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Minireview

Protein serine/threonine phosphatases; an expanding family

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Five protein serine/threonine phosphatases (PP) have been identified by cloning cDNA from mammalian and *Drosophila* libraries. These novel enzymes, which have not yet been detected by the techniques of protein chemistry and enzymology, are termed PPV, $PP2B_W$, PPX, PPY and PPZ. The complete amino acid sequences of PPX, PPY and PPZ and an almost complete sequence of PPV are presented. In the catalytic domain PPV and PPX are more similar to PP2A (57–69% identity) than PP1 (45–49% identity), while PPY and PPZ are more similar to PP1 (66–68% identity) than PP2A (44% identity). The cDNA for $PP2B_W$ encodes a novel Ca^{2+}/calmodulin-dependent protein phosphatase only 62% identical to PP2B in the catalytic domain. Approaches for determining the cellular functions of these protein phosphatases are discussed.

Protein phosphorylation; Gene family; Evolutionary conservation; Cellular regulation

1. INTRODUCTION

A large number of proteins in mammalian cells contain covalently bound phosphate. While in many cases the functional significance of this modification is unclear, it is nevertheless now obvious that the phosphorylation of proteins is the major general mechanism for the control of cellular functions in eukaryotic cells (reviewed in [1,2]). The steady state level of phosphorylation of any protein depends on the relative activities of protein kinases and phosphatases, but far more attention has been lavished on the former class of enzymes. It has been suggested that '1001 protein kinases' may exist [3] and several of these are known to be regulated by second messengers such as cyclic AMP (cyclic AMP-dependent protein kinase), cyclic GMP (cyclic GMP-dependent protein kinase), Ca^{2+} (calcium/calmodulin-dependent protein kinases) and diacylglycerol (protein kinase C).

2. IDENTIFICATION OF FOUR TYPES OF PROTEIN SERINE/THREONINE PHOSPHATASES

In contrast to the protein kinases, our understanding of the protein phosphatase involved in cellular regulation and the mechanisms that control their activities has developed more recently. Four major types of protein serine/threonine phosphatase catalytic subunits have been identified in eukaryotic cells by the techniques of protein chemistry and enzymology and termed protein phosphatases 1, 2A, 2B and 2C (PP1, PP2A, PP2B and PP2C). They can be distinguished by their action on phosphorylase kinase and by their sensitivity to certain activators and inhibitors. PP1 is potently inhibited by the thermostable protein inhibitors 1 and 2 and dephosphorylates the β subunit of phosphorylase kinase specifically, whereas type 2 protein phosphatases are unaffected by the inhibitor proteins and dephosphorylate the α subunit of phosphorylase kinase preferentially [4,5]. The three type 2 protein phosphatases can be distinguished in a number of ways, but most effectively by their requirement for divalent cations and their response to the tumour promoter okadaic acid [6–8] and liver toxin termed microcystin LR [9]. PP2A does not have an absolute requirement for divalent cations, whereas PP2B is a Ca^{2+}-dependent, calmodulin stimulated enzyme and PP2C is dependent on Mg^{2+} [4,5]. PP2A is inhibited by subnanomolar concentrations of okadaic acid and microcystin, whereas PP2B is over 1000-fold less sensitive and PP2C is resistant to these toxins [6–9]. PP1 is also extremely sensitive to okadaic acid and microcystin, although the K_i values are higher than those observed with PP2A [6–9].

PP1 and PP2A are known to dephosphorylate many proteins in vitro and are likely to have pleiotropic actions in vivo. For example, PP1 is involved in the control of glycogen metabolism [5], muscle contractility [5], and chromosome separation at mitosis (reviewed in [10]), while PP2A regulates enzymes involved in glycolysis, lipid metabolism and catecholamine synthesis (reviewed in [8]) and cdc2 kinase activation at mitosis [11]. PP2C also dephosphorylates many pro-

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teins in vitro [5] but, in contrast to PP1 and PP2A, none of its physiological roles are defined. The specificity of PP2B is much more restricted than PP1, PP2A and PP2C, and the most effective substrates so far identified are proteins that regulate other protein kinases and phosphatases [12].

The catalytic subunits of PP1 and PP2A are complexed to other proteins in vivo that are believed to play important roles in determining the subcellular location, specificity and regulatory behaviour of these enzymes (reviewed in [5,10]). The catalytic subunit of PP2B is complexed to a Ca^{2+} binding protein related in structure to calmodulin, and also interacts with calmodulin itself in the presence of Ca^{2+} (reviewed in [12]). In contrast, the catalytic subunit of PP2C is not known to interact with other proteins [5].

3. STRUCTURAL RELATIONSHIPS BETWEEN PROTEIN PHOSPHATASE CATALYTIC SUBUNITS

The structures of PP1, PP2A, PP2B and PP2C have been elucidated by cDNA cloning (reviewed in [10,13]). These studies have revealed that mammalian PP1 (~37 kDa) and PP2A (36 kDa) are about 50% identical in amino acid sequence in the catalytic domain comprising amino acids 29–299 of PP1α (Fig. 1). PP2B is a member of the same gene family, its catalytic domain being ~40% identical to PP1 and PP2A. It also possesses a long C-terminal extension containing regions that interact with the Ca^{2+} binding protein and with calmodulin, as well as a domain for suppressing activity in the absence of Ca^{2+} and calmodulin (reviewed in

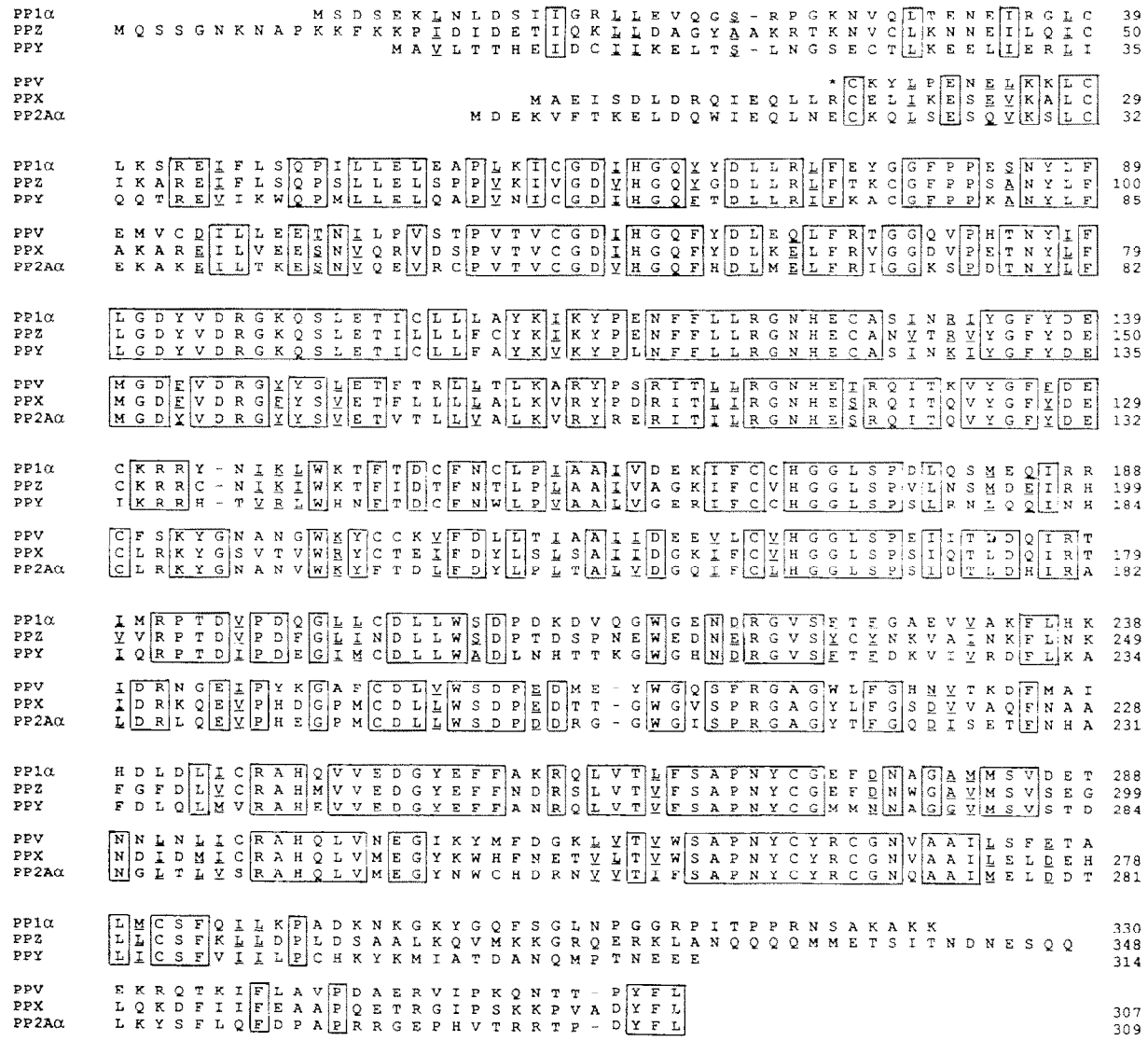

Fig. 1. Comparison of the amino acid sequences of protein serine/threonine phosphatases related to PP1 and PP2A. PPY and PPZ are compared with PP1, while PPX and PPV are compared with PP2A. Identities are boxed and the most conservative replacements are underlined. The sequences of PP1α and PP2Aα are derived from rabbit cDNA libraries [21,31]. cDNA encoding PPX and PPZ were obtained from rabbit liver and brain respectively, while cDNA for PPY and PPV were from *Drosophila* libraries. PPZ may possess the sequence MENVNDKNNNITDSKKDP-NEEFNDI N-terminal to the initiating methionine presented in the figure.

[10,14]). In contrast, PP2C (~42 kDa) is completely unrelated in structure [15]. The protein serine/threonine phosphatases therefore comprise two distinct gene families.

The structures of both PP1 and PP2A have remained remarkably constant through evolution and may be the most conserved of all known enzymes [14]. The amino acid sequences of PP1 from mammals and *Drosophila* show greater than 90% overall identity, while mammalian PP1 is over 80% identical to the corresponding enzymes in yeast and *Aspergillus* (reviewed in [10]). Similar observations have been made with PP2A [13].

At least two isoforms of PP1, PP2A, PP2B and PP2C are present in mammalian tissues that are the products of distinct genes. The isoforms of PP1 (PP1α, PP1β) show ~95% identity in the catalytic domain, the isoforms of PP2A (PP2Aα, PP2Aβ) ~98% identity, and the isoforms of PP2B (PP2Bα, PP2Bβ) 89% identity in the same region [16]. In *Drosophila*, three genes encoding isoforms of PP1 have been identified, two of which (PP1α_1 and PP1α_2) are extremely similar to mammalian PP1α (>94% identity in the catalytic domain), the third PP1β being extremely similar to mammalian PP1β(>94% identity) ([17], Dombrádi et al., submitted).

4. NOVEL PROTEIN SERINE/THREONINE PHOSPHATASE

In 1988, this laboratory reported the isolation of a cDNA from a rabbit liver library encoding part of the catalytic domain of a protein serine/threonine phosphatase related to, but distinct from, PP1 and PP2A. This putative phosphatase, termed PPX, showed more amino acid sequence identities with PP2A than PP1 [18]. However, PPX was not the hepatic form of PP2A, since both PP2A isoforms (PP2Aα and PP2Aβ) are expressed in liver, as well as in other tissues. PPX therefore appears to represent a novel phosphatase not previously recognised by the techniques of protein chemistry and enzymology. Fig. 1 shows the entire amino acid sequence of PPX, which has recently been obtained by sequencing a clone obtained from a different rabbit liver cDNA library. The complete amino acid sequence of PPX shows that it is 49% identical to PP1 and 69% identical to PP2A in the catalytic domain.

Last year our laboratory described a further cDNA clone isolated from a *Drosophila* head library, which encoded the complete amino acid sequence of a protein serine/threonine phosphatase, termed PPY, which in contrast to PPX was more similar to PP1 (66% identity in the catalytic domain) than PP2A (44% identity) [19]. This enzyme was not *Drosophila* PP1 since, as discussed above, all three isoforms of *Drosophila* PP1 are >94% identical to their mammalian equivalents in the same regions.

We now report the identification of cDNA clones encoding further novel protein serine/threonine phosphatases. The structure of an enzyme termed protein phosphatase Z (PPZ) was deduced from a full length cDNA isolated from a rabbit brain library, and the amino acid sequence is shown in Fig. 1. Like PPY, this enzyme is more similar to PP1 (68% identity) than PP2A (44% identity), but it is not the mammalian homologue of PPY, nor is it the brain isoform of PP1, since PP1α, cloned from both skeletal muscle and liver, is also present in brain as judged by Northern blotting [20,21].

Interestingly, our laboratory has isolated a further clone from the same rabbit brain library that encodes part of an enzyme that is 94% identical in amino acid sequence to PPZ, yet is the product of a distinct gene (E.F. da Cruz e Silva and P.T.W. Cohen, unpublished work). This demonstrates that at least two isoforms of PPZ (PPZα, PPZβ) are present in mammalian brain.

Had we realised that so many novel protein phosphatases were present in eukaryotic tissues we would have called the first one PPA and not PPX! The identification of yet further enzymes therefore necessitated going backwards through the alphabet. A cDNA was isolated from the rabbit brain library that encoded a putative Ca^{2+}/calmodulin-dependent protein phosphatase, PP2B$_w$, whose catalytic domain is only 62% identical to the other isoforms of PP2B (PP2Bα, PP2Bβ) [22], and is therefore likely to possess a distinct substrate specificity.

We have also recently isolated a cDNA from the *Drosophila* head library (D.J. Mann, V. Dombrádi and P.T.W. Cohen, unpublished results) comprising nearly all the coding region of an enzyme termed PPV which is more similar to PP2A (57% identity) than PP1 (45% identity) (Fig. 1). PPV is not the *Drosophila* homologue of PPX.

Comparison of these novel protein phosphatase structures with PP1 and PP2A (Fig. 1) shows that PP1, PPZ and PPY comprise one subfamily, while PP2A, PPX and PPV form another. Regions likely to be responsible for catalytic activity (amino acids 63–67, 90–97 and 121–126 of PP1 α), which were identified by comparison with the bacteriophage protein phosphatase ORF221 [23], are conserved in the novel protein phosphatases. The structural relationships between the phosphatases in the PP1/PP2A/PP2B gene family are depicted in Fig. 2.

The identification of five new members of the PP1/PP2A/PP2B family raises the question of why these phosphatases have not been isolated as enzymes. Several obvious possibilities spring to mind. Firstly, they might be present at very low levels in tissues, or have a specialised cellular or subcellular location. Secondly, it is possible that they are complexed to inhibitory subunits in tissue extracts, thereby preventing detection of their activity. Thirdly, they might have

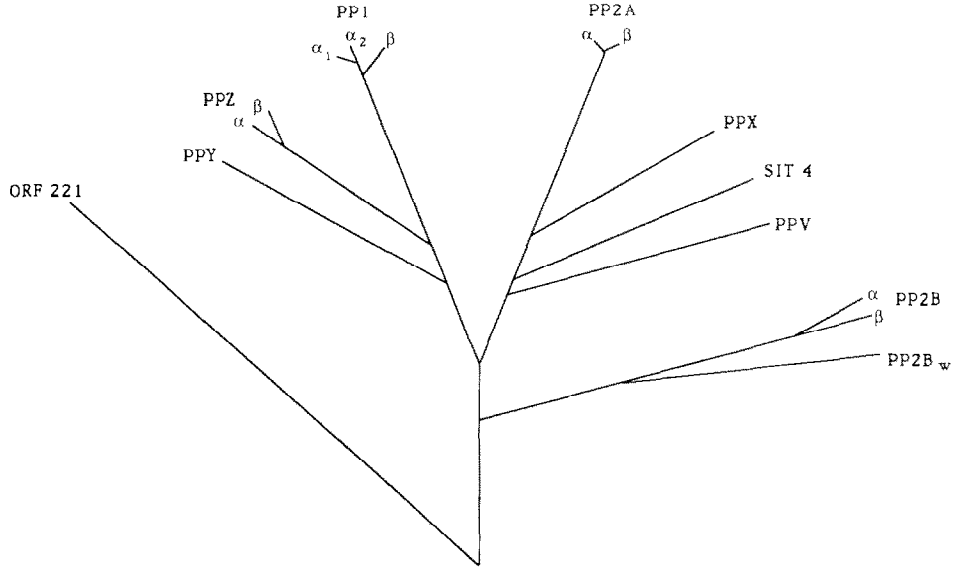

Fig. 2. Structural relationships between enzymes of the protein phosphatase 1/2A/2B family. In order to construct the diagram, identities in the catalytic domain (amino acids 29–299 of rabbit PP1α (Fig. 1) and the corresponding regions of the other protein phosphatases) were obtained from pairwise comparisons and the percent differences calculated. The branch lengths are proportional to the percent differences. Sequences used were PP1 ([17,21] and unpublished data). PP2A [32], PP2B [16,32], PPY [19], SIT 4 [33], $PP2B_w$ [22], ORF 221 [23], and PPV, PPX and PPZ (present work).

restricted substrate specificities and dephosphorylate very poorly the substrates normally used to assay PP1 and PP2A. We are currently using a number of approaches to elucidate the substrate specificities and physiological roles of PPV, $PP2B_w$, PPX, PPY and PPZ. The cDNA clones are being expressed in the insect baculovirus system that has already been used to express PP1α at high levels [24]. This will enable their enzymatic properties to be compared to PP1, PP2A and PP2B, and allow the production of polyclonal antibodies, which can be used, in conjunction with Northern blotting experiments and in situ cDNA hybridisation techniques, to elucidate the tissue and subcellular locations of the phosphatases. In addition, work to isolate each of these cDNAs in both *Drosophila* and yeast is in progress. This will allow the genes to be disrupted and the effect of such gene deletions on phenotype to be examined. The techniques introduced recently by Ballinger and Benzer [25] and Kaiser and Goodwin [26] may facilitate such experiments in *Drosophila*. An important role for PP1α in allowing chromosome separation at mitosis has recently been identified by the analysis of mutant *Drosophila* lacking this enzyme (Axton et al., submitted; Dombrádi et al., submitted). Isolation of homologous human cDNAs will allow the phosphatase genes to be mapped within the human genome and studied for segregation with human diseases. This has only been performed so far with PP1α [27].

Fifteen genes encoding protein serine/threonine phosphatase catalytic subunits have now been identified from their cDNA sequences in eukaryotes and one further protein phosphatase gene in the bacteriophage genome [23]. These probably do not include the mitochondrial enzymes that dephosphorylate the pyruvate dehydrogenase complex and branched chain keto acid dehydrogenase complex [28]. Furthermore a number of protein tyrosine phosphatases have recently been recognised [29,30]. However, although the number of protein phosphatases in cells is increasing rapidly, at the present time it seems unlikely that the target of '1001' catalytic subunits predicted for protein kinases [3] will be reached for protein phosphatases.

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