Volume 226, number 1, 176–178 FEB 05439 December 1987

A second catalytic subunit of type-2A protein phosphatase from rabbit skeletal muscle

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Received 13 November 1987

A cDNA clone encoding a second type-2A protein phosphatase catalytic subunit ($2A_\beta$) was isolated from a rabbit skeletal muscle cDNA library constructed in λgt10. The deduced protein sequence (309 residues, 35.59 kDa) was 97% identical to that of phosphatase $2A_\alpha$(309 residues, 35.58 kDa). At the nucleotide level, the two clones showed only 82% identity in the coding region. The results indicate the presence of at least two isoforms of protein phosphatase 2A in skeletal muscle.

Protein phosphatase; cDNA cloning; Nucleotide sequence; Amino acid sequence; Isozyme

1. INTRODUCTION

Four serine/threonine-specific protein phosphatases have been identified in mammalian tissues, and classified into two groups (type-1 and type-2) on the basis of certain properties [1–3]. Recently, we isolated and sequenced clones containing the entire coding region of a type-1 [4] and a type-2A [5] protein phosphatase from a rabbit skeletal muscle cDNA library constructed in phage λgt10 [6]. Here, we have isolated and sequenced a further clone from the same library that encodes a second type-2A protein phosphatase (termed $2A_\beta$), and compare it with the type-2A protein phosphatase ($2A_\alpha$) that was sequenced previously [5].

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00763

2. METHODS AND RESULTS

2.1. *Isolation of the cDNA clone*

We reported in [5] the identification of two clones that were positive with a 36 base pair oligonucleotide (3′-CTT GA^G_C CTG GTT ACC TAG CTT GTT GA^G_C TTG CTT ACG-5′) complementary to tryptic peptide T1 of protein phosphatase 2A (ELDQWIEQLNEC). One of these was isolated and sequenced to give the com-

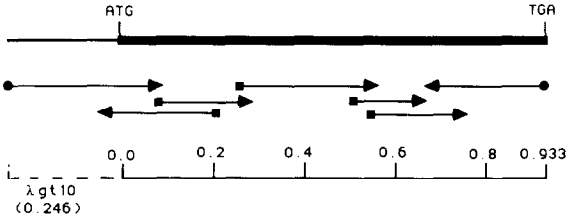

Fig.1. Strategy used to sequence the cDNA clone coding for protein phosphatase $2A_\beta$. The scale indicates the nucleotide position in kilobases from the 5′-end of the cDNA insert. The arrows indicate the direction and length of the DNA sequences obtained. Sequences were initiated with Bluescript primers (●) or specific oligonucleotide primers (■).

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

plete primary structure of a type-2A protein phosphatase [5], now termed $2A_\alpha$. The other clone had an internal *Eco*RI site yielding a 600 base pair insert, when digested with *Eco*RI. The rest of the insert remained attached to the λgt10 arms because the clone had also lost a terminal *Eco*RI site [5]. The isolation and sequence of this second clone is described below.

2.2. *Subcloning and cDNA sequencing*

The 600 base pair fragment was subcloned into the *Eco*RI site of Bluescript pKS-M13$^+$ (Stratagene Cloning Systems, San Diego, USA). The rest of the cDNA insert was recovered from the λgt10 arms as a *Hin*dIII and *Eco*RI fragment of about 1.2 kilobases, of which 246 base pairs 3′ to the *Hin*dIII site were derived from phage λgt10. This

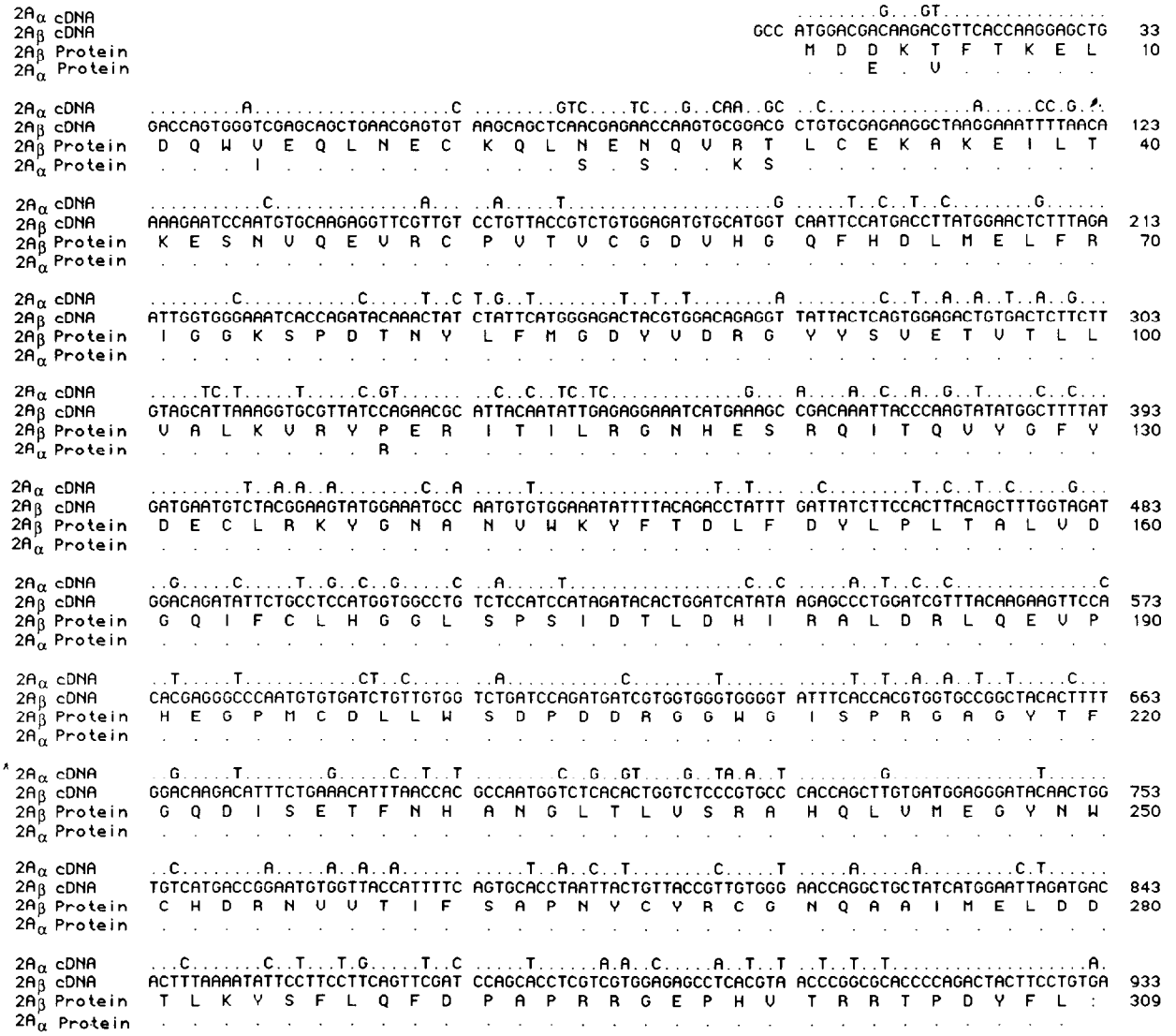

Fig.2. The cDNA and translated protein sequence of protein phosphatase $2A_\beta$. The structure of protein phosphatase $2A_\alpha$ is also shown where it differs from $2A_\beta$. The single letter code for amino acids has been used, and identities are indicated by dots. Sequencing was performed by the dideoxy chain termination procedure [7], [α-^{35}S]dATPαS and buffer gradient gels [8].

fragment was also subcloned into Bluescript pKS-M13$^+$, that had been digested with *Eco*RI and *Hin*dIII.

The sequencing strategy of the 1179 base pair *Hin*dIII/*Eco*RI fragment is shown in fig.1, and the nucleotide and deduced amino acid sequence in fig.2. The cDNA clone has three nucleotides preceding the putative initiating ATG codon and an open reading frame of 927 base pairs terminated by a TGA stop codon. The molecular mass of the protein calculated from the sequence is 35593 Da. The primary structure deduced from the cDNA is extremely similar to that of protein phosphatase $2A_\alpha$ (fig.2) and this enzyme has therefore been termed protein phosphatase $2A_\beta$.

The 600 base pair fragment which comprised the 3′-non-coding region containing the AATAAA signal for cleavage of the messenger RNA and addition of the poly(A)$^+$ tail was not sequenced in its entirety (not shown).

3. DISCUSSION

The nucleotide and deduced amino acid sequence of protein phosphatase $2A_\beta$ is compared with that of $2A_\alpha$ in fig.2. The proteins are of identical length (309 residues) and there are only eight amino acid differences (97% identity). Seven of the differences lie in the first 30 residues from the N-terminus (positions 3, 5, 14, 24, 26, 29 and 30) and the eighth is at residue 108. Of these, four are very conservative changes, Asp for Glu, Val for Ile, Arg for Lys and Thr for Ser. These findings establish that both enzymes are type-2A protein phosphatase catalytic subunits.

At the nucleotide level there are 130 differences in the coding region between phosphatase $2A_\alpha$ and $2A_\beta$ (82% identity), which are spread throughout the cDNA sequence (fig.2). Furthermore, the 3′-non-coding regions are completely different. Thus protein phosphatases $2A_\alpha$ and $2A_\beta$ must be the products of distinct genes.

ACKNOWLEDGEMENTS

This work was supported by Grants from the Wellcome Trust, University of Dundee, and Medical Research Council, London (to P.T.W.C.) and The Royal Society (to Professor Philip Cohen). O.B.C.S. was the recipient of a Postgraduate Studentship from the Medical Research Council. We thank Mr Alastair I.H. Murchie for the synthesis of oligonucleotide primers.

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