The structure of protein phosphatase 2A is as highly conserved as that of protein phosphatase 1

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cDNA coding for protein phosphatase 2A (PP2A) has been isolated from *Drosophila* head and eye imaginal disc libraries. *Drosophila* PP2A mRNA is expressed throughout development, but is most abundant in the early embryo. The cDNA hybridises to a single site on the left arm of the second chromosome at position 28D2-4. The deduced amino acid sequence (309 residues) of *Drosophila* PP2A shows 94% identity with either rabbit PP2Aα or PP2Aβ, indicating that PP2A may be the most conserved of all known enzymes.

Protein phosphatase; cDNA cloning; Nucleotide sequence; Amino acid sequence; Sequence homology; *Drosophila melanogaster*

1. INTRODUCTION

Many extracellular stimuli elicit diverse intracellular responses through phosphorylation-dephosphorylation cascades, ranging from the control of metabolism [1] to the modulation of synaptic efficiency [2-4]. The phosphorylation state of a protein is controlled by protein kinases and phosphatases, and there have been major advances in understanding the structure and regulation of the latter class of enzymes in recent years (reviewed in [5,6]). The protein serine/threonine phosphatases comprise two distinct families, protein phosphatases 1, 2A and 2B (PP1, PP2A and PP2B) being homologous to one another, while protein phosphatase 2C (PP2C) is a structurally distinct protein.

PP1 and PP2A show 41% amino acid sequence identity and these enzyme activities have been found in all eukaryotic cells examined to date (reviewed in [5,6]). Furthermore, their specificities and sensitivity to inhibitors, such as inhibitor 1, inhibitor 2 and okadaic acid, have been remarkably conserved in organisms as diverse as mammals, *Drosophila* [7,8], yeast [9] and higher plants [10]. In the case of PP1, the structural basis for these biochemical similarities has emerged from cDNA cloning studies, which have revealed that it is the most conserved of all enzymes so far examined [11]. Mammalian and *Drosophila* PP1 show ~90% sequence identity [12,13], while comparison of the mammalian and yeast [14] or mammalian and *Aspergillus* [15] enzymes reveals >80% identity. The extreme structural conservation of PP1 may be a reflection of its crucial and multiple roles in vivo, implying an ability to interact with many substrate proteins, and/or regulatory subunits [6].

In this paper, we report the isolation of cDNA encoding a PP2A catalytic subunit from *Drosophila melanogaster* and the determination of its sequence. These studies have revealed that the structure of PP2A is as highly conserved as that of PP1.

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2. MATERIALS AND METHODS

2.1. *Isolation of cDNA clones*

A *Drosophila* head cDNA library constructed in λgt11, kindly provided by Dr Y. Citri [16], was screened under high stringency hybridisation conditions (0.75 M sodium chloride, 75 mM sodium citrate pH 7.0, 0.1% polyvinylpyrrolidone, 0.1% Ficoll 400, 0.1% bovine serum albumin at 67°C) with a 0.96 kb *Hpa*II/*Rsa*I fragment which comprised 20 bases of the non-coding region 5′ to the initiating ATG and the nucleotide sequence encoding amino acids 1-303 of the rabbit PP2A catalytic subunit [17]. A *Drosophila* eye imaginal disc cDNA library constructed in λgt10, kindly provided by Dr D. Kalderon and Professor G.M. Rubin, was screened under low stringency hybridisation conditions [18] with a 0.57 kb *Eco*RI/*Sma*I fragment encoding amino acids 108-295 of rabbit PPX [18]. The probes were labelled to a specific activity of 2×10^9 dpm/μg by random hexanucleotide priming [19] and further purified by spun column chromatography [20]. Filters were hybridised overnight with 2×10^6 dpm/ml of the probe and washed with 0.3 M sodium chloride, 30 mM sodium citrate pH 7.0 at 67°C (high stringency) or 55°C (low stringency). Positive recombinant phages were purified by CsCl density centrifugation [21] and DNA was isolated by phenol/chloroform or formamide extraction of the phages.

2.2. *Subcloning and sequence determination*

DNA from the λ phage recombinants was digested with *Eco*RI to release the cDNA inserts which were subcloned into the Bluescript pKS$^+$-vector. DNA sequencing was performed either on single-stranded DNA, or on double-stranded supercoiled plasmid DNA by the dideoxy chain termination method [22], using [α-^{32}P]dATP or

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(a)

(b)

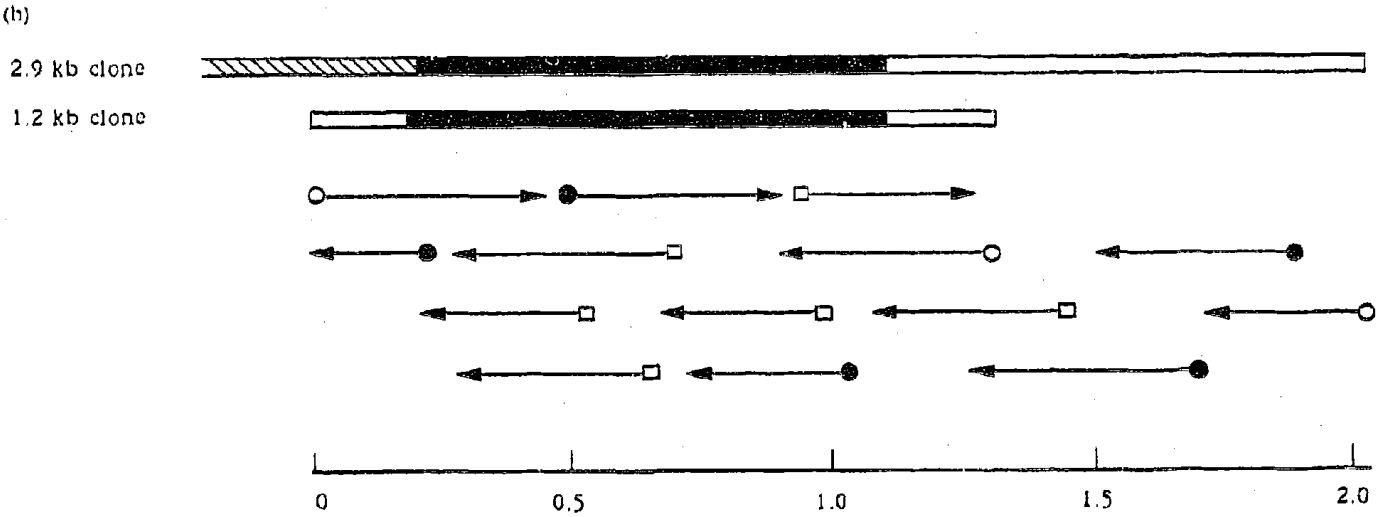

Fig. 1. Structure of *Drosophila* protein phosphatase 2A. (a) Nucleotide and predicted amino acid sequence of *Drosophila* protein phosphatase 2A catalytic subunit. A putative polyadenylation signal AATAAA is underlined. (b) Strategy used to sequence *Drosophila* clones coding for the catalytic subunit of protein phosphatase 2A. Black bars indicate the coding region, open bars the non-coding regions and the hatched bar represents a cloning artefact. The horizontal arrows show the direction and length of sequences obtained with specific oligonucleotide primers (□) and with Bluescript primers on the native (○) or deleted (●) clones. The scale is in kilobase pairs.

[α-^{35}S]dATP and Sequenase (US Biochemical Corp., Cleveland, Ohio, USA). Progressive unidirectional deletions of the inserted DNA were constructed using Erase-a-Base kit (Promega, Madison, Wisconsin, USA). Alternatively, restriction fragments were removed and the religated truncated recombinants were sequenced using Bluescript primers. Oligonucleotide sequencing primers were synthesized by Dr O. Goldberg at the Weizmann Institute of Science, and Mr A.D.H. Murchie at Dundee University.

2.3. *RNA preparation, Northern blot analyses and in situ hybridisation*

These procedures were carried out as described previously [12]. Three probes were employed for mRNA hybridisation. A coding region fragment (nt 486–805) was labelled by random hexanucleotide priming [19] while the other two probes consisted of 'run off' transcripts complementary to either the 5′ non-coding region or the 3′ non-coding region and part of the coding region. For preparation of the 5′ probe, the 1.2 kb clone was truncated from the 3′ end to the *Bgl*II at nt 24 and religated. For the 3′ probe, the 2.9 kb cDNA clone cleaved with *Bgl*II at nt 486. Both probes were then synthesized from the T3 promoter in Bluescript using [α-^{32}P]UTP and Stratagene (San Diego, CA, USA) RNA transcription kit (catalogue number 042003340). The probes used for in situ hybridisation were coding region fragments comprising nt 486–805 and nt 24–485, which were labelled by random priming [19].

3. RESULTS

3.1. *Sequence of cDNA encoding the* Drosophila *PP2A catalytic subunit*

Screening of 2×10^5 recombinants of the head library with the 0.96 kb rabbit PP2A cDNA probe yielded a positive clone with an insert size of 2.9 kb (Fig. 1b), while screening of 1.8×10^5 recombinants of the eye imaginal disc library with the 0.57 kb rabbit PPX cDNA probe yielded a positive clone with an insert size of 1.2 kb (Fig. 1b). The latter clone yielded a 5′ non-coding region of 188 nucleotides, an open reading frame of 927 nucleotides followed by 133 nucleotides of 3′ non-coding sequence (Fig. 1a). The 2.9 kb clone contains the coding region starting from nucleotide 22 and 377 nucleotides of 3′ non-coding region. The 133 nucleotides immediately after the stop codon are identical in the 2.9 kb and 1.2 kb clones. In the 2.9 kb clone, the sequence 5′ to the coding region nucleotide 22 contains several stop codons, but no initiating methionine, indicating a cloning artefact, and for this reason the 2.9 kb clone was not fully sequenced. The sequence of the 3′ end is presented in Fig. 1a. Northern blotting was used to demonstrate that the 1.2 kb clone contained the correct 5′ non-coding region and start of the coding region. An *Eco*RI/*Bgl*II fragment (nt −188 to +24) hybridised to the same 1.5 kb and 2.3 kb mRNA species as coding region fragments (see below).

The nucleotide sequence of the coding region of *Drosophila* PP2A shows 74% identity to the nucleotide sequences of both PP2Aα and PP2Aβ from rabbit skeletal muscle [23].

3.2. *Amino acid sequence of the* Drosophila *PP2A catalytic subunit*

The deduced translation product of the *Drosophila* cDNA is 309 amino acids (Fig. 1), identical in size to rabbit PP2A. The predicted molecular mass of *Drosophila* PP2A is 35.42 kDa, assuming that the initiating methionine is not removed or acetylated.

3.3. *Expression during* Drosophila *development*

Restriction fragments from the coding region (nt 486–805) and the 5′ non-coding region (nt −188 to 24) of the *Drosophila* PP2A cDNA recognized two transcripts of 2.3 kb and 1.5 kb (data not shown), whereas 'run off' transcripts of the 2.9 kb clone, which are likely to be mainly from the 3′ end of the 3′ non-coding region, hybridised predominantly to the 2.3 kb transcript under stringent hybridisation conditions (Fig. 2). It is therefore likely that the 1.5 kb and 2.3 kb transcripts are produced from the same PP2A gene, the 1.5 kb mRNA being terminated with the polyadenylation signal AATAAA at nt 1042–1047 (Fig. 1a). Both the 1.5 kb and 2.3 kb transcripts were detected throughout *Drosophila* development, but were notably more abundant in early embryos.

3.4. *Chromosomal localization of* Drosophila *PP2A by in situ hybridisation to polytene chromosomes*

Coding region fragments of *Drosophila* PP2A cDNA hybridised to one site in the salivary gland

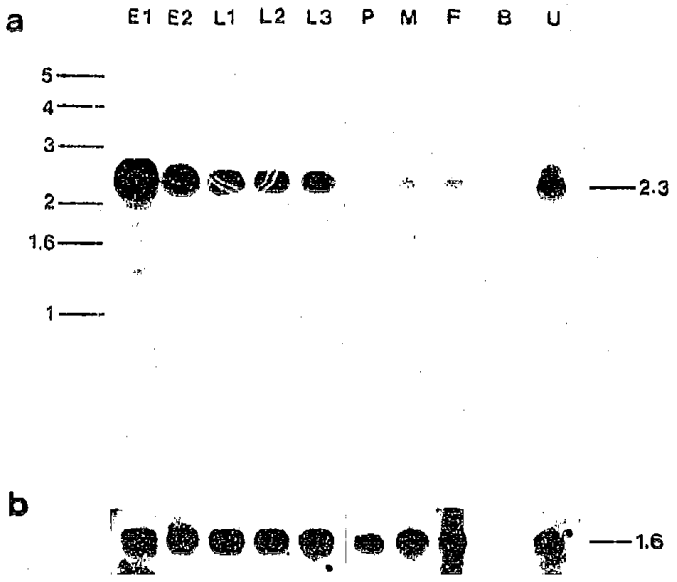

Fig. 2. Expression of the protein phosphatase 2A gene during *Drosophila* development. E1, 0–4 h embryo; E2, 4–22 h embryo; L1, 1st instar larva; L2, 2nd instar larva; L3, 3rd instar larva; P, pupa; M, adult male; F, adult female; B (blank), no RNA; U, unfertilised eggs. The size of the mRNA transcript (on the right) and the marker DNA fragments (on the left) are given in kilobases. (a) Northern blot probed with the 3′ non-coding region of *Drosophila* PP2A cDNA. (b) The same blot stripped and reprobed with pDmras64B to control for variation in loading of the poly(A)-rich RNA. The 1.6 kb Dmras64B transcript is present at a constant proportion of the total poly(A)-rich RNA throughout development [24].

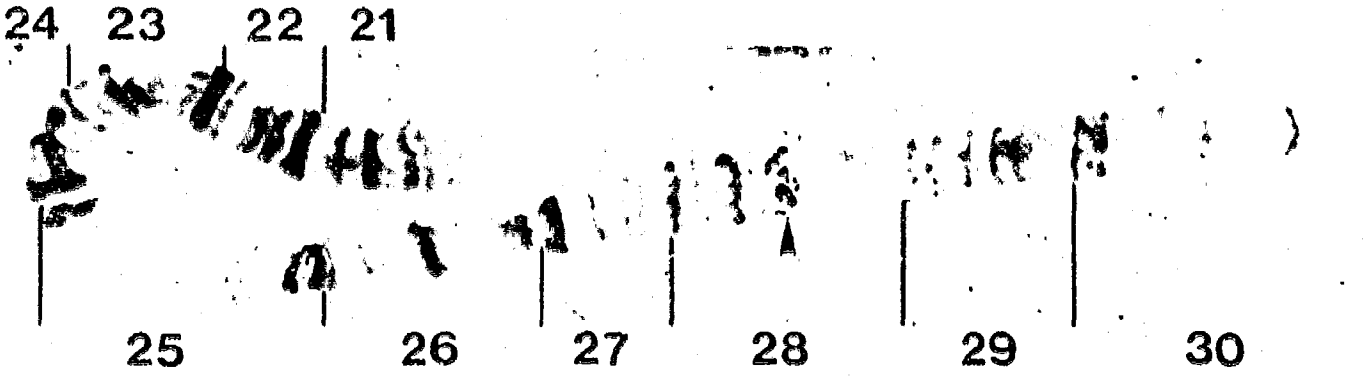

Fig. 3. In situ hybridisation of *Drosophila* protein phosphatase 2A cDNA to salivary gland polytene chromosomes at 28D2-4 on chromosome 2.

chromosomes (Fig. 3). The signal was located on the left arm of chromosome 2 at position 28D2-4.

4. DISCUSSION

Comparison of the encoded amino acid sequence of *Drosophila* PP2A cDNA with those of rabbit shows very high conservation of structure (Fig. 4). The amino acid sequence identity between *Drosophila* PP2A and both PP2Aα and PP2Aβ of rabbit is 94% and the similarity rises to 97–98% if conservative substitutions are included. Most of the differences are near the N-terminus, and the identity is 98% over the region comprising amino acids 50–309. The conservation of structure from *Drosophila* to mammals is slightly higher than that observed for PP1 [12,13], indicating that PP2A may be the most conserved enzyme known, even more constant in structure than histone 2A. Only histones 3 and 4 and calmodulin show higher evolutionary conservation [11]. PP2A is thought to regulate multiple functions in vivo including several metabolic pathways, protein synthesis, DNA replication (reviewed in [5]) and the cell cycle. Studies using the inhibitor okadaic acid in *Xenopus* oocyte interphase extracts demonstrated that PP2A is involved in suppressing activation of cdc2 protein kinase [25]. Consistent with a role in cell division, abundant transcripts of PP2A are present throughout the life cycle of *Drosophila*, and it is of interest that transcripts are most abundant in the very early 0–4 h embryo (Fig. 2), at which time a series

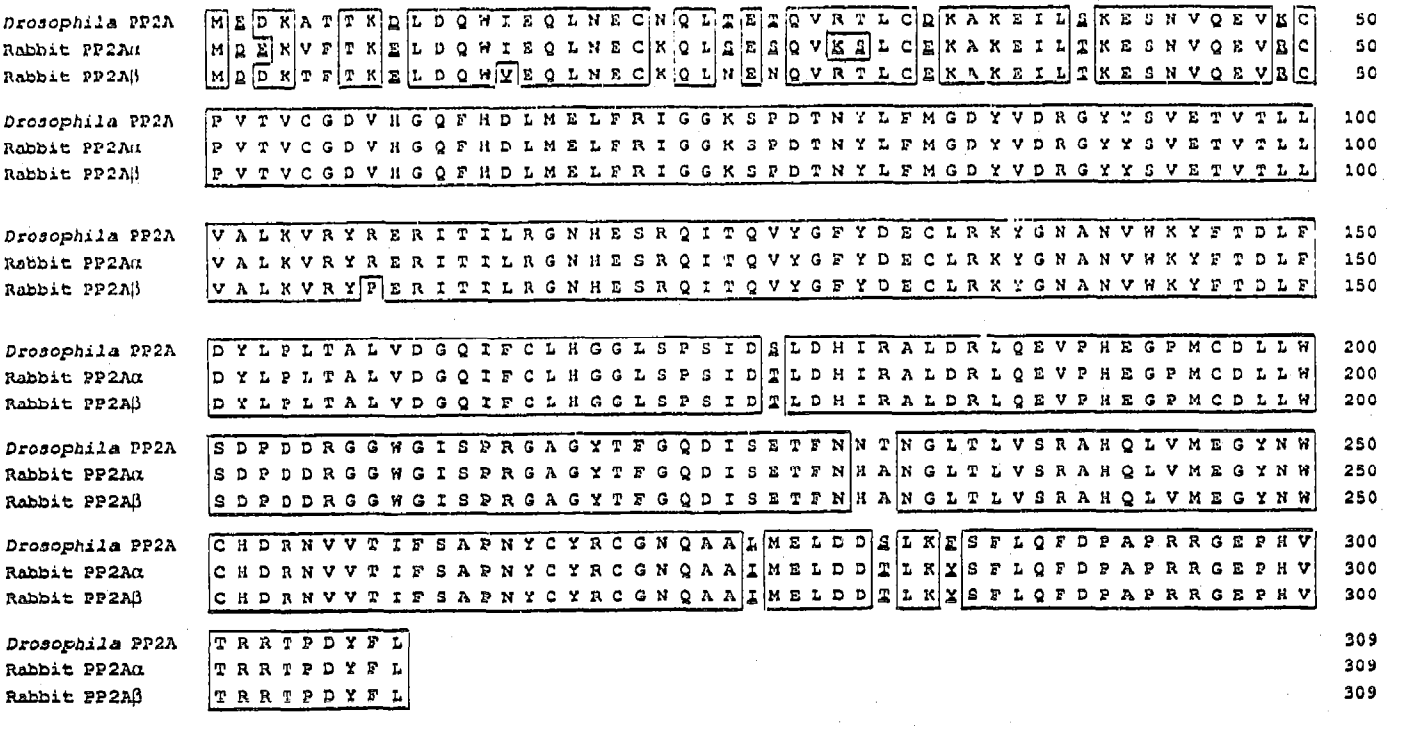

Fig. 4. Comparison of the deduced amino acid sequence of the catalytic subunit of *Drosophila* protein phosphatase 2A with rabbit protein phosphatase 2Aα and β catalytic subunits. Identities are boxed and conservative substitutions are underlined.

of rapid cell divisions take place (reviewed in [26]). However PP1, which is known to play a role in the separation of sister chromatids at a later stage of mitosis in *Drosophila* [27,28], does not appear to be elevated substantially in very early embryos ([12] and L. Alphey and P.T.W. Cohen, unpublished data).

Only one isoform of PP2A has been isolated from *Drosophila* so far. In mammals, two isoforms are known, PP2Aα and PP2Aβ, which differ in only 8 amino acids, 7 of which are within 30 residues of the N-terminus (the 8th being at residue 108). Amino acids 14 and 108 of *Drosophila* PP2A are as in rabbit PP2Aα, while amino acids 29 and 30 are as in PP2Aβ, the other four residues being different from both PP2Aα and PP2Aβ. Since only one chromosomal location was identified with the *Drosophila* PP2A cDNA, it appears likely that there is only one gene in *Drosophila*. A second PP2A gene, if it exists, would have to be at the same locus or have a rather different nucleotide sequence. Finally, identification of the hybridising site of *Drosophila* PP2A at 28D2-4 will facilitate mutagenesis of this locus and the isolation of mutants defective in PP2A activity using the recently reported techniques [29,30].

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