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Identification of a novel protein phosphatase catalytic subunit by cDNA cloning

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A cDNA encoding a novel protein phosphatase catalytic subunit (protein phosphatase X) has been isolated from a rabbit liver library. It codes for a protein having 45% and 65% amino acid sequence identity, respectively, to the catalytic subunits of protein phosphatase 1 and protein phosphatase 2A from skeletal muscle. The enzyme is neither the hepatic form of protein phosphatase 1 or 2A, nor is it protein phosphatase 2B or 2C. The possible identity of protein phosphatase X is discussed.

Protein phosphatase; cDNA cloning; Nucleotide sequence; Amino acid sequence; Sequence homology

1. INTRODUCTION

A wide variety of cellular functions are controlled by changes in the phosphorylation states of intracellular proteins. At any instant, the level of phosphorylation of a protein represents a steady state determined by the relative activities of protein kinases and protein phosphatases. Four types of serine/threonine-specific protein phosphatase have been identified in mammalian tissues by protein chemical and enzymatic criteria and termed protein phosphatases 1, 2A, 2B and 2C [1]. The amino acid sequences of two forms of protein phosphatase 1 [2,3] and two forms of protein phopshatase 2A [4–9] have recently been elucidated by cDNA cloning. The two species of protein phosphatase 1 (1_α and 1_β) only differ at their extreme N-termini and appear to be generated by alternative splicing or differential transcription of the mRNA from a single gene. The two forms of protein phosphatase 2A ($2A_\alpha$ and $2A_\beta$) are 97% identical, and of the eight amino acid differences, seven are located within 30 residues of the N-terminus. However, there are approximately 130 nucleotide differences spread throughout the coding region and the 3′-non-translated regions show no homology. These results together with Southern blotting of the genomic DNA demonstrate that protein phosphatases $2A_\alpha$ and $2A_\beta$ are distinct gene products [5,7,8,10].

We have recently screened liver and brain libraries with cDNA coding for protein phosphatase 1 in order to examine whether the hepatic and neuronal forms of this enzyme are identical or distinct from those in skeletal muscle. During these studies we isolated a cDNA clone coding for a hepatic protein phosphatase catalytic subunit homologous, yet distinct, from protein phosphatases 1 and 2A. The sequence of this clone is presented in this paper and its possible role is discussed.

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00980

2. MATERIALS AND METHODS

2.1. *Construction of a cDNA library from rabbit liver*

Total RNA was isolated from the livers of 14-day-old rabbits by phenol extraction as described previously [11]. Poly(A)$^+$

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RNA was selected by oligo(dT) chromatography [12] and used as a template with a cDNA synthesis system (Amersham International, Bucks, England). After addition of *Eco*RI linkers, and size fractionation on Sepharose CL-4B to remove fragments smaller than 500 base pairs, the cDNA was ligated into the *Eco*RI site of phage λgt10, packaged in vitro and used to infect *E. coli* C600Hfl [13]. The resultant library contained 3.3 $\times 10^5$ independent clones and was used without amplification.

2.2. *Screening of the cDNA library*

Screening of the liver cDNA library was performed on duplicate nitrocellulose filters. Prehybridisation was carried out in 0.75 M NaCl/5 mM EDTA/0.05 M sodium phosphate, pH 7.4/0.1% polyvinylpyrrolidone/0.1% Ficoll/0.1% bovine serum albumin/0.5% SDS and 100 μg/ml denatured herring sperm DNA at 60°C for 2 h. Hybridisation was performed under the same conditions for 18 h with the addition of 10^6 cpm of the appropriate cDNA probe, labelled by random hexanucleotide priming [14]. Filters were washed in 0.3 M NaCl/0.03 M sodium citrate/0.1% SDS, pH 7.0, at 60°C. Isolation of positive clones and purification of their DNA were carried out as in [11].

2.3. *Subcloning and sequencing*

cDNA inserts of clones that were positive with the protein phosphatase 1 or 2A probes were subcloned into the *Eco*RI site of Bluescript pKS-M13$^+$ (Stratagene Cloning Systems, San Diego, USA). DNA sequencing was performed directly on the double-stranded plasmid DNA using the dideoxy chain termination procedure [15], [α-^{35}S]dATPαS and Bluescript or oligonucleotide primers. In addition, restriction fragments were removed and the religated truncated recombinants were sequenced using Bluescript primers. To overcome ambiguities often encountered with GC-rich DNA, most reactions were also performed in the presence of 7-deaza-2′-dGTP [16].

3. RESULTS

3.1. *Screening of the liver cDNA library*

Screening of 6×10^4 recombinants of the rabbit liver cDNA library yielded one clone that was positive with the 2.5 kb full-length cDNA probe for skeletal muscle protein phosphatase $2A_\alpha$ [4] and two that were positive with the 1.5 kb full-length cDNA probe for skeletal muscle protein phosphatase 1_β [2]. Restriction digests of the recombinant phage DNA indicated that the insert size of the former was 1.4 kb, while those of the latter two were both 1.0 kb.

3.2. *Sequence analysis of cDNA coding for protein phosphatase 2Aβ*

Sequence analysis of the insert, which hybridised to the phosphatase 2A probe, showed that it encoded protein phosphatase $2A_\beta$. All 718 coding nucleotides (encoding amino acids 73–309) were identical with those reported in [5] for skeletal muscle phosphatase $2A_\beta$ with the exception of the third base specifying amino acid 267, which was T in liver and C in muscle. The 3′-non-coding region

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CTCCTGGGAAACCTGCCTTTCTTTGTGGAAGTATACCTGGCTTTTTAAAATATATATATATATATATTTA   70

AAAGCAAACAAAGCAACAGTAATCTATGTGTTTCTGTAACAAATTGGGATCTGTCTTGGCATTAAACCAC  140

ATCATGGACCAAAATGTGCCATACTAATGATGAGCATTTAGCACAATTTGAGACTGAAATTTAGTACACT  210

ATGTTCTAGATTGGTCAGTCTAACAGTTTGCCTGCTGTATTTGTAGTAACCATTTTCCTCTGGACTGTTC  280

AAGCAAAAAAAAAAGGTAACTAATTTGTTCCATCTCCTTTTGCGCTTATTTGGAAATTTAGTTATAGTGT  350

TTAACTGGCATGGATTAATAGAGTTGGAGTTTTATTTTTTAAAATATTCACAAGCTAACATCCAATTAAC  420

CCATTACCCTTTATTTTATTGAAATGTGTAATTAACTTAACTGAAGAAAGTCTTCTTGGGAGTATGTTGT  490

CATAACATTTAAAGAAATTTTCCTTTCATTTAAGCTAAATTACTGTTTTATGTTGATCTGCATATTTCTG  560

TATATTTGTCACAACAATGCTTGCATCCTATTTGGTGTACTGAGCAAATAAACTTTCCATTTTAAACAAA  630

AAAAAAA                                                                  637
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Fig.1. Nucleotide sequence of the 3′-non-coding region of the cDNA for liver protein phosphatase 2Aβ.

of the liver clone is shown in fig.1. The skeletal muscle counterpart (not shown) differed in the deletion of TA between based 55 and 61 and the addition of TT after base 470. These minor differences between the muscle and liver clones may be due to allelic variation.

3.3. *Sequence analysis of cDNA coding for a novel protein phosphatase*

The two clones which hybridised to the phosphatase 1 probe were identical and the strategy adopted for their sequencing is illustrated in fig.2. The nucleotide and deduced amino acid sequences are shown in fig.3. The clones encode the central and C-terminal sections of a novel protein phosphatase, termed protein phosphatase X, which is only 45% identical to protein phosphatase 1_α or 1_β (60% homologous if conservative substitutions are included) and 65% identical to protein phosphatase $2A_\alpha$ or $2A_\beta$ (78% homologous) (fig.4). At the nucleotide level the identity of protein phosphatase X with phosphatases 1 and 2A is 57 and 60%, respectively, in the coding region. The 3'-non-coding region of protein phosphatase X was not homologous to those of protein phosphatases 1 or 2A.

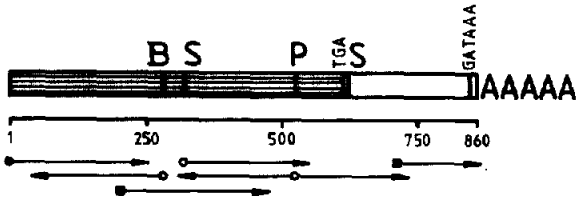

Fig.2. Partial restriction map and strategy used to sequence the cDNA coding for protein phosphatase X. The letters B, S and P mark the sites for the cleavage by restriction enzymes *Bam*HI, *Sac*II and *Pst*I, respectively. The scale indicates the nucleotide position from the 5'-end of the cDNA. The arrows show the direction and length of the DNA sequences obtained. Sequences were initiated with Bluescript primers for the intact insert (●) and restriction fragments (○) or with specific oligonucleotide primers (■).

4. DISCUSSION

In this paper we have sequenced a cDNA clone that codes for a protein phosphatase (protein phosphatase X) that is homologous to the catalytic subunits of protein phosphatase 1 and protein phosphatase 2A. The finding that protein phosphatase X is only 45% identical to protein phosphatase 1 suggess that it is most unlikely to represent the hepatic form of this enzyme. The re-

```
CCTGACCGCATCACTCTGATCCGGGGCAAC CACGAGAGCCGGCAGATCACCCAGGTCTAC GGCTTCTACGATGAGTGCCTGCGCAAGTAC  90
 P  D  R  I  T  L  I  R  G  N   H  E  S  R  Q  I  T  Q  V  Y   G  F  Y  D  E  C  L  R  K  Y  30

GGCTCGGTGACCGTGTGGCGCTACTGCACC GAGATCTTCGACTACCTCAGCCTGTCGGCC ATCATCGACGGCAAGATCTTCTGCGTGCAC 180
 G  S  V  T  V  W  R  Y  C  T   E  I  F  D  Y  L  S  L  S  A   I  I  D  G  K  I  F  C  V  H  60

GGGGGCCTCTCCCCCTCCATCCAGACGCTG GACCAGATCAGGACGATTGACCGGAAGCAA GAGGTGCCGCACGACGGCCCCATGTGCGAC 270
 G  G  L  S  P  S  I  Q  T  L   D  Q  I  R  T  I  D  R  K  Q   E  V  P  H  D  G  P  M  C  D  90

CTCCTCTGGTCGGATCCCGAAGACACAACA GGCTGGGGCGTGAGTCCCCGCGGGGCCGGC TACCTTTTTGGCAGTGATGTGGTGGCTCAG 360
 L  L  W  S  D  P  E  D  T  T   G  W  G  V  S  P  R  G  A  G   Y  L  F  G  S  D  V  V  A  Q  120

TTCAACGCGGCCAACGACATTGACATGATC TGCCGCGCCCACCAACTGGTGATGGAGGGT TACAAGTGGCATTTCAACGAGACGGTGCTC 450
 F  N  A  A  N  D  I  D  M  I   C  R  A  H  Q  L  V  M  E  G   Y  K  W  H  F  N  E  T  V  L  150

ACCGTGTGGTCGGCGCCCAACTACTGCTAC CGCTGTGGGAATGTGGCAGCCATCCTGGAG CTGGACGAGCATCTGCAGAAGGACTTCATC 540
 T  V  W  S  A  P  N  Y  C  Y   R  C  G  N  V  A  A  I  L  E   L  D  E  H  L  Q  K  D  F  I  180

ATCTTCGAGGCCGCTCCCCAGGAGACCCGG GGCATCCCCTCCAAGAAGCCCGTGGCCGAC TACTTCCTGTGACCCCGGCCCGCGGACCAC 630
 I  F  E  A  A  P  Q  E  T  R   G  I  P  S  K  K  P  V  A  D   Y  F  L  *                       203

TGTGACTCTGCCCTCTTCCCCAGGTGGAGGCTGGGCAAGGGGGGGCGCCCAGCTCTGCTCCCCCCACCCCGAGGGCGCTCGGAGGGCGAGGA 722

CTTCGTGGAGAGGTTGGAGCCTAGCGCCACGCTCCCTCTCTTCTCCCCACTTGAACCATGAAGTTCCCAGTAATTTTTTTGTTTTCTTTTTT 814

TCCTTTTTTTTTTTTTTGGTTGGTTTTTAGATAAACGTTTTGAGGT (A)~98                                          ~958
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Fig.3. The cDNA and translated protein sequence of protein phosphatase X.

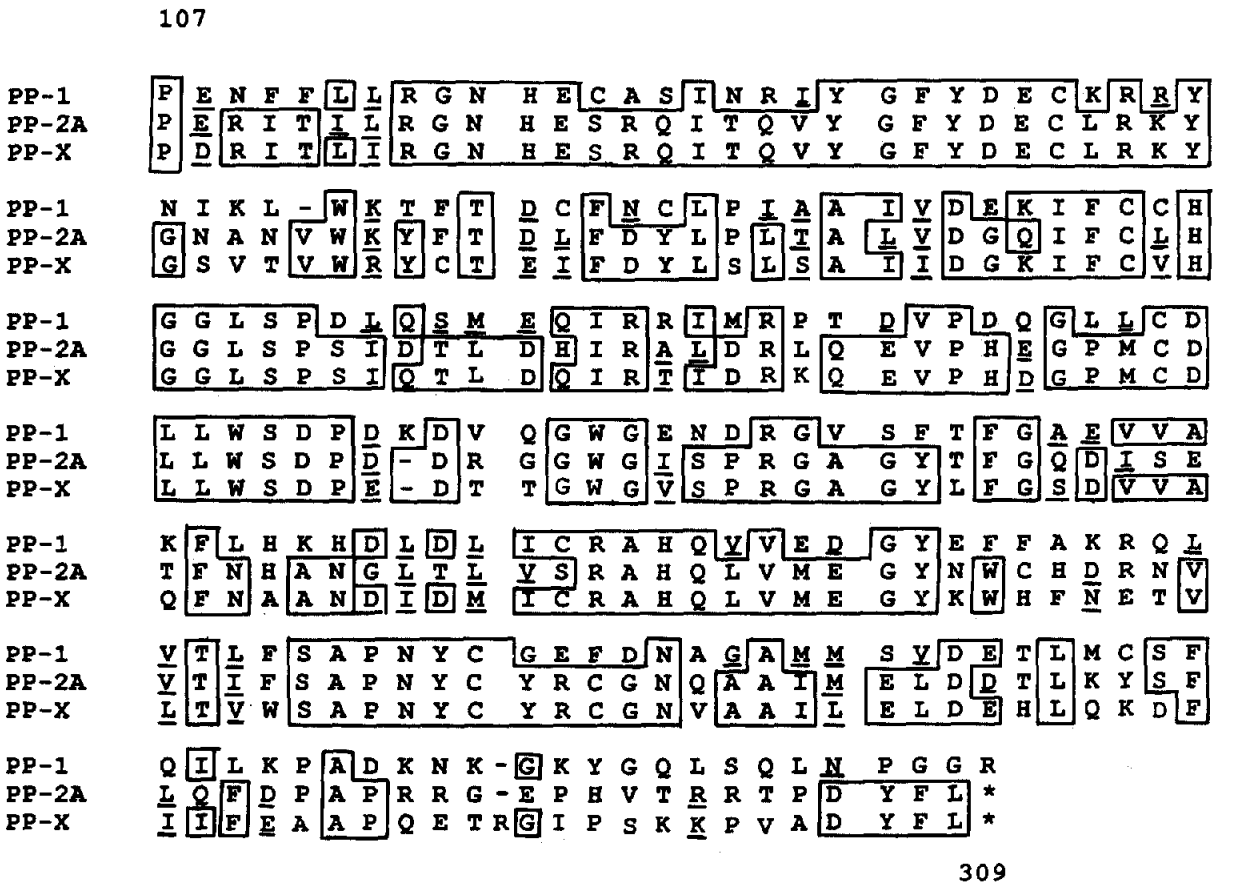

Fig.4. Comparison of the amino acid sequence of protein phosphatase X to those of protein phosphatase 1 and protein phosphatase 2A. Identical residues are boxed and conservative replacements are underlined. Protein phosphatase 1_α and 1_β are identical in this region. The numbering system is that of protein phosphatase $2A_\beta$. Protein phosphatase $2A_\alpha$ differs from $2A_\beta$ in this section by replacement of R for P at position 108.

cent isolation of cDNA coding for the calmodulin-regulated protein phosphatase (phosphatase 2B) has shown that protein phosphatase X is not this enzyme ([17] and Klee, C.B., personal communication). Similarly, phosphatase X is not protein phosphatase 2C because peptide sequences of the latter totalling 250 residues (≈65% of the sequence) have failed to reveal any homology with protein phosphatases 1 and 2A [18].

Since the clone coding for protein phosphatase X was isolated from a liver cDNA library, it could be argued that it is the hepatic form of protein phosphatase 2A, but this is not the case, since the two isoforms of phosphatase 2A ($2A_\alpha$ and $2A_\beta$) present in rabbit skeletal muscle and pig kidney epithelial cells are also present in liver. A clone coding for phosphatase $2A_\beta$ was isolated from the rabbit liver library in the present work, while a phosphatase $2A_\alpha$ clone has been isolated from a rat liver library [9]. cDNA clones for phosphatases $2A_\alpha$ and $2A_\beta$ were also both found in a human liver library [8].

Protein phosphatase X is, nevertheless, most closely related to protein phosphatase 2A. The sequence terminates at exactly the same position as phosphatase $2A_\alpha$ and $2A_\beta$, and has a single amino acid insertion and a single deletion at the two positions where protein phosphatases $2A_\alpha$ and $2A_\beta$ have deletions and insertions with respect to protein phosphatase 1 (fig.4). However, it should also be noted that protein phosphatase X has an amino acid addition with respect to both protein

phosphatases 1 and 2A near the C-terminus. Several high molecular mass forms of protein phosphatase 2A have been isolated from mammalian tissues in which the catalytic subunits are complexed to other proteins. In the case of three of these forms, termed $2A_0$, $2A_1$ and $2A_2$, peptide mapping and immunological studies have indicated that the catalytic subunits are very similar or identical to $2A_\alpha/2A_\beta$ [19] (which would not be distinguishable by these criteria). However, the catalytic subunits of other phosphatase 2A-like enzymes have not yet been subjected to structural analysis. Protein phosphatase X may therefore represent the catalytic subunit of one of these enzymes, such as 'polycation-stimulated protein phosphatase M' [20], or enzymes detected in nuclei [21] and mitochondria [22] that were classified as type-2A phosphatases. We are currently attempting to answer these questions by raising an antipeptide antibody specific for protein phosphatase X.

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