Identification by molecular cloning of two cDNA sequences from the plant *Brassica napus* which are very similar to mammalian protein phosphatases-1 and -2A

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Two clones encoding protein phosphatase (PP) catalytic subunits have been isolated from a *Brassica napus* cDNA library screened with rabbit muscle PP1α and PP2Aα cDNAs. The deduced protein sequences are very similar to those of mammalian PP1α and PP2Aα (72% and 79% overall identity, respectively) indicating that they are the plant homologues of PP1α and PP2Aα. This high degree of similarity provides a molecular explanation for the remarkable conservation of the catalytic and regulatory properties between animal and plant protein phosphatases and supports the concept that PP1 and PP2A may be the most highly conserved of known enzymes.

Plant; Protein phosphorylation; Protein phosphatase; cDNA cloning; Sequence homology; *Brassica napus*

1. INTRODUCTION

Recently, 3 of the 4 major protein phosphatases (PP1, PP2A and PP2C) present in animal cells (reviewed in [1]) were identified in the cytoplasmic compartment of both mono- and dicotyledonous plants and their properties were found to be virtually indistinguishable from the corresponding enzymes in mammalian tissues [2,3,4]. Similarities between plant and animal PP1 and PP2A included substrate specificity (using mammalian phosphoprotein substrates), and differential sensitivity to the mammalian heat stable inhibitor proteins (inhibitor-1 and inhibitor-2), the dinoflagellate toxin, okadaic acid, and the cyanobacterial hepatotoxin, microcystin-LR.

PP2A in spinach leaves has been shown to catalyse the in vivo dephosphorylation and activation of sucrose-phosphate synthase in response to light [5]. PP2A can also dephosphorylate quinate dehydrogenase from carrot cells [4] and phospho*enol*pyruvate carboxylase from *Bryophyllum fedschenkoi* [6] in cell-free assays. By analogy with mammals and yeast, it is anticipated that both PP1 and PP2A will prove to be critical to the regulation of many aspects of plant growth and metabolism.

In view of the biochemical similarities between PP1 and PP2A from mammals and plants and because of the high degree of sequence conservation among PP1 and PP2A cDNAs from mammals, *Drosophila* and fungi (reviewed in [7]), we searched for PP1 and PP2A clones in a *Brassica napus* (oilseed rape) library by screening with probes derived from PP1α or PP2Aα rabbit skeletal muscle cDNAs.

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2. MATERIALS AND METHODS

An amplified λgt10 cDNA library was constructed using mRNA from developing seeds of *Brassica napus*, in collaboration with Dr R. Safford (Unilever Research, Colworth Laboratories, Sharnbrook, Bedford). This library was screened, as described in [8] except that the hybridization temperature was 55°C, with a 0.76 kb *SmaI/NaeI* cDNA probe encoding amino acids 43-298 of rabbit skeletal muscle PP1α [9] and a 0.93 kb *HpaII/RsaI* cDNA fragment comprising 20 bases prior to the initiating ATG up to nucleotides encoding amino acid 303 of rabbit skeletal muscle PP2Aα [10]. By this method a single putative PP1 and a single putative PP2A clone were chosen for purification and further analysis. Rapid isolation of the λDNA, followed by *Eco*RI digestion showed the cDNA inserts of the PP1 and PP2A clones to be approximately 1.3 kb and 1.2 kb, respectively. The inserts were subcloned into Bluescript pKS-M13$^+$ and a nested deletion library was constructed using exonuclease III digestion (Pharmacia Nested Deletion Kit, Uppsala, Sweden). The plant protein phosphatase sequences were determined by the dideoxy chain-termination method [11].

3. RESULTS AND DISCUSSION

The sequencing strategies, nucleotide sequences and deduced amino acid sequences are shown in Figs 1 and 2. The PP1-like cDNA is composed of 1344 nucleotides and has an open reading frame which starts at nucleotide 222 and codes for 264 amino acids. Since no initiating methionine codon was detected and a stop codon immediately precedes nucleotide 222, it is likely that there has been a rearrangement at the 5′ end during construction of the cDNA library. Accordingly the amino acid sequence (255 residues) of the *Brassica napus* PP1-like clone which is presented in Figs 1 and 3

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commences with the amino acid at which homology with the mammalian PP1 is evident. The PP2A-like cDNA clone has 1232 nucleotides and an open reading frame which starts at the first nucleotide and codes for 309 amino acids. No initiating methionine is present, and it may be that the *Brassica napus* PP2A-like clone is longer than its mammalian counterpart (Fig. 3). Repeated screening of the cDNA library, using either probe under the original or less stringent conditions, resulted only in the isolation of clones identical to the two initially obtained.

Regions that are conserved in all the known protein phosphatases, including the bacteriophage phosphatase ORF221 have been identified [21]. These regions are

(a)

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                                                    TCTGGGTATTG TAATCCCTCTCTCTCCGATCATCGATTCAC   41
GATGAAATTTGCATAGTTTGTGGGTGAAAG TAGCAAACTTTAGAGTTTCTCTGTGAATTT AAGCATCTGGGTATTGTAATCCCTCTCTCT  131
CTGATCATCGATTCATGATGAAATTTGCAT AGTTGGGGGGTGAAAGTACCAAACTTCAGG GTTTCTCTTTGAACTTGAGCATCTGAGTAA  221

CGTTTTCTTTGGCTTTGTTGGAGCTCAGGT GATATCCATGGCCAGTATCAAGATCTTCTG AGGCTATTCGAGTACGGAGGCTACCCTCCT  311
 - - - - - - - - - - G  D I H G Q Y Q D L L  R L F E Y G G Y P P

TCAGCCAACTTTCTCTTCCTCGGCGACTAC GTTGACAGAGGCAAGCAAAGTCTCGAGACC ATTTGTCTCCTTCTCGCTTACAAGATTCGT  401
 S A N F L F L G D Y  V D R G K Q S L E T  I C L L L A Y K I R

TACCCATCAAAGATTTATCTCTTGAGAGGC AACCACGAGGACGCTAAGATCAACAGGATT TACGGATTCTACGACGAGTGCAAACGGAGA  491
 Y P S K I Y L L R G  N H E D A K I N R I  Y G F Y D E C K R R

TTCAATGTGCGCCTCTGGAAGATATTTACC GATTGTTTCAACTGTTTGCCCGTAGCTGCA CTCATCGACGACAAGATCTTGTGTATGCAC  581
 F N V R L W K I F T  D C F N C L P V A A  L I D D K I L C M H

GGTGGCTTGTCACCGGAGCTGGATAATTTG AATCAGATTCGAGAGATTCAGAGGCCTACG GAGATTCCAGACAGTGGTCTTCTTTGTGAT  671
 G G L S P E L D N L  N S I R E I Q R P T  E I P D S G L L C D

TTGCTTTGGTCTGATCCTGATCAGAAGATT GAAGGGTGGGCTGATAGTGATCGAGGCATC TCTTGTACTTTTGGAGCTGATAAAGTCGCT  761
 L L W S D P D Q K I  E G W A D S D R G I  S C T F G A D K V A

GAGTTCTTGGATAAGAATGATCTTGACCTC ATTTGCCGTGGCCATCAAGTAGTGGAAGAT GGTTACGAGTTCTTCGCAAAAAGGAGATTA  851
 E F L D K N D L D L  I C R G H Q V V E D  G Y E F F A K R R L

GTGACGATATTCTCAGCTCCGAACTATGGT GGGGAGTTTGATAACGCTGGTGCATTACTG AGCGTGGACGAGTCTCTTGTTTGCTCTTTC  941
 V T I F S A P N Y G  G E F D N A G A L L  S V D E S L V C S F

GAGATTATGAAACCTGCCCTAGCTTCTAGC AGCGGACATCCTCTCAAGAAGGTGCCGAAA ATGGGGAAGTCTTAGTCCTCACTCTTAAGA 1031
 E I M K P A L A S S  S G H P L K K V P K  M G K S

TAACGATTCCATAGCTCAAGCAAGGCTCCA CATACGGACTTCCACTATGGTTTGAATGCA GTGAAGTACCCAAAGGGTCCTTAAATCATC 1121
ACCAAACAACTTCTGTATAAAAAAAAAAGAG TGATAGCAAAAAACACACTTCGTTTCCTGT CTTCTGCCTCTGGAGATTTGTACCTCCTTG 1211
GTAGTCTATTTTTCTTTCTTCTTTTAACCC TTTTAGTTTAAGCTAGAGTGTATCTCGTAG TTTTTTAGTTCTTGCCTGTTACTTTTTTCT 1301
TGTTCTACAAACCCCTTTGCATATCATCAA TGTACAACGTTTT                                                  1344
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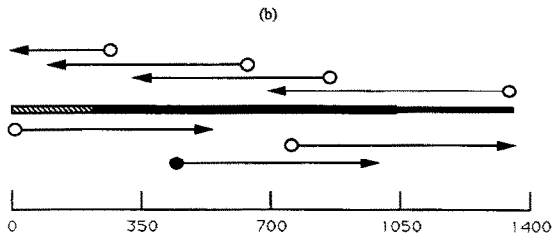

Fig. 1. *Brassica napus* protein phosphatase 1 (a) cDNA and deduced amino acid sequences of the catalytic subunit. The amino acid sequence begins at the start of the homology with mammalian PP1, the dashes indicate the untranslated start of the reading frame that is within the cloning artefact. (b) Strategy used to sequence the clones. The coding sequence is denoted by the thick filled bar and the non-coding region by a thin bar. The hatched bar represents a cloning artefact. The scale shows the nucleotide position from the 5′ end of the cDNA insert. The arrows indicate the direction and length of DNA sequences obtained. Using complete or partially deleted cDNA inserts, sequencing was initiated with specific oligonucleotide primers (●) or Bluescript primers (○) on the native or deleted clones.

also highly conserved in the plant PP1-and PP2A-like clones. In particular, 22 residues that are conserved in all protein phosphatases [7,21] are present in the deduced amino acid sequences of the plant clones except for a substitution (alanine to glycine) at position 185 in the plant PP1-like clone. This strongly suggests that these two plant clones represent plant homologues of PP1 and PP2A.

The plant PP1 showed 72% overall identity to rabbit PP1 but only 49% identity to rabbit PP2A excluding additions and deletions (Fig. 3). If conservative substitutions are taken into account, the overall similarity between the plant and mammalian PP1 is 82%. The plant PP2A showed 79% overall identity to rabbit PP2A and only 43% identity to rabbit PP1. The overall similarity between the mammalian and plant PP2A rises to 86% if conservative substitutions are included (Fig. 3). The structure of PP1 and PP2A have thus been highly conserved since the separation of plant and animal kingdoms. The plant PP1 also shows ap-

(a)

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TCGATTCCGACGGACGCAACTCTGGATCTC GATGAGCAGATCTCTCAGCTCATGCAGTGC AAGCCTCTCTCCGAGCAACAGGTTAGAGCA   90
 S  I  P  T  D  A  T  L  D  L   D  E  Q  I  S  Q  L  M  Q  C   K  P  L  S  E  Q  Q  V  R  A

TTATGCGAGAAAGCTAAGGAGATCTTGATG GATGAAAGCAATGTTCAGCCTGTCAAAAGC CCTGTGACAATCTGTGGTGATATTCATGGA  180
 L  C  E  K  A  K  E  I  L  M   D  E  S  N  V  Q  P  V  K  S   P  V  T  I  C  G  D  I  H  G

CAGTTCCATGATCTTGCAGAGCTTTTCCGT ATTGGAGGAATGTGCCCTGATACCAACTAT CTATTTATGGGTGATTATGTCGATCGTGGA  270
 Q  F  H  D  L  A  E  L  F  R   I  G  G  M  C  P  D  T  N  Y   L  F  M  G  D  Y  V  D  R  G

TATTATTCCGTCGAAACTGTTACGCTGTTA GTTGGCTTGAAAGTACGGTATCCTCAGCGA ATCACTATTCTTAGAGGAAACCATGAAAGT  360
 Y  Y  S  V  E  T  V  T  L  L   V  G  L  K  V  R  Y  P  Q  R   I  T  I  L  R  G  N  H  E  S

CGTCAGATTACTCAGGTGTATGGATTTTAT GATGAATGCCTGCGCAAGTATGGTAATGCG AATGTTTGGAAGTATTTTACTGACCTCTTC  450
 R  Q  I  T  Q  V  Y  G  F  Y   D  E  C  L  R  K  Y  G  N  A   N  V  W  K  Y  F  T  D  L  F

GACTATCTTCCACTGACAGCCTTGGTGGAA TCGGAAATATTCTGCCTTCACGGTGGTTTG TCACCGTCTATCGAGACCCTTGACAACATT  540
 D  Y  L  P  L  T  A  L  V  D   S  E  I  F  C  L  H  G  G  L   S  P  S  I  E  T  L  D  N  I

AGGAACTTTGACCGAGTTCAAGAAGTTCCA CATGGAGGACCGATGTGCGACTTACTATGG TCTGATCCTGATGACAGATGTGGGTGGGGA  630
 R  N  F  D  R  V  Q  E  V  P   H  G  G  P  M  C  D  L  L  W   S  D  P  D  D  R  C  G  W  G

ATCTCTCCTCGTGGTGCCGGATATACATTT GGTCAGGACATTTCGAACCAATTCAACCAC AGCAACAGCTTAAAACTTATCAGTCGAGCG  720
 I  S  P  R  G  A  G  Y  T  F   G  Q  D  I  S  N  Q  F  N  H   S  N  S  L  K  L  I  S  R  A

CATCAACTGGTTATGGATGGTTACAACTGG GCACATGAGGCCAAAGGTGGTACTATTTTC AGTGCCCCAAACTATTGTTATCGTTGTGGA  810
 H  Q  L  V  M  D  G  Y  N  W   A  H  E  A  K  G  G  T  I  F   S  A  P  N  Y  C  Y  R  C  G

AACATGGCCTCTATTCTTGAGGTCGATGAC TGCAGAAACCACACCTTCATTCAGTTTGAA CCAGCCCCAAGGAGAGGAGAACCAGATGTG  900
 N  M  A  S  I  L  E  V  D  D   C  R  N  H  T  F  I  Q  F  E   P  A  P  R  R  G  E  P  D  V

ACCCGAAGGACACCTGACTACTTCCTATAA ACAAAACTCACCTCCTCTCCAGCTGCAAAG TCCGGTTGTTGGTTTTTTGAAGATCTCTGG  990
 T  R  R  T  P  D  Y  F  L
CTTATTCCATTTGCAACGCCTTGTCTTCTG AGTGAGGTTGCGTTTCTTGAAATCGATTTA GCCTTCTACCTTGGAATAGTGTAATATAGG 1080
CAATGAGGTCAATGAACTATAAAACCTAGT CCCATGAATGTTTGTGTTTGGCTGAAATTA TTTAGCTCCCTTCTTGCATCAGAATCTATT 1170
TCTTCTCAGATATTATTTTGACTTAATTTC ATGTTAATTCAATTTATGCTCGTGCCGCTC GTGCCG                          1232
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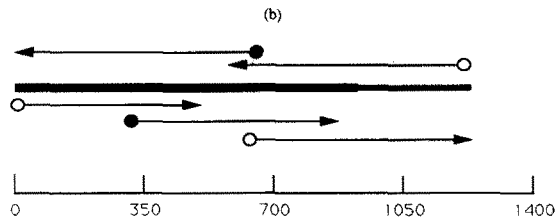

Fig. 2. *Brassica napus* protein phosphatase 2A (a) cDNA and deduced amino acid sequences of the catalytic subunit. (b) Strategy used to sequence the clones. The coding sequence is denoted by the thick filled bar and the non-coding region by a thin bar. The scale shows the nucleotide position from the 5′ end of the cDNA insert. The arrows indicate the direction and length of DNA sequences obtained. Using complete or partially deleted cDNA inserts, sequencing was initiated with specific oligonucleotide primers (●) or Bluescript primers (○) on the native or deleted clones.

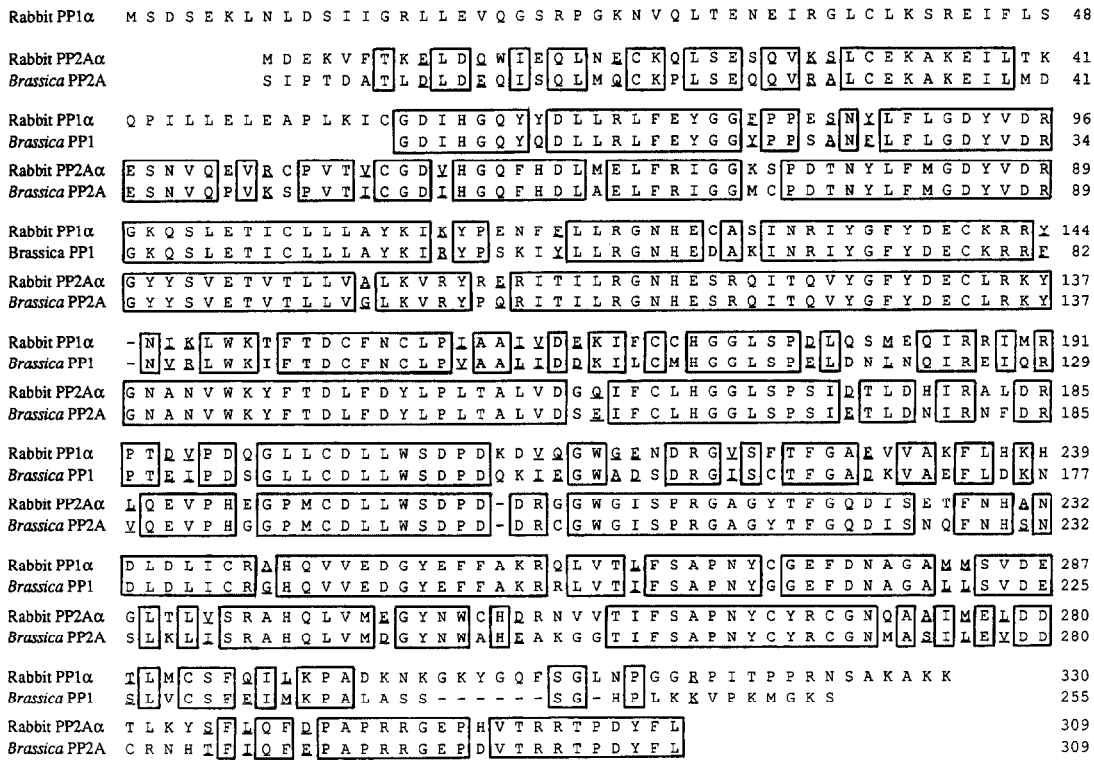

Fig. 3. Comparison of primary structures of *Brassica napus* and rabbit protein phosphatases. *B. napus* PP1 is compared with rabbit PP1α, while *B. napus* PP2A is compared with rabbit PP2Aα. Identities are boxed and conservative substitutions are underlined.

proximately 70% sequence identity to PP1 from *Drosphila melanogaster* [12], *Aspergillus niger* [13], *Schizosaccharomyces pombe* [14,15], and *Saccharomyces cerevisiae* [14], while the plant PP2A shows approximately 80% sequence identity with PP2A from *D. melanogaster* [16] and *S. cerevisiae* [17].

Table I

Comparison of the rates of change of plant PP1 and PP2A with those of other plant proteins. Plant proteins have been compared with the counterpart in the animal kingdom

	Millions of years for 1% change
Histone H4	500
Histone H3	330
Calmodulin	100
α-Tubulin	59
Ubiquitin	50
PP2A	48
PP1	36
Histone H2A	33
GAPDH	27
Cytochrome *c*	23
Aldolase	22

Proteins considered to be highly conserved in the different kingdoms have been chosen: they are maize and rabbit aldolase [22], wheat and rabbit calmodulin [23], wheat and rabbit cytochrome *c* [24], tobacco and rat cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [25,26], wheat and rat histone H2A [27,28], pea and calf histone H3 and H4 [29], *Arabidopsis thaliana* and human α-tubulin [30], barley and human ubiquitin [31], *Brassica napus* and rabbit PP1 and PP2A, this study. The divergence of the plant and animal kingdoms is taken to be 1000 million years [18]. Although the precise values of the rates of change differ from those calculated when mammalian and *Drosophila* PP1 and PP2A are compared [32] the overall order of the conserved proteins is very similar.

The time for a 1% change in amino acid sequence can be calculated to be approximately 36 and 48 million years for PP1 and PP2A, respectively, if the time of divergence of the plants and animals is taken to be 1000 million years ago [18]. If the rate of change of the plant protein phosphatases is compared with that of other plant proteins it can be seen that the protein phosphatases have a rate of change slower than the cytosolic enzymes glyceraldehyde-3-phosphate dehydrogenase and aldolase, the electron carrier cytochrome *c*, and also the structural protein histone H2A (Table I). This very slow rate of change suggests that any major changes in the structure of the protein phosphatases would be highly detrimental to any eukaryotic organism.

Although only four classes of serine/threonine protein phosphatase have been detected in eukaryotic cells by biochemical methods, cDNA cloning has revealed several novel protein phosphatases, namely PPV, PPX, PPY, and PPZ [20]. Whether they encode protein phosphatases with specialized functions is not yet known. The plant PP1 and PP2A isolated here are more similar to rabbit PP1 and PP2A, respectively, than they are to the novel protein phosphatases. It is not yet known if PPV, PPX, PPY and PPZ are present in plants. If not, it may be that they perform animal-specific functions.

Two isoforms of PP1, namely PP1α and PP1β, have been found in rabbit [19] and mouse [14] and three in *Drosophila* (PP1α_1, PP1α_2 and PP1β) [12,19]. The characteristic amino acid differences between PP1α and PP1β are preserved in all three organisms, implying that these amino acids confer distinct biological functions [19]. However, *B. napus* PP1, *A. niger* bim G, *S. pombe* dis 2^+/bws 1^+ and *S. cerevisae* DIS2S1 contain 'marker' amino acids for both the α and β isoforms. Thus, plants and lower eukaryotes do not share the same specialisation of function of PP1α and PP1β isozymes which appears to occur in multicellular animals.

The high degree of identity exhibited by the plant and animal PP1 and PP2A sequences not only supports the observed remarkable similarities in catalytic properties [2] but also suggests that the surface regions involved in the binding of regulatory subunits are also conserved. Indeed, the catalytic subunits of PP1 and PP2A are complexed to other proteins in extracts of plant tissues (R.W. MacKintosh, unpublished results). Investigation of whether these other subunits have important roles in determining the subcellular location and modulation of the specificity and regulatory behaviour of these enzymes, as in animals [1], will be a prerequisite to understanding the control of dephosphorylation processes in plants. Since the structures of the protein phosphatases from plant and animals are very similar it is expected that the study of signal transduction in plants may benefit from molecular genetic analyses of protein phosphatases in animals.

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