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The *Schizosaccharomyces pombe pla1* gene encodes a poly(A) polymerase and can functionally replace its *Saccharomyces cerevisiae* homologue

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ABSTRACT

We have isolated the poly(A) polymerase (PAP) encoding gene *pla1* [for poly(A) polymerase] from the fission yeast *Schizosaccharomyces pombe*. Protein sequence alignments with other poly(A) polymerases reveal that *pla1* is more closely related to *Saccharomyces cerevisiae* PAP than to bovine PAP. The two yeast poly(A) polymerases share significant sequence homology not only in the generally conserved N-terminal part but also in the C-terminus. Furthermore, *pla1* rescues a *S.cerevisiae PAP1* disruption mutant. An extract from the complemented strain is active in the specific *in vitro* polyadenylation assay. In contrast, recombinant PLA1 protein can not replace bovine PAP in the mammalian *in vitro* polyadenylation assay. These results indicate a high degree of conservation of the polyadenylation machinery among the evolutionary diverged budding and fission yeasts.

INTRODUCTION

The 3′-ends of almost all eukaryotic mRNAs are generated by endonucleolytic cleavage of a primary transcript and subsequent polyadenylation of the upstream cleavage product [for review, see (1,2)]. Both steps are accomplished by a multiprotein complex. In the budding yeast *Saccharomyces cerevisiae* four 3′-processing activities have been separated: cleavage factor I (CFI), cleavage factor II (CFII), polyadenylation factor I (PFI) and poly(A) polymerase (PAP). Cleavage can be reconstituted *in vitro* with CFI and CFII, whereas polyadenylation requires PAP, PFI and CFI (3). Polyadenylation is specific in the presence of Mg^{2+} and only RNAs containing specific 3′-processing signals can be polyadenylated. In the absence of the specificity factors PFI and CFI, PAP can polyadenylate an RNA only very inefficiently and without substrate specificity. This unspecific activity is enhanced when Mg^{2+} is replaced by Mn^{2+} (4).

Poly(A) polymerase has been cloned from *S.cerevisiae* (5) and from various vertebrates including human (6), bovine (7,8) and *Xenopus* (9). The vertebrate PAPs are highly homologous to each other throughout their entire sequence. *S.cerevisiae* PAP is encoded by the essential gene *PAP1*. *PAP1*-encoded protein and its bovine counterpart are 47% identical within the first 395 amino acids. Mutational analysis of bovine PAP revealed a catalytic domain within the conserved N-terminal part (10). The high conservation of this domain suggests the same function also in other poly(A) polymerases. The C-terminal regions are not conserved on the primary sequence level. Nevertheless, recent results show that both C-termini contain an RNA-binding domain. In both cases a nuclear localization signal (NLS) was found to be essential also for RNA-binding (10,11).

In spite of these similarities, bovine PAP is not able to rescue a *PAP1* deletion mutant (12) and *S.cerevisiae* PAP can not replace the bovine enzyme in the mammalian *in vitro* polyadenylation system. The reason for this might be the inability of both polymerases to interact with the specificity factors of the respective other species. *S.cerevisiae* PAP has been shown to directly interact with the FIP1 protein, a subunit of PFI (13). Bovine PAP forms a complex with the mammalian cleavage and polyadenylation specificity factor CPSF on the substrate RNA (14). Other differences between mammalian and *S.cerevisiae* polyadenylation are the poly(A) tail length and the signals on the RNA directing 3′-processing (15,16). In *S.cerevisiae*, poly(A) tails consist of 60–70 A residues whereas mammalian poly(A) tails reach a length of ~250 A residues.

In summary, many similarities, but also several differences, have been found between pre-mRNA 3′-processing in mammals and in *S.cerevisiae*. It is not known whether or not *S.cerevisiae* is a typical representative of the lower eukaryotes with regard to 3′-processing. Some evidence for a strong conservation of 3′-end formation among lower eukaryotes comes from a study dealing with the 3′-processing signals of *S.cerevisiae* and the distantly related fission yeast *Schizosaccharomyces pombe*. It was found that *in vitro*, the *S.pombe URA4* transcript is correctly processed in a *S.cerevisiae* extract and that *S.pombe* can efficiently process the *S.cerevisiae CYC1* transcript *in vivo* (17). This finding was surprising because *S.pombe* and *S.cerevisiae* are evolutionarily distant. In fact, several *S.pombe* genes show about the same degree of homology with their counterparts in *S.cerevisiae* and with those in higher eukaryotes (18). On the other hand, the fact that the 3′-end formation signals can be exchanged between the two highly diverged yeast species does not mean that the *trans*-acting factors are conserved as well. For example, the conservation could be limited to the RNA-binding sites of the specificity factors. The cloning of the *S.pombe* poly(A) polymerase appeared to be a reasonable starting point to find out whether the 3′-processing factors of *S.pombe* are more closely related to those of mammals or those of *S.cerevisiae*.

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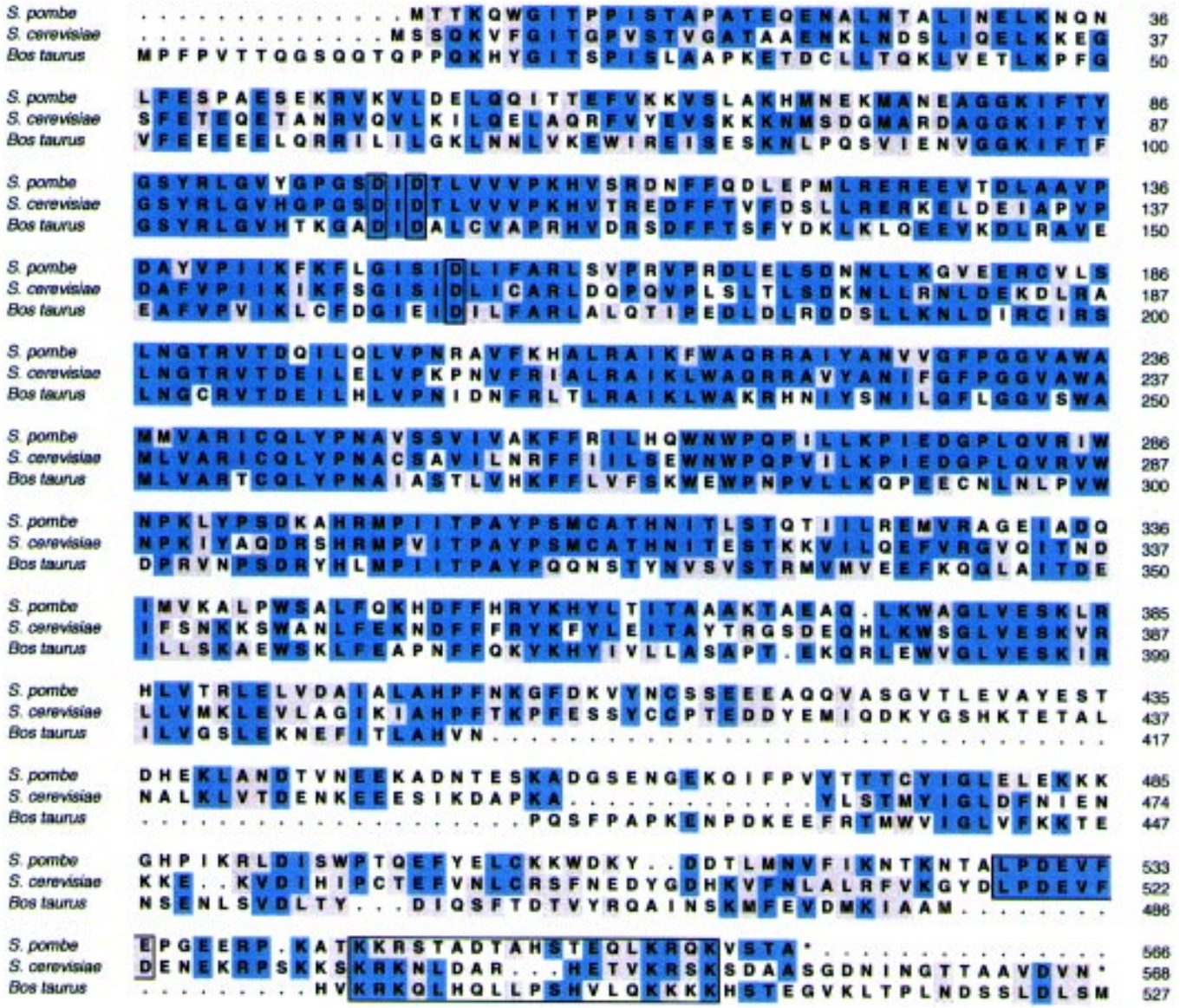

Figure 1. Multiple peptide sequence alignment of three poly(A) polymerases: *PAP1* (*S.cerevisiae*), *pla1* (*S.pombe*) and bovine PAP (*Bos taurus*). Sequence identities are displayed as blue boxes, similarities are depicted as purple boxes. The sequence of bovine PAP (total length 739 amino acids) is only shown partially. The alignment of *PAP1*, *pla1* and amino acids 1–417 of bovine PAP was done with the program PILEUP. The remaining part of bovine PAP was aligned manually to the other two sequences. The following features are shown in black frames: the three aspartate residues relevant for catalysis in bovine PAP (10) and the corresponding aspartates of *PAP1* and *pla1*, the NLS1 of bovine PAP and the putative NLS of the two yeast enzymes, and the conserved LPDEVF(D/E) sequence motif (see text for further details). The peptide sequences of *PAP1* and bovine PAP are accessible in the SWISSPROT database under the numbers P29468 and P25500 respectively.

Here we report the cloning of *pla1*, a poly(A) polymerase encoding gene from the fission yeast *S.pombe*. We find that the protein sequence of *pla1* is more similar to the *S.cerevisiae* PAP than to bovine PAP. Furthermore, we show that *pla1* rescues a *S.cerevisiae PAP1* disruption mutant, whereas recombinant PLA1 protein can not replace bovine PAP in the mammalian *in vitro* polyadenylation system.

METHODS AND METHODS

PCR on genomic *S.pombe* DNA

The amino acid sequences of two conserved peptides of *S.cerevisiae* PAP and bovine PAP were used to design two degenerate PCR primers: 5′-CCGTCGACGGIAARATHTTYAC-3′ (R = A or G, Y = C or T, H = A or C or T) from the peptide sequence GKIFT (in Fig. 1: amino acids 82–86 in *PAP1*) and 5′-CCGGATCCA-CIARCATNGCCCA-3′ from the peptide sequence WAMLV (amino acids 236–240 in *PAP1*). The 50 µl PCR mixture contained: 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% Triton X-100, 0.2 mM deoxynucleoside triphosphates, 250 pmol PCR primers, 4 ng genomic *S.pombe* DNA and 1 U *Taq* DNA polymerase (Perkin Elmer). Twenty-five cycles were carried out, with annealing at 48°C for 30 s and extension at 72°C for 2 min. A PCR product of the expected size (476 bp) was reamplified and subcloned into the *Sal*I–*Bam*HI site of the Bluescript KS vector (Stratagene) and sequenced with Sequenase (Amersham Life Science Inc.).

Isolation of cDNA clones

A random-primer labelled probe was synthesized from the subcloned PCR fragment. The probe was used to screen a *S.pombe* cDNA plasmid library (vector: pFL61, see Table 1). The cDNA library was a gift from Dr François Lacroute (Gif-sur-Yvette, France). 2.5×10^4 colonies were screened on nitrocellulose filters according to standard procedures (19).

Table 1. Yeast plasmids

Name	Description
pFL61	2μ-based vector with a *URA3* marker (34)
pFL61-pla1	pFL61 containing *pla1* in its *Not*I site under the control of the PGK promoter
pFLT-pla1	Derivative of pFL61-pla1 in which the *Bgl*II *URA3* cassette has been replaced by the *Bgl*II *TRP1* cassette
pHCp50	Contains *URA3* and the genomic *Hin*dIII fragment of *PAP1*; rescues a chromosomal *PAP1* disruption (26)
pHCBS3	Contains *TRP1* and the genomic *Hin*dIII fragment of *PAP1*; rescues a chromosomal *PAP1* disruption (26)

Sequence analysis

Protein sequences were analyzed with GCG software programs. Sequence databases were searched with the programs FASTA and TFASTA (20). For sequence alignments the program PILEUP was used. The program BESTFIT was used to calculate the percentages of identity. The nucleotide sequence of *pla1* has been deposited in the EMBL database under the accession number X79705. The email server at the EMBL was used for the secondary structure prediction (21,22).

Western blots

Proteins were separated on 9% polyacrylamide gels (23) and blotted to nitrocellulose membranes. The blots were blocked in TN Tween [10 mM Tris–HCl (pH 8.0), 150 mM NaCl, 0.05% Tween 20] containing 5% non-fat milk. The same buffer was also used for the antibody incubations. Peroxidase-conjugated swine anti-rabbit immunoglobulins were used as secondary antibodies. Chemiluminescence (ECL kit, Amersham) was used for detection. Affinity-purified antibodies directed against *S.cerevisiae* PAP were a gift from Pascal Preker.

Yeast strains, media and genetic methods

Yeast media were prepared as described elsewhere (24). Yeast transformations were carried out following the lithium acetate procedure (25). The strain used in this study was *S.cerevisiae* JL17-3A (*Matα, ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3 can1-100 pap1::LEU2*, pHCp50) (26).

In the plasmid shuffling experiment, JL17-3A was transformed either with pFLT-pla1, pHCBS3 (positive control, see Table 1) or pFLT (negative control). Transformants were transferred to SD-Leu plates containing 5-fluoroorotic acid (5-FOA) to select for cells having lost pHCp50 (27). The strain carrying pFLT-pla1 instead of pHCp50 is called JL17PLA, the one carrying pHCBS3 is called JL17PAP.

Bacterial expression of histidine-tagged recombinant proteins

The open reading frame of *pla1* was amplified by PCR with two primers: 5′-CGGGATCCATGACTACCAAGCAATGG-3′ and 5′-CGGGATCCTTATGCCGTTGAAACTTT-3′. The reaction was carried out with *Pfu* DNA polymerase (Stratagene) according to the manufacturer's instructions. The PCR product was subcloned into the *Bam*HI site of the histidine-tag expression vector pQE9 (Qiagen). *Escherichia coli* M15 was used to overexpress pQE9-pla1. The expression was induced by adding 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) to a 500 ml LB culture. The induced culture was grown overnight. After harvesting by centrifugation, the cells were resuspended in 50 ml TK buffer [50 mM Tris–HCl (pH 8.0) , 100 mM KCl] and frozen at –80°C. The cells were thawed after addition of 100 ml lysis buffer [50 mM Tris–HCl (pH 8.0), 100 mM KCl, 15% glycerol, 1.5 mM phenylmethylsulfonyl fluoride (PMSF), 0.6 mg/ml lysozyme, 15 μg/ml DNase I, 3 μg/ml leupeptin, 3 μg/ml pepstatin]. After sonication and centrifugation the supernatant was filtered and batch absorbed to 2 ml of a 50% slurry of nickel–nitrilotriacetic acid agarose (Ni–NTA, Qiagen), which had been equilibrated with GTK buffer [50 mM Tris–HCl (pH 8.0), 100 mM KCl, 10% glycerol]. After 30 min the suspension was poured into a column. The column was washed with 10 ml GTK buffer containing 10 mM imidazole. The recombinant protein was eluted with 5× 1 ml GTK buffer containing 100 mM imidazole. The pool of recombinant protein was loaded on a 1 ml DEAE–Sepharose column. The column was washed with 2× 1 ml GTK buffer containing 150 mM KCl and with 2× 1 ml GTK buffer containing 500 mM KCl. Recombinant PLA1 protein eluted in the 150 mM step.

In vitro polyadenylation assays

Unspecific polyadenylation was done as described previously (28). Specific polyadenylation of the *in vitro* transcript pG4-CYC1 pre (13) was performed as described previously (29). Specific polyadenylation of the L3 precleaved RNA (30) was done as described (14). The reaction products were analyzed on denaturing polyacrylamide gels. Purified CPSF was a gift from Silvia Barabino and Andreas Jenny. Recombinant bovine PAP and *S.cerevisiae* PAP were gifts from Georges Martin.

Yeast extract preparation

Yeast extracts were prepared as described previously (31) with the modifications described below. Cells were harvested at OD 2.5–4. Spheroplasts were homogenized in the presence of the protease inhibitors PMSF (1 mM), pepstatin (0.7 μg/ml) and leupeptin (0.4 μg/ml). Potassium acetate was used instead of KCl. Whole cell extracts were brought to 45% ammonium sulphate saturation. The same protease inhibitors (at the same concentrations) as described above were also used in the dialysis buffer.

Alternatively, yeast extracts for *in vitro* 3′-processing were prepared by freezing the cells in liquid nitrogen and homogenizing them with a mortar and pestle as described previously (32). The

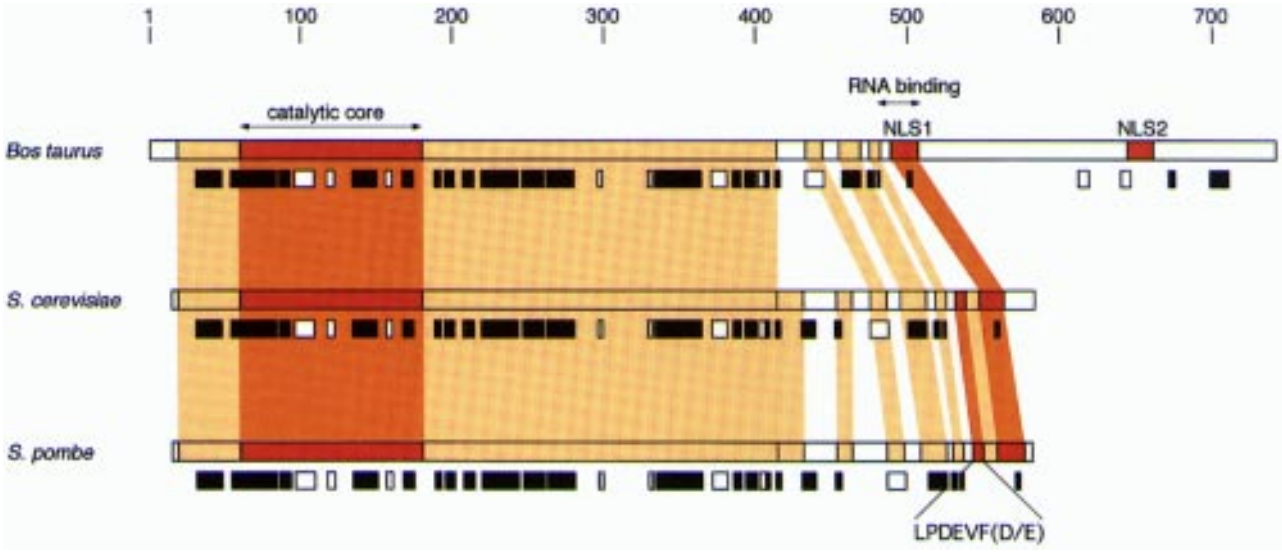

Figure 2. Schematic sequence organization of three poly(A) polymerases: bovine PAP (Bos taurus), *PAP1* (*S.cerevisiae*) and *pla1* (*S.pombe*). The scale on top shows the position in number of amino acids. The locations of the catalytic core and the RNA-binding region in bovine PAP are indicated with double arrows. The following features are shown as dark red boxes: the catalytic core and the corresponding regions of *PAP1* and *pla1* , the nuclear localization signals of bovine PAP and the putative NLS of *PAP1* and *pla1*, the LPDEVF(D/E) sequence motif. Regions with a similarity >40% are indicated as light red boxes. The boxes below the bars show a secondary structure prediction of the alignment of all three sequences. Black boxes correspond to α helices, white boxes indicate β strands.

ammonium sulphate precipitation and the dialysis were done as described above.

RESULTS

Isolation of a *S.pombe* cDNA clone with significant homology to poly(A) polymerases of other species

In order to clone the poly(A) polymerase of *S.pombe* two degenerate primers were derived from the peptide sequences GKIFT and WAMLV, which are conserved in *S.cerevisiae* PAP and bovine PAP. The primers were used for PCR on genomic *S.pombe* DNA. A band corresponding to the expected size was subcloned and sequenced. The putative peptide sequence of the PCR product was found to be 70% identical to *S.cerevisiae* PAP and 58% identical to bovine PAP. The amplified DNA could therefore be used as a probe for screening a *S.pombe* cDNA library. Three positive clones with a 2.1 kb insert and identical restriction pattern were obtained. The insert of one of them (*pla1*) was subcloned into the *Not*I site of the Bluescript KS vector and sequenced on both strands. Sequencing of *pla1* revealed a putative ORF of 566 amino acids corresponding to a 64 kDa protein. As there are stop codons in front of the start ATG we conclude that *pla1* is a full length clone. The *pla1* protein sequence contains a putative bipartite nuclear localization signal (33) at its C-terminus (amino acids 545–562).

Sequence comparisons

Database searches with *pla1* yielded high scores with poly(A) polymerases but not with any other protein sequence (data not shown) suggesting that *pla1* encodes a poly(A) polymerase. An alignment of the *pla1* amino acid sequence with that of *S.cerevisiae* PAP and bovine PAP reveals a high degree of identity, especially in the N-terminal part. In the first 400 amino acids, *pla1* is 62% identical to *S.cerevisiae* PAP and 47% identical to bovine poly(A) polymerase. The alignment in Figure 1 shows blocks of complete conservation in all three sequences in the N-terminal part but also stretches that are highly conserved only among the two yeast enzymes, for example in residues 308–316 (the numbers refer to the *pla1* sequence). The region recently identified as the catalytic core of bovine PAP (10; see Figs 1 and 2, residues 59–179) is quite well conserved in *pla1* and within this part the three aspartates involved in catalysis in the mammalian enzyme (10) are conserved (see Fig. 1). Although the C-termini are less conserved, there is significant homology among the two yeast poly(A) polymerases (35% identity). One of the most striking common C-terminal features of *pla1* and *PAP1* is a predominantly acidic stretch (amino acids 528–538) which is followed by a predominantly basic region, containing the putative NLS. The sequence LPDEVF(D/E) is conserved among *pla1* and *PAP1* but is absent in the C-terminus of the bovine poly(A) polymerase (Figs 1 and 2). The C-terminal portions of *pla1* and bovine PAP are 23% identical. The overall identity of *pla1* and *S.cerevisiae* PAP is 55%, whereas *pla1* and bovine PAP are only 38% identical. From these data we conclude that *pla1* is more closely related to *S.cerevisiae* PAP than to its mammalian counterpart.

Bacterial expression of *pla1*

The ORF of *pla1* was expressed with a histidine-tag in *E.coli* and the recombinant protein was purified as described in Materials and Methods. On a SDS–polyacrylamide gel the recombinant protein appears as a band of ~67 kDa which is in good agreement with the calculated molecular mass (64 and 1.4 kDa for the histidine tag).

In an unspecific polyadenylation assay a specific activity of 8×10^5 U/mg was measured. The activity of PLA1 protein is in the same range as that of *S.cerevisiae* PAP (1.3×10^6 U/mg).

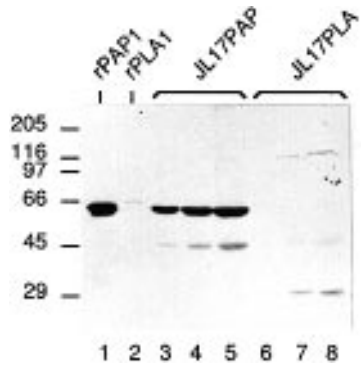

Figure 3. Immunoblot analysis of an extract from the *S.cerevisiae PAP1* deletion mutant rescued by the *S.pombe* poly(A) polymerase (JL17PLA). The blot was probed with an antibody directed against *S.cerevisiae* PAP (1:1000 dilution). Lane 1, 30 ng recombinant PAP1 protein; lane 2, 30 ng recombinant PLA1 protein; lanes 3, 4 and 5: 10, 30 and 100 µg protein of an extract from JL17PAP; lanes 6, 7 and 8: 10, 30 and 100 µg protein of an extract from JL17PLA. Abbreviations: rPAP1, recombinant PAP1 protein; rPLA1, recombinant PLA1 protein. The molecular mass (in kilodalton) and migration position of protein size markers are indicated on the left.

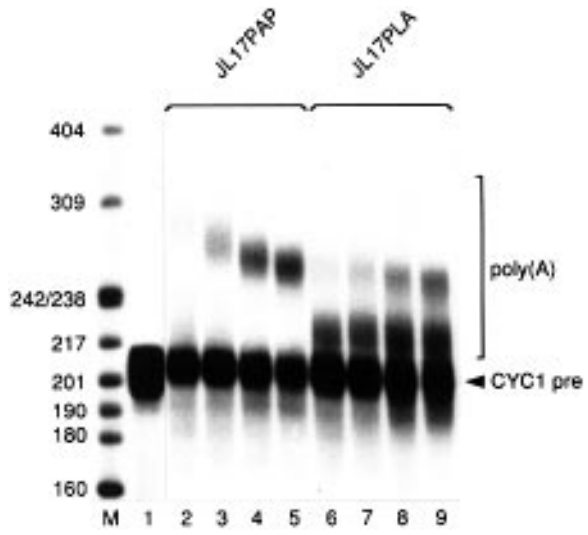

Figure 4. Polyadenylation of the CYC1 precleaved RNA *in vitro*. Lane 1, no protein was added to the assay. Lanes 2, 3, 4 and 5: 12, 24, 48 and 72 µg of an extract from JL17PAP were used. Lanes 6, 7, 8 and 9: 12, 24, 48 and 72 µg of an extract from JL17PLA were used. The migration position and size of markers (in number of nucleotides) are indicated on the left. The migration position of the substrate (CYC1 pre) and polyadenylated RNA are indicated on the right.

Complementation of a *S. cerevisiae PAP1* deletion mutant

By complementation of the *S.cerevisiae PAP1* deletion mutant JL17-3A we addressed the question whether *pla1* can functionally substitute for *PAP1*. In JL17-3A the chromosomal copy of *PAP1* is replaced by *LEU2* (26). The strain is rescued by pHCp50 which contains *PAP1* and *URA3*. JL17-3A was transformed with pFLT-pla1. Transformants were transferred on a SD-LEU plate containing 5-FOA. 5-FOA kills cells that contain a wild-type copy of *URA3* (27). Therefore, survival depends on the loss of pHCp50 and on the ability of *pla1* to take over the essential function of *PAP1*. Viable colonies were obtained in the plasmid shuffling with pFLT-pla1. These cells grew slightly slower than those rescued by pHCBS3 (160 min doubling time versus 95 min). To exclude the possibility that the growing colonies had maintained pHCp50 (for example by mutating the *URA3* marker), we prepared an extract from the *S.cerevisiae* strain expressing the *S.pombe* poly(A) polymerase (JL17PLA) and probed it on a western blot with a polyclonal antiserum directed against *S.cerevisiae* PAP. Figure 3 shows that the extract does not contain *S.cerevisiae* PAP (lanes 6–8) whereas PAP can easily be detected in a control extract from JL17PAP (lanes 3–5). A significant amount (30 ng) of recombinant PLA1 protein (lane 2) is only visible as a very faint band due to a crossreaction with anti-PAP antibodies. It is thus not surprising that PLA1 protein could not be detected in the extract from JL17PLA cells.

The complementation of the *S.cerevisiae PAP1* deletion with the *S.pombe pla1* gene shows that PLA1 protein can fulfil the essential function of a poly(A) polymerase in *S.cerevisiae*. Similar results were obtained when a temperature sensitive allele of *PAP1* was complemented by pFL61-pla1 (results not shown).

In vitro complementation

In order to test whether PLA1 protein can also have a specific poly(A) polymerase activity *in vitro* we made an extract from JL17PLA and tested it in a specific polyadenylation assay. The substrate for this reaction was the CYC1 precleaved RNA which ends at its natural polyadenylation site and can be polyadenylated by a *S.cerevisiae* wild-type extract (13). Figure 4 shows a polyadenylation assay, in which extracts from JL17PAP and JL17PLA were titrated. The extract from JL17PLA (lanes 6–9) produces essentially the same reaction products as the wild-type (lanes 2–5). The polyadenylation reaction seems to be slightly less efficient with the extract from JL17PLA than with the wild-type extract, but in both cases the poly(A) tails reach approximately the same length (compare lanes 5 and 9). The long poly(A) tails in lanes 2–4 are probably due to inefficient poly(A) length control in reactions with low amounts of wild-type extract. This effect is less pronounced for JL17PLA (lanes 6–8). In the reactions with the extract from JL17PLA a band of intermediate length can be seen. This band represents oligoadenylated precursor RNA, as confirmed by binding to poly(U)–Sepharose (results not shown). The intermediate band may be caused by decreased stability of the polyadenylation complex in the presence of PLA1, leading to a premature release of the polyadenylation product. In any case, we conclude that PLA1 can replace the *S.cerevisiae* poly(A) polymerase in a specific polyadenylation assay.

To test whether PLA1 protein can also act in concert with the mammalian cleavage and polyadenylation factor CPSF, we examined the ability of PLA1 protein to polyadenylate the adenoviral L3 precleaved RNA in the presence of CPSF. Figure 5 shows that bovine poly(A) polymerase can polyadenylate L3 precleaved RNA in the presence of CPSF (lane 2), but not when CPSF is absent (lane 3). In contrast, *S.cerevisiae* PAP is not stimulated by CPSF (compare lanes 4 and 5). The same is also observed for recombinant PLA1 protein (compare lanes 6 and 7). This result shows that PLA1 is not able to replace bovine PAP and

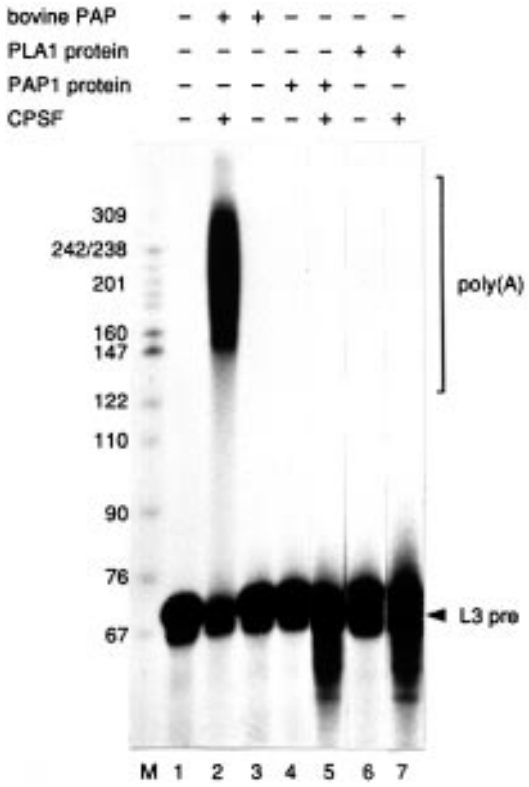

Figure 5. Polyadenylation of the L3 precleaved RNA *in vitro*. Proteins were added as indicated on the top. One ng of a recombinant poly(A) polymerase was used per reaction. The size (in number of nucleotides) and migration position of the markers are indicated on the left. The migration position of the substrate (L3 pre) and polyadenylated RNA are indicated on the right.

suggests that the *S.pombe* poly(A) polymerase does not interact specifically with CPSF.

DISCUSSION

In this paper we report the cloning of the *S.pombe* gene *pla1* which is highly homologous to the *S.cerevisiae* poly(A) polymerase gene *PAP1* and is capable of complementing a *S.cerevisiae PAP1* deletion mutant.

Recombinant PLA1 protein is active in unspecific polyadenylation. Thus, *pla1* encodes a poly(A) polymerase. Sequence comparisons with other poly(A) polymerases show that *pla1* is more closely related to *PAP1* (62% identity within the first 400 amino acids) than to bovine PAP (47% identity in the N-terminal part). The two yeast poly(A) polymerases differ approximately equally from their bovine homologue on the primary sequence level. The same tendencies can also be seen in an alignment of the C-termini. Whereas it was not possible so far to find a significant sequence homology between the C-terminus of *PAP1* and any other poly(A) polymerase, the C-termini of *pla1* and *PAP1* are 35% identical. This identity is significant because alignments of randomized versions of the two C-termini always yielded identities below 25%.

A high similarity of the two yeast PAPs is not only observed on the sequence level but also with regard to the ability of *pla1* to rescue the *S.cerevisiae PAP1* deletion mutant. An extract prepared from JL17PLA efficiently polyadenylates the CYC1 precleaved RNA and the products are comparable to those obtained with a wild-type *S.cerevisiae* extract. Extracts from mutants affected in essential polyadenylation factors are inactive in specific polyadenylation *in vitro* (13,29), although they contain a functional PAP. The reason for this might be the very inefficient manner by which poly(A) polymerases elongate RNA substrates in the absence of specificity factors (28). Therefore, in order to produce normal poly(A) tails, PLA1 protein has to interact with the *S.cerevisiae* polyadenylation specificity factors (PFI, CFI or yet unidentified additional components). This suggests that similar factors exist in *S.pombe* and argues for a conservation of the polyadenylation machinery in the two yeast species.

In contrast to *pla1* bovine PAP can not rescue a *S.cerevisiae PAP1* null allele (12) and none of the yeast polymerases is able to replace bovine PAP *in vitro* in the mammalian polyadenylation system. Consequently, there must be an important difference between the two yeast polymerases and bovine PAP. Because the *S.cerevisiae* poly(A) polymerase and its bovine homologue differ mostly in their C-terminal parts, the C-termini have been suggested to contain domains involved in species specific functions such as interactions with specificity factors (5,8). One striking feature common to both yeast PAPs is the sequence LPDEVF(D/E) which is located upstream of the putative NLS. This sequence is absent in the mammalian poly(A) polymerase, which suggests that LPDEVF(D/E) may have a function that is specific for yeast PAPs. Interestingly, a recent study showed that a truncated version of *PAP1* ending at amino acid 525 could still rescue a *PAP1* deletion mutant whereas deletion of additional 12 amino acids, including LPDEVFD, was lethal (11). These authors also showed that a predominantly basic region overlapping the NLS is involved in RNA-binding. A similar basic region is also present in *pla1* and is aligned with the RNA-binding domain of *PAP1* in Figure 1 (amino acids 537–547 in *pla1*). The NLS1 of bovine PAP (amino acids 489–507) was also found to be essential for RNA-binding (10). This indicates that the involvement of a NLS in RNA-binding is a general feature of poly(A) polymerases.

It is surprising that the 525 amino acid truncation of *PAP1* is able to complement the null allele, although it is severely affected in RNA-binding (11). A possible explanation for this is the interaction of PAP with the specificity factors, which might still allow the mutant polymerase to establish the contact to its RNA substrate. This would require that also the truncated PAP forms a stable complex with the specificity factors. Further deletion of 12 amino acids (position 514–525 in *PAP1*) may destroy the residual activity of the enzyme by affecting the complex stability. It is tempting to speculate that the conserved LPDEVFD sequence motif could be involved in the formation of the polyadenylation multiprotein complex.

The region comprising the first 18 amino acids of *PAP1* has been implicated in the interaction with polyadenylation specificity factors (11). Because PLA1 must be able to interact with the *S.cerevisiae* polyadenylation specificity factors, a strong conservation of the extreme N-termini would be expected. Thus, it is surprising that the extreme N-terminus is not completely conserved among the two yeast polymerases. In fact *pla1* and bovine PAP show about the same degree of identity to *PAP1* within this stretch. On the other hand, the similarity of this region is clearly higher than random. This may serve as an argument for a functional conservation of this part. However, all N-terminal truncations of bovine PAP tested so far were found to be inactive even in unspecific polyadenylation (10). It is therefore still

unclear whether or not the involvement of the extreme N-terminus in specific protein–protein interactions is a general characteristic of poly(A) polymerases. This and other questions concerning the functions of specific domains of poly(A) polymerases can be answered by further mutational analysis and by the determination of the crystal structure of these enzymes.

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