Vol. 170, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1990 Pages 169-175

PRODUCTION OF SHORTER mRNA FOR PROTEIN PHOSPHATASE 2Aβ BY ALTERNATIVE POLY(A) ADDITION[+]

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Received May 21, 1990

Summary: Two types of cDNA clones of protein phosphatase 2Aβ, *PP-2AβL* and *PP-2AβS*, were isolated from a rat testis cDNA library. *PP-2AβL* was 1.8 kb in length and corresponded to the 2.0 kb mRNA that is the major species expressed in somatic tissues. *PP-2AβS* was 1.3 kb in length and corresponded to the 1.4 kb mRNA that is the major species expressed in germ cells of the testis. Nucleotide sequencing of these two clones revealed that their open reading frames were identical. The only difference between these two cDNAs was at the 3' non-coding region due to alternative poly(A) addition: the 1.8 kb cDNA had a consensus poly(A) addition signal, AATAAA, but the 1.3 kb cDNA had an unusual poly(A) addition signal, probably with the sequence AATATA. These data suggest that in germ cells there may be some mechanism that utilizes the specific poly(A) addition signal.

Phosphorylation and dephosphorylation of serine and threonine residues of various proteins are the principal mechanisms regulating almost all cellular processes. Protein phosphatases (PPs) that are inhibited by inhibitor-1 or -2 have been classified as PP-1 enzymes, whereas those that are insensitive to these inhibitors have been classified as PP-2 enzymes. PP-2 enzymes are further subdivided into PP-2A, PP-2B and PP-2C

[+]The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and Gene Bank Nucleotide Sequence Database under the accession number D90111.

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0006-291X/90 $1.50

enzymes according to their divalent cation requirements (1). Recent studies on fungi have revealed that PP-1 enzymes play important roles in mitosis and that chromatid disjunction is impaired in PP-1 deficient mutants (2-5). On the other hand, the catalytic subunit of PP-2A was shown to enhance replication of SV40 *in vitro*, possibly due to dephosphorylation of the SV40 large T antigen (6). These data suggest that specific protein phosphatases are involved in stage specific events in the cell cycle and DNA replication.

We have found that 2.0 kb mRNA for *PP-2Aβ*, one of the catalytic subunits of PP-2A, was detected as a major species in somatic tissues, whereas 1.4 kb mRNA was predominantly expressed in the testis. Further analysis by fractionating the testicular cells indicated that this shorter transcript is mainly expressed in meiotic prophase, suggesting that this shorter mRNA plays a special role in meiosis (7).

In this study, to clarify the mechanism regulating production of the shorter mRNA we isolated cDNA clones of *PP-2Aβ* and obtained two types: one corresponded to the 2.0 kb mRNA expressed as the major species in somatic tissues (*PP-2AβL*) and the other corresponded to 1.4 kb mRNA expressed predominantly in germ cells (*PP-2AβS*).

MATERIALS AND METHODS

Isolation of cDNA clones A rat testis cDNA library, constructed in λgt10 (8, 9), was screened with a 0.94 kb *Pvu*II fragment, a part of the coding region of a rat *PP-2Aβ* cDNA clone (10). Plaque hybridization was carried out at 42 °C in a solution of 50 % formamide, 0.65 M NaCl, 0.1 M sodium PIPES (pH 6.8), 5x Denhardt's solution, 0.1 % SDS, 5 mM EDTA, 10 % dextran sulfate, salmon sperm DNA (100 μg/ml) and a probe labeled by the random priming method (Amersham) using [α-^{32}P]dCTP, followed by four washes with 2x SSC and 0.1 % SDS at 50 °C for 20 min each time. Positive clones were purified and cloned into the *Eco*RI site of Bluescript pKS-M13^{+} (Stratagene) (11).

Sequence analysis of cDNA clones A series of overlapping deletion mutants were obtained by treatment with Exonuclease III and Mung Bean Nuclease (11). Both DNA chains were sequenced by the dideoxy chain-termination method using a 7-deaza-Sequenase II kit (USB) (12).

Genomic DNA analysis using the polymerase chain reaction (PCR) Samples of 500 ng of rat genomic DNA and 5 ng of *PP-2AβL* cDNA clone were amplified by the PCR as described (13) with the primers indicated in Fig. 2. Forty cycles of the PCR were performed. Samples of one tenth of the amplified DNAs were subjected to electrophoresis on 2 % 'Nu-sieve' agarose (FMC).

Differential hybridization using oligomers as probes Total RNAs from tissues were extracted by the single-step total RNA isolation method (14). Samples of 10 μg of RNAs were fractionated in formaldehyde/agarose gel and transferred to a nitrocellulose membrane as described (15). Forty-mer oligonucleotides corresponding to the 3' non-coding region of the *PP-2Aβ* cDNAs shown in Fig. 2 were end-labeled with [γ-^{32}P]ATP by T_4 polynucleotide kinase and used as probes. Hybridization was performed under the same conditions as plaque hybridization but without dextran sulfate.

RESULTS

Isolation of two kinds of cDNA clones for *PP-2Aβ* mRNA

Six positive cDNA clones were obtained from $2x10^5$ cDNA clones derived from a rat testis cDNA library. The restriction sites of *Pvu*II, *Acc*I and *Bgl*II were mapped. Four of the six cDNA clones were suggested to correspond to the 1.4 kb mRNA for *PP-2Aβ* which was expressed predominantly in germ cells and were named *PP-2AβS* (Fig. 1). The other two clones seemed to correspond to the 2.0 kb mRNA for *PP-2Aβ* which was major mRNA in somatic tissues and were named *PP-2AβL* (Fig. 1). The restriction map of *PP-2AβS* was the same as that of a *PP-2Aβ* cDNA clone previously isolated from a rat liver cDNA library (10). Restriction mapping of *PP-2AβS* and *PP-2AβL* suggested that they differed only in the length of the 3' untranslated region (Fig. 1).

PP-2AβS produced by alternative poly(A) addition in testis

The nucleotide sequence was determined by the strategy described in Materials and Methods. The sequence of *PP-2AβS* was the same as that of the *PP-2Aβ* cDNA that we previously cloned from a rat liver cDNA library (10). The nucleotide sequences of *PP-2AβS* and *PP-2AβL* upstream of nucleotide 1008 were identical (Fig. 2). A poly(A) tail followed just downstream of nucleotide 1008 in *PP-*

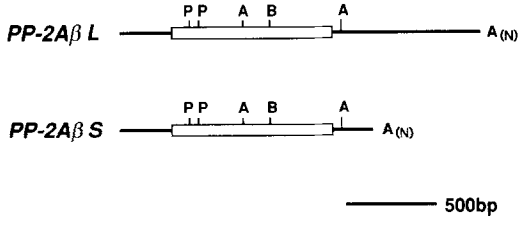

Figure 1. **Restriction maps of *PP-2Aβ* cDNA clones from rat testis.** The sizes of *PP-2AβL* and *PP-2AβS* were 1.8 kb and 1.3 kb, respectively. Open bars indicate the open reading frames and closed bars represent non-coding regions of the cDNAs. Restriction enzymes: P, *Pvu*II; A, *Acc*I; B, *Bgl*II.

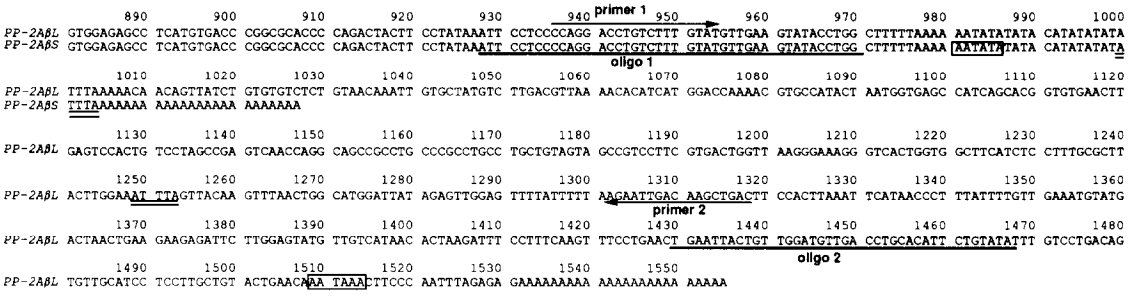

Figure 2. **Nucleotide sequence of 3' non-coding regions of *PP-2AβL* and *PP-2AβS* cDNAs.** Open boxes denote possible poly(A) addition signals. The regions complementary to the synthetic oligomers used as probes are underlined. The destabilization signals are doubly underlined. Arrows denote PCR primers.

2AβS, but more than 500 bp downstream of nucleotide 1008 in *PP-2AβL*. The *PP-2AβL* has a consensus polyadenylation signal, AATAAA, 23 nucleotides upstream of the poly(A) addition site (Fig. 2). In *PP-2AβS*, AATATA located 23 nucleotides upstream of the poly(A) tail (Fig. 2) seemed to be the polyadenylation signal, as reported previously (10).

To confirm that *PP-2AβL* was not produced by recombination with an unrelated sequence during the process of cDNA cloning, we

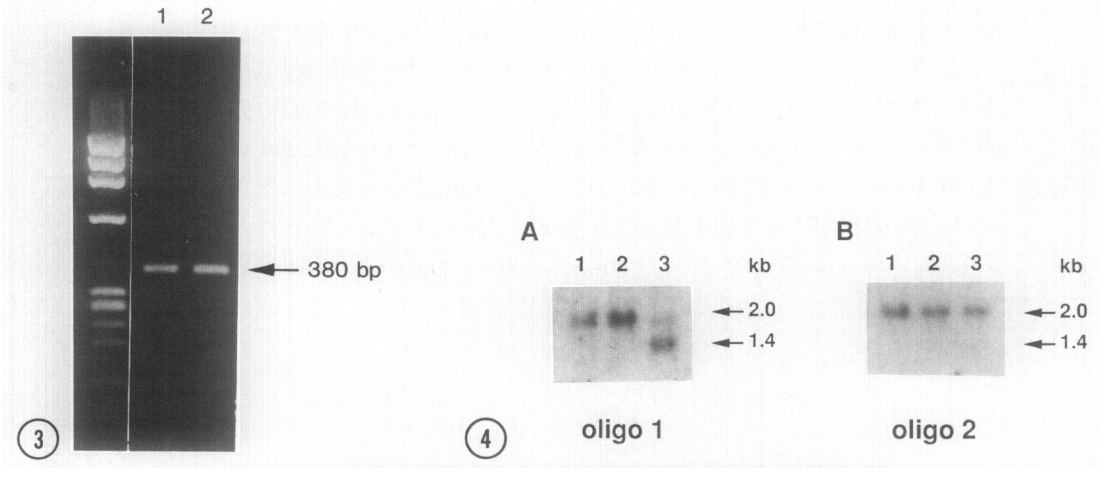

Figure 3. **Genomic analysis using PCR.** PCRs were performed as described in Materials and Methods. Amplified DNAs were separated by gel electrophoresis, stained with ethidium bromide and photographed in UV light using a red filter. lane 1, genomic DNA; lane 2, *PP-2AβL*.

Figure 4. **Differential hybridization using synthetic oligomers as probes.** Samples of 10μg of total RNA from normal rat heart, brain and testis were blotted and hybridized with end-labeled synthetic oligo 1 (A) and oligo 2 (B). lane 1, heart; lane 2, brain; lane 3, testis.

analyzed the genomic DNA using the PCR. The primers indicated in Fig. 2 were used for amplification of the genomic sequence of interest by PCR. As shown in Fig. 3, genomic DNA and *PP-2AβL* both produced DNA fragments of 380 bp. We obtained further confirmation by differential hybridization with oligo 1 and oligo 2 as probes (Fig. 2). Oligo 1 detected both 2.0 kb and 1.4 kb mRNA for *PP-2Aβ*, but oligo 2 detected only the 2.0 kb band (Fig. 4). These data proved that the cDNAs that we cloned represent two species of mRNA, and that *PP-2AβS* and *PP-2AβL* corresponded to 1.4 kb and 2.0 kb mRNA, respectively.

These results show that shorter mRNA for *PP-2Aβ*, which is present predominantly in the testis, is produced by alternative poly(A) addition.

DISCUSSION

In this study, we cloned and analyzed two types of cDNAs, *PP-2AβS* and *PP-2AβL*, from rat testis. Interestingly, *PP-2AβS* and *PP-2AβL* were produced by alternative poly(A) addition. In *PP-2AβL*, a consensus poly(A) addition signal, AATAAA, was found 23 bp upstream of the poly(A) tail, whereas in *PP-2AβS*, an unusual poly(A) addition signal, AATATA (16), was found 23 bp upstream of the poly(A) tail. Differential hybridization using oligoprobes clearly demonstrated that *PP-2AβS* is the cDNA corresponding to the 1.4 kb mRNA for *PP-2Aβ*, which is predominantly expressed in the testis, whereas *PP-2AβL* corresponded to the 2.0 kb mRNA expressed as the major species in somatic tissues (7). A small amount of the shorter mRNA is also expressed in somatic tissues, and, in fact, we previously obtained a cDNA clone of this from a rat liver cDNA library (10). *PP-2AβL* has two destabilization signals, ATTTA (17), in the 3' non-coding region, whereas *PP-2AβS* has only one (Fig. 2). Thus this shorter mRNA may have a longer half life.

Shorter species of mRNAs for the regulatory subunits of cAMP dependent protein kinase, RIα, RIIα and RIIβ, have been reported to be expressed only in the testis (18, 19). Recently, a smaller 1.5 kb transcript and longer 3.0 kb transcript of human RIα were shown to be generated from the same gene by the use of different poly(A) addition signals and the smaller one was found to have fewer destabilization signals (19). This report together with the present results suggest that there is a testis specific mechanism that recognizes another poly(A) additional signal efficiently, and that this may be important for mRNA stability in germ cells.

Interestingly, smaller sized mRNAs for other genes such as *pim-1* and *c-raf* have also been shown to be expressed only in the testis (20, 21). We previously reported that 1.8 kb shorter mRNA of *dis2*m1, encoding a catalytic subunit of PP-1, was a major species in testicular germinal cells, whereas 2.6 kb mRNA was the major species in somatic tissues (7).

For polyadenylation of mRNA precursors, multiple factors, such as poly(A) polymerase, cleavage factor and snRNP are required (22-25). Some of them may be involved in testis specific poly(A) addition.

Acknowledgments: This work was supported by a Grant-in-Aid from the Ministry of Health and Welfare for a Comprehensive 10-Year Strategy of Cancer Control, Japan. K. Sasaki is the recipient of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research. We also thank Dr. K. Ikeda for his support.

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