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Molecular cloning and chromosomal localization of a novel *Drosophila* protein phosphatase

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A 1.0 kilobase cDNA coding for the complete amino acid sequence of a putative protein phosphatase (314 amino acid residues, molecular mass 36 kDa) has been isolated from a *Drosophila* head cDNA library. The cDNA hybridises to a single site on the right arm of the second chromosome at cytological position 55A1-3. The deduced sequence of the protein, designated protein phosphatase-Y, is homologous to the catalytic subunits of *Drosophila* and rabbit protein phosphatase-1α (64 and 59% identity, respectively) and rabbit protein phosphatase-2A (39% identity). These and other comparisons demonstrate that this novel enzyme is not the *Drosophila* counterpart of mammalian protein phosphatases 1, 2A, 2B, 2C or X.

Protein phosphatase; cDNA cloning; Nucleotide sequence; Amino acid sequence; (*Drosophila melanogaster*)

1. INTRODUCTION

Reversible protein phosphorylation is a post-synthetic modification that is used widely in eukaryotic cells to transduce extracellular stimuli [1]. The degree of phosphorylation of a specific protein depends on protein phosphatases as well as protein kinases. Four serine/threonine-specific protein phosphatases have been characterised by enzymatic and physiochemical methods and termed PP-1, PP-2A, PP-2B and PP-2C [1]. Molecular cloning has been used successfully to reveal the primary structures of the catalytic subunits of these enzymes. Two forms of PP-1 were found in rabbit and *Drosophila* [2-6]. PP-2A has been sequenced from mammalian sources [7-13] and two isozymes have been detected in rabbit [7,8], porcine [11] and human tissues [13]. The partial sequence of murine PP-2B [14] and a novel rabbit enzyme termed PP-X [9] have also been determined. The latter is more similar to PP-2A than to any other phosphatase. Here, we report on the identification by recombinant DNA techniques of another novel phosphatase in *Drosophila*, which has greater similarity with PP-1 than PP-2A.

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Abbreviations: PP, protein phosphatase; kb, kilobase; SSC, 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0

The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y07510

2. EXPERIMENTAL

2.1. *Screening*

A *Drosophila* head cDNA library, constructed in λgt10, was screened with a 0.76 kb *SmaI/NaeI* rabbit PP-1 cDNA fragment [3] coding for amino acids 43-298, and with a 51-base synthetic oligonucleotide [2] coding for amino acids 152-168 of rabbit skeletal muscle PP-1α. The cDNA probe was labelled with [γ^{32}P]dCTP using the random oligonucleotide priming method [15] to a specific activity of $\sim 2 \times 10^9$ dpm/μg and further purified by spun column chromatography [16]. The labelled cDNA was hybridised at a concentration of 0.5 ng/ml to recombinant DNA on nitrocellulose filters as described [9]. The filters were washed with 0.2 × SSC containing 0.1 % SDS at 65°C. The oligonucleotide probe was labelled with [γ^{32}P]ATP by T_4 polynucleotide kinase [16] to a specific activity of $\sim 4 < 10^6$ dpm/pmol. Hybridization was carried out with 0.8 pmol/ml

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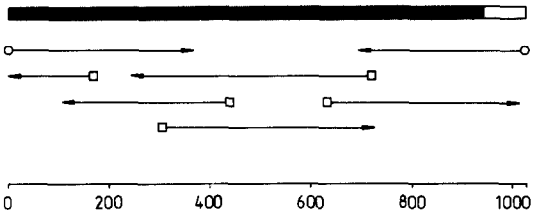

Fig.1. Strategy used to sequence the cDNA coding for *Drosophila* protein phosphatase-Y. The scale indicates the nucleotide position in base pairs starting from the 5'-end. The arrows show the direction and length of the sequences obtained with Bluescript primers (○) and specific oligonucleotide primers (□).

labelled oligonucleotide according to [17]. The filters were washed in 6 × SSC at 50°C.

2.2. *Subcloning and sequencing*

The inserts of two clones selected by hybridisation to the rabbit cDNA, but which did not hybridise to the oligonucleotide probe, were subcloned into Bluescript pKS$^+$ vector (Stratagene Cloning Systems, San Diego, CA). DNA sequencing was performed on the denatured double-stranded plasmid preparations by the dideoxy chain-termination method [18] using synthetic oligonucleotide primers (fig.1), modified T_7 DNA polymerase (US Biochemical Corp., Cleveland, OH), [α^{35}S]dATP and buffer gradient gel electrophoretic separation [19]. 75% of the sequence was also determined with 7-deaza-2'-dGTP in order to resolve compressions in the G-C rich regions [20].

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                                                    ATAAT      5

ATGGCCGTCTTAACTACTCATGAAATAGAT TGCATTATAAAGGAGCTCACATCTCTGAAT    65
 M  A  V  L  T  T  H  E  I  D   C  I  I  K  E  L  T  S  L  N    20

GGAAGTGAGTGCACATTAAAGGAGGAACTA ATCGAGAGACTCATTCAACAGACTCGTGAA   125
 G  S  E  C  T  L  K  E  E  L   I  E  R  L  I  Q  Q  T  R  E    40

GTGATCAAATGGCAACCGATGCTGCTGGAA CTTCAGGCTCCGGTCAATATATGCGGCGAT   185
 V  I  K  W  Q  P  M  L  L  E   L  Q  A  P  V  N  I  C  G  D    60

ATTCATGGCCAGTTTACAGATCTCTTGAGG ATTTTCAAGGCATGCGGCTTTCCACCCAAA   245
 I  H  G  Q  F  T  D  L  L  R   I  F  K  A  C  G  F  P  P  K    80

GCCAACTATTTATTTCTCGGTGACTATGTG GACCGAGGCAAGCAATCGCTGGAAACGATT   305
 A  N  Y  L  F  L  G  D  Y  V   D  R  G  K  Q  S  L  E  T  I   100

TGTTTGCTATTTGCTTACAAAGTTAAATAT CCGCTAAATTTCTTTTTGCTTCGCGGCAAT   365
 C  L  L  F  A  Y  K  V  K  Y   P  L  N  F  F  L  L  R  G  N   120

CACGAGTGCGCCAGTATAAATAAAATTTAC GGATTTTACGACGAGATCAAACGTAGACAC   425
 H  E  C  A  S  I  N  K  I  Y   G  F  Y  D  E  I  K  R  R  H   140

ACTGTCCGTTTGTGGCACAATTTCACGGAT TGCTTCAACTGGCTTCCGGTGGCCGCGTTG   485
 T  V  R  L  W  H  N  F  T  D   C  F  N  W  L  P  V  A  A  L   160

GTGGGCGAGCGCATCTTCTGCTGCCACGGA GGACTGAGTCCATCGCTCCGGAATTTGCAA   545
 V  G  E  R  I  F  C  C  H  G   G  L  S  P  S  L  R  N  L  Q   180

CAGATCAATCATATTCAGCGACCCACTGAT ATTCCGGATGAGGGTATTATGTGCGATCTC   605
 Q  I  N  H  I  Q  R  P  T  D   I  P  D  E  G  I  M  C  D  L   200

CTTTGGGCGGATCTAAATCACACCACCAAA GGCTGGGGTCACAACGATCGCGGTGTGAGC   665
 L  W  A  D  L  N  H  T  T  K   G  W  G  H  N  D  R  G  V  S   220

TTCACCTTCGATAAGGTCATAGTTCGGGAT TTTCTTAAAGCCTTCGACTTGCAACTTATG   725
 F  T  F  D  K  V  I  V  R  D   F  L  K  A  F  D  L  Q  L  M   240

GTTCGCGCCCATGAGGTTGTGGAGGATGGA TACGAGTTCTTTGCCAACCGACAGCTGGTC   785
 V  R  A  H  E  V  V  E  D  G   Y  E  F  F  A  N  R  Q  L  V   260

ACCGTATTCTCGGCCCCCAACTATTGCGGT ATGATGAACAATGCCGGCGGAGTGATGAGT   845
 T  V  F  S  A  P  N  Y  C  G   M  M  N  N  A  G  G  V  M  S   280

GTTAGCACAGACTTGATCTGCTCCTTCGTC ATTATTCTACCGTGTCACAAATACAAAATG   905
 V  S  T  D  L  I  C  S  F  V   I  I  L  P  C  H  K  Y  K  M   300

ATTGCGACTGATGCCAACCAAATGCCGACT AACGAAGAGGAG                    947
 I  A  T  D  A  N  Q  M  P  T   N  E  E  E                     314

TGATTTTGTTTTTCATACATGTATATATCA CTTCGTGCAATTAAATTCGTACAATAACAA 1007

TTCTTTTTATGTAGCTGATATATC                                      1031
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Fig.2. Nucleotide sequence and predicted amino acid sequence of *Drosophila* protein phosphatase-Y.

Table 1

Comparison of the amino acid sequence of *Drosophila* PP-Y to other known protein phosphatase sequences

Enzyme	Number of amino acids compared	Identity (%)	Homology (%)	References
Drosophila				
PP-1α	300	61	70	[5]
PP-1β	129	64	74	[6]
Rabbit				
PP-1α	314	59	68	[3]
PP-2Aα	309	39	52	[7]
PP-2Aβ	309	39	52	[8]
PP-X	203	38	51	[9]
Murine				
PP-2B	111	32	47	[14]

Fig.3. Comparison of animal protein phosphatase sequences. Amino acids that are variable among different species and isoenzymes are represented by asterisks. Residues identical in PP-Y and at least one of the compared protein phosphatases are boxed. The sequences were obtained from the following sources: PP-1 rabbit muscle [2–4, 26], *Drosophila* head [5,6]; PP-Y *Drosophila* head (this paper); PP-2A, rabbit muscle [7,8] and liver [9], bovine adrenal [10], porcine kidney [11], rat liver [12], human liver [13].

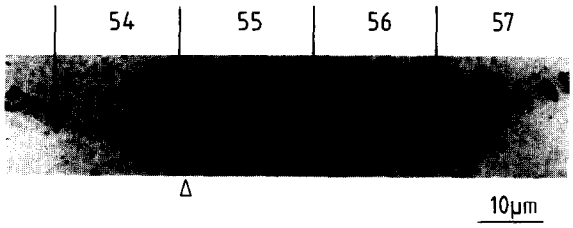

Fig.4. Localization of *Drosophila* protein phosphatase-Y by in situ hybridisation to salivary gland polytene chromosome 2. The arrow indicates the site of hybridisation at 55A 1-3.

2.3. *In situ hybridisation*

The entire PP-Y cDNA insert (fig.2) was labelled with [^{3}H]dCTP and [^{3}H]TTP by the random oligonucleotide priming technique [15] to 2×10^7 dpm/μg specific activity. The probe was hybridised to polytene chromosome preparations obtained from the salivary gland of female Canton S strain *D. melanogaster* larvae as described [5].

3. RESULTS

Out of 216 000 plaque forming units of a *Drosophila* head cDNA library we found 26 clones that hybridised to a 0.76 kb rabbit PP-1 cDNA probe but were not detected by a synthetic oligonucleotide coding for a rabbit PP-1 tryptic peptide. From these, two clones containing 1.0 kb inserts were purified and sequenced (fig.1). These clones were identical and contained the full-length coding region of a protein of 314 amino acids. The 5′-noncoding region is extremely short and the 3′-noncoding region has 3 additional potential stop codons starting at positions 989, 1002 and 1023, one of which is in-frame with the stop codon at 948 (fig.2). The predicted molecular mass of the encoded protein is 36 026 Da assuming that there are no posttranslational modifications. The deduced amino acid sequence is distinct from any known PP sequence (table 1), but nevertheless contains most of the polypeptide stretches that are highly conserved among the members of the PP-1/PP-2A family (fig.3). This clone hybridises in situ to polytene chromosomes at a single locus on the right arm of the second chromosome at cytological position 55A1-3 (fig.4) on the standard maps [21].

4. DISCUSSION

We predict that the protein encoded by the clone described here (fig.2) and designated PP-Y is a new member of the PP family. Although isolated with the aid of a PP-1 cDNA probe, it probably does not represent a closely related isoenzyme of PP-1 for the following reasons. (i) The amino acid sequence of PP-Y is distinct from PP-1α (table 1, fig.3) and a recently sequenced fragment of *Drosophila* PP-1β, a new PP-1 isoenzyme (table 1). On the other hand, PP-1α and PP-1β are very closely related and both are highly conserved (92% identity for PP-1α) from mammals to *Drosophila* [6]. (ii) The nucleotide sequence of the PP-Y coding region shows only 63% identity to that of *Drosophila* PP-1α [5]. (iii) The *Drosophila* PP-1 genes were located on the right arm of chromosome 3 and on the X-chromosome by in situ hybridisation [5], while the PP-Y gene mapped on the second chromosome. (iv) All published PP-1 nucleotide sequences are G-C-rich especially at the third positions of the codons [2–5], in contrast to PP-Y which has an average G-C content (47% in the coding region and 55% in the third positions of the codons).

It is unlikely that PP-Y is a type-2 PP, since it has only about 50% amino acid homology to PP-2A and PP-2B (table 1), nor did we find any homology with the sequenced peptides of PP-2C (comprising ~65% of the total sequence) [22]. *Drosophila* PP-2A has been sequenced and localized at a different cytological position (Orgad, S., Dudai, Y. and Cohen, P.T.W., unpublished result), and the molecular mass of the PP-2B catalytic subunit [23] is much higher than that predicted for PP-Y. Furthermore, the amino acid sequence of PP-Y is different from that of PP-X, a novel hepatic PP identified by molecular cloning (table 1). These considerations indicate that PP-Y is a related but quite distinct protein.

Since PP-Y was found in a head cDNA library and has the highest homology to PP-1 catalytic subunits it is possible that PP-Y is a neuronal form of PP-1. Alternatively, it could be an enzyme related to smooth muscle phosphatase IV which has a substrate specificity similar to PP-1, but is unaffected by inhibitor-2, a protein which inhibits PP-1 specifically [24]. A further possibility is that it may be highly specific for a substrate that has yet to be identified, like the protein phosphatase reported to be specific for ribosomal protein S6 [25]. Expression of the clone and subsequent analysis will be necessary to identify its enzymatic properties and physiological role(s).

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