyadenylation leads to translational activation of mRNA and deadenylation to its translational inactivation but not necessarily to its degradation (Wickens, 1992). It has been proposed that in animals, a divergent, oocyte-specific isoform of PABP (not yet identified) mediates such polyadenylation-dependent translational activation events (Wickens, 1990, 1992; Bachvarova, 1992). In this regard, the ability of Arabidopsis *PAB5* to activate translational initiation in a poly(A)-dependent manner in yeast, as well as its exclusive expression in plant reproductive tissues, and particularly its maternal expression in the embryo sac and possibly in the egg cell (Figures 7N and 7O), is quite striking and suggests that plants also may utilize regulation of polyadenylation status as a means of translational control during reproductive development.

Although there is no conclusive evidence for translational control mediated by cytoplasmic polyadenylation in plants, several recent reports have shown that plants do modulate the poly(A) tail length of specific mRNAs during development, including a heat shock protein (*HSP21*) transcript (Osteryoung et al., 1993), S RNase transcripts (McClure, 1993), and several other major transmitting tissue mRNAs in tobacco (Wang et al., 1996). During reproductive development in plants, partially characterized examples of operating translational activation and repression mechanisms that could be mediated by deadenylation are ubiquitin synthesis (Callis and Bedinger, 1994) and expression of *Zmc13* mRNA (Mascarenhas, 1993) in maize. Also, early stages of pollen germination and growth in various plant species are associated with a massive activation of protein synthesis, which apparently is independent of transcription (Mascarenhas, 1993). Although a direct connection between translational activity and polyadenylation status of a particular mRNA species has not been conclusively demonstrated in any of these cases, this kind of behavior would be expected for mRNA substrates of *PAB5* acting as a potential poly(A)-dependent translational regulatory factor.

Another possible function of *PAB5*, suggested by its ability to activate poly(A) shortening in yeast, could be in stage- and/or tissue-specific mRNA deadenylation, leading to the mRNA decay pathway. The deadenylation of β-1,3-glucanase and MG15 mRNA species in tobacco pistils is accompanied by the decline in their steady state levels (Wang et al., 1996). In contrast, endonucleolytic cleavage turns out to be the earliest step in the decay of ribulose bisphosphate carboxylase small subunit mRNA in soybean (Tanzer and Meagher, 1995), suggesting that a linkage between deadenylation and decay is not essential.

Our findings that the Arabidopsis poly(A) binding protein encoded by *PAB5* can rescue a PABP-deficient yeast strain and is able to activate poly(A) tail shortening as well as translational initiation provide direct evidence that many of the basic functions of eukaryotic PABPs have been conserved throughout evolution. They also suggest that plants, like yeast and animals, may possess a deadenylation-dependent pathway of mRNA degradation. Furthermore, plants, like yeast, are likely to have a similar requirement for a functional PABP for efficient translational initiation. The large family of diverse, differentially expressed *PABP* genes in Arabidopsis (and most likely other flowering plants) may provide additional levels of regulation of gene expression that may be advantageous for organisms with complex developmental pathways. We propose, based on yeast complementation data and the presence of a diverse PABP family in Arabidopsis, that plant PABPs possess the ability to regulate stability and translation of mRNA. Future work will focus on testing the possibility that *PAB5* is a post-transcriptional factor regulating gametogenesis and/or early development.

METHODS

Plant Material

Arabidopsis thaliana ecotype RLD was used for transformation. Columbia and Landsberg *erecta* recombinant inbred lines (Lister and Dean, 1993) were obtained through the Arabidopsis Biological Resource Center at Ohio State University (Columbus, OH).

Plasmid Constructs

pYPAB5 was constructed as follows. First, the 1.8-kb fragment of the poly(A) binding protein *PAB5* cDNA, encoding amino acids 38 to 625 of 668–amino acid PAB5 protein (Belostotsky and Meagher, 1993), was recloned from pET-15b into pBluescript SK+ (Stratagene) as a XbaI-BamHI fragment to create pPAB5-1.8SK. It was then moved into pAS135 (Sachs and Davis, 1989) by digesting with XbaI, filling in with the Klenow fragment of DNA polymerase I, redigesting with SalI, and inserting into pAS135 that was cut with SmaI and SalI so that the *PAB5* cDNA was under the control of the β-galactosidase (*GAL1*) promoter. The resulting pYPAB5 plasmid was used for complementation and also served as a template for random polymerase chain reaction (PCR) mutagenesis (below).

To construct pYPAB5DKpn, pPAB5-1.8SK was digested with XbaI and KpnI, thus truncating the *PAB5* coding region at amino acid 462. Ends of the fragment were made blunt by using T4 DNA polymerase, and it was inserted into the SmaI site of pAS135MS. pAS135MS (pAS135MultiStop) is a pAS135 derivative created by inserting a double-stranded oligonucleotide containing stop codons in all three reading frames into the SphI site of pAS135 to prevent translational readthrough from the *PAB5* deletion construct. Thus, the resulting construct pYPAB5DKpn encodes amino acids 38 to 462 of PAB5, driven by the *GAL1* promoter, and is followed by a short piece of pAS135 polylinker and a stop codon.

The p*PAB5*–β-glucuronidase (*GUS*) translational fusion construct was created by inserting ~2 kb of the XhoI-NcoI fragment containing the promoter and leader of the *PAB5* gene (NcoI site was made blunt with the Klenow fragment) into pBI101.2 (Jefferson et al., 1987) digested with SalI and BamHI (BamHI site blunted with the Klenow fragment). Both NcoI and BamHI sites were regenerated after ligation. The NcoI site in p*PAB5–GUS* overlaps with the translational start codon of the deduced PAB5 protein, which is in frame with the *GUS* coding region in the final p*PAB5-GUS* construct. The structure of the junction between the *PAB5* fragment and the coding region was verified by sequencing. Plasmid pAS85 has been described by Sachs et al. (1987), pAS135 and pAS137 by Sachs and Davis (1989), and pRP590 by Muhlrad et al. (1994).

PCR Mutagenesis of *PAB5*

PCR mutagenesis/in vivo recombination of the *PAB5* cDNA was performed essentially as described by Muhlrad et al. (1992), using pYPAB5 as a template, except that equal deoxynucleotide triphosphate concentrations were used and the Mn^{2+} concentration range was 0.4 to 0.6 mM. The primers used for PCR were GAL1L (5′-CCTCTATACTTTAACGTC-3′, sense primer) and mp18 (−20) sequencing primer (5′-GTAAAACGACGGCCAGT-3′, antisense primer). PCR conditions were as follows: initial denaturation for 5 min at 95°C, followed by 35 cycles at 47, 95, and 72°C (1 min each step), with the final extension at 72°C for 5 min. One of several mutant alleles characterized with a ts phenotype, *PAB5*$^{11\text{-}7ts}$, complements PABP-deficient yeast for growth at 30°C but does not complement growth at 37°C. Wild-type *PAB5* complements well at both 30°C (Figure 3) and 37°C (data not shown).

Yeast Strains and Techniques

Yeast strains used in this study are listed in Table 1. The plasmid-shuffling technique was described by Sikorski and Boeke (1991). Temperature-shift and carbon source–shift experiments as well as the analysis of polysomal profiles and poly(A) tails were done essentially as described by Sachs and Davis (1989). Routine yeast manipulations were performed according to standard protocols (Guthrie and Fink, 1991). The magnitude of the polysome profile differences shown in Figure 4 was reproducible in independent experiments. RNase H cleavage using MFA2/pG- and PGK1/pG-specific oligonucleotides oRP140 and oRP141, respectively, as well as polyacrylamide RNA gel blotting, were performed according to Caponigro and Parker (1995).

Cleaved Amplified Polymorphic Sequence Mapping of *PAB5*

The MboII restriction length polymorphism in the promoter region of *PAB5* between Columbia and Landsberg *erecta* ecotypes of Arabidopsis was displayed by a PCR method based on cleaved amplified polymorphic sequences (Konieczny and Ausubel, 1993), using the sense primer 5PM (5′-AGTATCATCAAAATCGAGAGATTG-3′) and the antisense primer PEXTN (5′-GATGCAACCGCCGCAGCCATAGCGATAAGA-3′). DNA samples were prepared for PCR from young seedlings, according to Edwards et al. (1991). PCR conditions were as follows: initial denaturation at 95°C for 5 min, then 39 cycles at 50, 72, and 95°C for 1 min each, with the final 10-min extension at 72°C. Eighty-eight recombinant inbred lines (Lister and Dean, 1993) were used to obtain segregation data. Strain distribution patterns were analyzed by using the Plant RI Manager program developed and kindly provided by Kenneth Manley (Roswell Park Cancer Institute, Buffalo, NY). Map positions of *PAB5* and the linked markers discussed were determined relative to markers on a previously integrated map (Hauge et al., 1993).

Arabidopsis Transformation

Plasmid pPAB5-GUS was introduced into *Agrobacterium tumefaciens* LBA4404, and Arabidopsis root explants (cv RLD) were transformed according to the published procedure of Marton and Browse (1991), with modifications developed in our laboratory that are described in detail elsewhere (An et al., 1996).

Histochemical Staining

Detection of GUS activity in transgenic Arabidopsis tissues was performed essentially as described by Jefferson et al. (1987), except that acetone pretreatment was included, as suggested by Hemerly et al. (1993). Staining of ovules, although reproducible overall, was more variable than that of anthers, due to less efficient substrate penetration. This problem was somewhat alleviated by cutting siliques open before staining. Significant levels of *GUS* reporter gene activity were also detected in stipules, but this expression could not be confirmed by RNA gel blots, quantitative reverse transcriptase–PCR, or whole-mount in situ hybridizations (data not shown) and was therefore considered artifactual. However, exactly the same sort of discrepancy concerning expression in stipules was encountered by Larkin et al. (1993) with the promoter of the *GLABROUS* gene fused to *GUS*, raising the possibility that a stipule-specific enhancer may be contained within the *GUS* coding region and/or that stipules are transcriptionally indiscriminate.

Staining with 4′,6-diamidino-2-phenylindole and aniline blue was performed according to Regan and Moffatt (1990), and photographs were taken on a photomicroscope (Carl Zeiss, Inc., Hanover, MD) by using UV epifluorescence optics and Kodak 400 or Ektachrome 160T film.

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