Phosphoprotein Phosphatase Genes of Arabidopsis thaliana

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Phosphoprotein phosphatases are highly conserved enzymes found in fungous and animal cells. These enzymes have multiple functions in those cells, but little is known about phosphoprotein phosphatases in plants. We have now studied what kinds of and how many phosphoprotein phosphatase genes are present in a model higher plant, Arabidopsis thaliana, by DNA hybridization, polymerase-chain-reaction, and gene cloning procedures. Genomic Southern-blot hybridization analysis suggested that the A. thaliana genome contains multiple copies of genes encoding serine/threonine-specific phosphoprotein phosphatases similar to those of fungi and animals: five to eight copies of the type 1 phosphatase (PP1) genes, two to three copies of the type 2A phosphatase (PP2A) genes, and probably one copy of the type 2C phosphatase (PP2C) gene. Besides, a putative tyrosine-specific phosphoprotein phosphatase gene appeared to exist, being considerably diverged from those of fungi and animals. Among these genes one PP1 cDNA and one PP2A cDNA were isolated from an A. thaliana cDNA library, and their sequences were determined and characterized.

KEY WORDS: Protein phosphatase / Signal transduction / Higher plant / Phosphorylation / Dephosphorylation

1. INTRODUCTION

Protein phosphorylation is a central regulatory mechanism for cellular functions in all eucaryotic cells.1-4 Over the past decade it has become apparent that phosphoprotein phosphatases are as important as protein kinases in a variety of control mechanisms including signal transduction, metabolism, growth, and differentiation (for reviews see refs. 5 and 6). With regard to the substrate specificity, phosphoprotein phosphatases are categorized into two kinds, serine/threonine (Ser/Thr)-specific and tyrosine (Tyr)-specific phosphatases.

Ser/Thr-specific phosphatases are further divided into two types, type 1 (PP1) and type 2 (PP2) that are sensitive and insensitive, respectively, to the cellular inhibitors 1 and 2. The PP2 class of phosphoprotein phosphatases is subdivided into three groups termed PP2A, PP2B, and PP2C from their divalent cation dependency. PP2A requires no divalent cation for its activity, while PP2B and PP2C need Ca²⁺/calmodulin and Mg²⁺, respectively. All these phosphoprotein phosphatases except for PP2C appear to exist in a cell as hetero oligomers that consist of catalytic subunits (PP1c, PP2Ac, or PP2Bc) and regulatory subunits.⁵ The primary structure of the catalytic subunits belonging to the same subfamilies is highly conserved among various organisms.⁵ Furthermore, the catalytic subunits of the different subfamilies are significantly similar to one another. For instance, the amino acid sequence of PP1c is about 50% and 40% identical to those of PP2Ac and PP2Bc, respectively.⁵

Tyr-specific phosphatases (PTP) are classified for their architecture into two types, the
membrane-spanning receptor-type and the non-receptor-type, the catalytic domain of which is high
gly conserved between two types and over species.\textsuperscript{5} In addition, there is a novel type of
PTP, Cdc25, that specifically dephosphorylates the cell-cycle-controlling Cdc2 kinase.\textsuperscript{6} The
similarity between Cdc25 and typical PTP is limited within very short regions probably
essential for their activity.\textsuperscript{7}

Biochemical and structural characterization of phosphoprotein phosphatases described
above has been carried out chiefly with animals and more recently with fungi. Particularly,
distinct evidence has been obtained exclusively with fungi, showing that PP1 and PP2A are
essential because of their fundamental functions such as the regulation of cell division.\textsuperscript{8,9} By
contrast, little is known about phosphoprotein phosphatases in plants except for the pre-
ence in several plant species of phosphatase activities similar to PP1, PP2A, and PP2C of
animals and fungi. Neither PP2B-like nor PTP-like activities have been detected in plants so
far examined.\textsuperscript{10-12} As a step toward shedding a light on features underlying cascades of pro-
tein phosphorylation/dephosphorylation in higher plants, we have now studied what kinds of
and how many phosphoprotein phosphatase genes are present on the \textit{Arabidopsis thaliana}
chromosome using DNA hybridization, polymerase-chain-reaction (PCR), and gene cloning
procedures.

2. MATERIALS AND METHODS

\textit{General methods.} As described were procedures for transformation with \textit{Escherichia coli}, pre-
paration of plasmid and phage DNA, DNA cleavages with restriction endonucleases, repair
synthesis, ligation, \textsuperscript{32}P-labeling of DNA with random primers, gel electrophoresis with agar-
ose and polyacrylamide, and gel blotting to a membrane filter.\textsuperscript{13}

\textit{DNA sequencing.} Long DNA fragments to be sequenced were separately inserted into the
M13mp18 vector,\textsuperscript{14} and their unidirectional deletions were generated by cleavages with re-
striction endonucleases. Each deletion derivative was sequenced by the chain-termination
method with T7 DNA polymerase (United States Biochemical Corp.).\textsuperscript{15} Sequencing of short
DNA fragments were done by cloning in the pUC18 vector.\textsuperscript{15}

\textit{Polymerase-chain-reaction (PCR).} Oligodeoxyribonucleotides used for PCR primers were synthe-
sized by a Beckman System 1 Plus DNA Synthesizer. Their nucleotide sequences were
5'-AAGTACCC (T/A) GAGAACTTCTT-3' (forward) and 5'-TC (A/G) TCGAACTC (A/T) CC
(A/G)CAGTA-3' (reverse) that correspond to internal portions of a rabbit PP1 (KYPENFF
at amino acid residues 113-119 and YCGEFDN at amino acid residues 272-278).\textsuperscript{16,17} Other sets
of primers specific for PTP were 5'-GG(A/T)GACTTCTGCGTATG-3' (forward), 5'-CCAGC
(A/G)GA (A/G)CA (A/G)TGAC-3' (reverse 1), and 5'-GCAGCGGATGAAGATGGTTC-3'
(reverse 2) that correspond to internal portions of human LAR (GDFWRM at amino acid re-
sidues 1406-1411 and VHCSAG at amino acid residues 1520-1525) and \textit{Schizosaccharomyces
pombe pph1} (QRMKMV at amino acid residues 517-523), respectively.\textsuperscript{18,19} PCR reaction mix-
tures (total volume of 100 \(\mu l\)) contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM
MgCl₂, 0.01% gelatin (w/v), 0.1 \(\mu g\) of an \textit{A. thaliana} cDNA mixture, 0.2 mM each of the
four dNTPs, 2.5 units of \textit{Thermus aquaticus} DNA polymerase (Takara Shuzo Co.), 50 pico-
moles each of the forward and the reverse primers. The reactions were done by two consecu-
tive modes in a thermal cycler (Perkin Elmer/Cetus Corp.): the first mode was 10 cycles of 1
min at 92°C (denaturation), 1 min at 42°C (annealing), and 1 min at 70°C (extension); and the
second mode was 25 cycles under the same conditions except annealing temperature at 54°C.

Cloning of PCR fragments for PP1. After treatment with 0.4 units/ml of Klenow fragment of
DNA polymerase I (Takara Shuzo Co.) at 37°C for 30 min, PCR reaction products were
separated by polyacrylamide gel electrophoresis. DNA fragments of about 480 base-pair (bp)
long were eluted from the gels, and then inserted at the HindII site of pUC18.

Isolation of plant DNA and RNA. The plant used was Arabidopsis thaliana (Columbia ecotype).
Its cultivation was done under the standard growth conditions at 22°C where 18-h illumina-
tion and 6-h darkness alternated. Plant DNA and RNA were isolated from whole tissues us-
ing cetyltrimethylammonium bromide, and poly(A)⁺ RNA was separated from poly(A) RNA
through oligo(dT)-cellulose (Pharmacia) column chromatography.

Yeast and animal phosphoprotein phosphatase clones. The following clones were used as probes
in Southern-blot hybridization and library screening experiments. PP2A, the 1.2-kilobase-pair
(kbp) EcoRI fragment of Brassica napus PP2A cDNA from pKS-M13′PP2A; PP2C, the 2.35
kbp Smal fragment of rat PP2C cDNA from pST-1121; pyp1, the 1.5 kbp PCR-amplified cDNA
of S. pombe pyp1 (corresponding to the amino acid residues 24-523); cdc25, the 0.95 kbp
BamHI-BglII fragment of pCDC25; and HPTP a, the 2.7 kbp BamHI-HindIII fragment of
psp65/HPTP a. respectively.

Membrane hybridization. Four conditions of different stringency (high, moderate, low, and
poor stringency) were used for hybridization. Under the high-stringency conditions, hy-
bridization was performed at 42°C for 20 h in a solution containing 50% formamide, 5X
SSC (1 X SSC = 150 mM NaCl-15 mM sodium citrate), 5 X Denhardt's solution, 0.1 mg/ml
calf thymus DNA, 0.5% SDS, and a probe DNA (about 5 X 10⁶ cpm/μg of DNA). The filter
was washed at room temperature, twice for 30 min in 2X SSC-0.1% SDS, once for 30 min at
room temperature in 0.1X SSC-0.1% SDS, and once for 30 min at 65°C in the same solution.
For three other conditions, the formamide concentration and the last washing conditions were
changed as follows: moderate stringency, 40% formamide, washing once at 50°C in the same
solution; low stringency, 20% formamide, washing once at room temperature in the same
solution; and poor stringency, 10% formamide, washing twice at room temperature in 0.5X
SSC-0.1% SDS.

Northern-blot hybridization. Plant total RNA was fractionated on a 1.2% agarose gel containing
1.8% formaldehyde with running buffer of 20 mM MOPS Good's buffer (pH 7.0)-5 mM
EDTA-8 mM sodium acetate that was previously treated with diethyl pyrocarbonate. RNA
was transferred to an Immobilon-N PVDF membrane filter (Millpore Corp.), and hybridiza-
tion was carried out under the conditions of moderate stringency. The autoradiograms were
generated by a Fujix BA 100 Bio-Image Analyzer (Fuji Photo Film).

Screening of cDNAs encoding PP1 and PP2A. An A. thaliana cDNA library constructed with
the λgt101 vector was screened under the hybridization conditions of either high or low
stringency.

Transformation of A. thaliana root explants. A. thaliana root explants were prepared by the
standard procedures, and then infected with Agrobacterium tumefaciens LBA4404 carrying
the pBI211 binary vector or its derivatives. Km⁺ transformant calli were selected, and then
mature plants were regenerated as described.
3. RESULTS

*Type 1 phosphoprotein phosphatase genes of A. thaliana*

To see if the *A. thaliana* genome contains PP1-like genes, PCR was run on a mixture of cDNA derived from whole plant tissues. The set of PCR primers used had degenerate sequences corresponding to the two highly conserved regions of PP1 (see Materials and methods). Gel electrophoresis of the PCR products gave the major band at about 480 bp position that roughly coincides with the size predicted from the primer locations (493 bp). These PCR fragments were inserted into pUC18, and the resulting clones were sequenced. Two out of them (PCR-1 and PCR-2) contained open reading frames intimating phosphoprotein phosphatases. Their putative translation products were close to each other (82% identity), and also to the corresponding positions of member enzymes of animal and fungous PP1c (78%-80%; see below). Thus these two PCR fragments appeared to be portions of *A. thaliana* PP1 cDNAs. To know the entire structure of PP1, an *A. thaliana* cDNA library constructed with the λgt10 vector was screened with a PCR-2 probe. From about $5.5 \times 10^5$ recombinants, six positive clones were obtained and sequenced. The longest clone contained an open reading frame of 957 bp, predicting a protein of 318 amino acid residues (35 kDa) (Fig. 1-A). The amino acid identity in its translation product with animal and fungous PP1c, PP2Ac, and PP2Bc occurred at 80%, 45%, and 40%, respectively, as shown in Fig. 2. The hydropathy profile revealed this protein is a soluble one (Fig. 3). These results implied that the cloned DNA fragment is derived from an *A. thaliana* counterpart of PP1c, and thereby named AT-PP1. Northern-blot hybridization analysis using the PCR-2 segment as a probe and total RNA from whole tissues gave a unique hybridization signal at about 1.3-kilobase (kb) position (Fig. 4). Therefore, it seems that the majority of AT-PP1 transcripts are matured to mRNA in a unique manner, probably without alternative splicings.

*Existence of a multi-gene family of type 1 phosphoprotein phosphatase*

To find out whether AT-PP1 gene homologs are dispersed over the *A. thaliana* genome or not, genomic Southern-blot hybridization analysis (*BamHI* and *EcoRI* digests) was done with PCR-1 and PCR-2 probes. Under the conditions of high stringency, each probe made one or two specific DNA fragments visible. Under the conditions of low stringency, on the other hand, several additional weak bands were detected. One such band probed with PCR-1 corresponded to the clear band probed with PCR-2 under the high-stringency conditions, and vice versa (Fig. 5-A). These results support the view that the *A. thaliana* genome contains multiple genes of PP1 with structural variations: presumably five to eight copies of PP1 genes including pseudogenes if any.

*Type 2A phosphoprotein phosphatase genes of A. thaliana*

Although an attempt was made to isolate PP2A cDNA clones by the PCR method as in the case of PP1, no candidate clone was obtained. As a next effort, using a truncated cDNA clone derived from the putative PP2A gene of *B. napus* as a probe, genomic Southern-blot hybridization analysis (*BamHI* and *EcoRI* digests) was done under the three different conditions. Although no signal band appeared under the high-stringency conditions, a unique signal band was detected under the moderate-stringency conditions, and two or more signal
bands under the low-stringency conditions (Fig. 5-B). These results are interpreted as that at least two to three copies of PP2A-like genes are present on the A. thaliana genome.

To confirm it, an A. thaliana cDNA library was screened under the conditions of low stringency with the B. napus partial PP2A cDNA probe. By screening about $5.5 \times 10^5$ recombinants, four positive clones were obtained. From their nucleotide sequence analysis, it was found that the longest clone (787 bp insert) had an open reading frame whose translation product was 93% identical to that of the used probe, and about 80% identical to fungous and animal PP2Ac members (Fig. 1-B and Fig. 2). This product also shared about 50% identity with PP1c members and 45% identity with a rat PP2Bc enzyme. Therefore, this product is likely to be an A. thaliana PP2A homolog (AT-PP2A).

Other types of phosphoprotein phosphatase genes of A. thaliana

To know if the A. thaliana genome contains type 2C phosphatase gene homologs, we carried out genomic Southern-blot hybridization analysis with a rat PP2C cDNA probe (Fig. 5-C). Only under the conditions of low stringency we detected a unique hybridization signal, intimating there is at least one PP2C-like gene in the A. thaliana genome.

The presence or absence of tyrosine-specific phosphoprotein phosphatases (PTP) was tested by the PCR method using various sets of PCR primers corresponding to the well-conserved regions among fungous and animal PTP members (see Materials and methods). However, no PCR fragment encoding PTP enzymes was produced. As a next effort, using yeast pyp1, yeast cdc25, and human PTPa clones as probes, genomic Southern-blot hybridization was done as above. We detected faint signal bands only with the yeast probes and only under the hybridization conditions of poor stringency. In addition, both yeast probes visualized a common restriction fragment as can be seen in Fig. 5-D. Since the similarity between pyp1 and animal PTPs is slightly higher than that between cdc25 and animal PTPs,6 these results imply that the observed signal is derived from a novel type of PTP, a cdc25 homolog, and that the A. thaliana genome contains no gene encoding typical types of PTP enzymes, though considerably diverged PTP-related genes may be present.

Trial for constructing transgenic plants carrying an antisense AT-PP1 gene

A large portion of AT-PP1 cDNA (corresponding to amino acid residues 19-221) was cloned into pBI121 in the antisense orientation under control of the cauliflower mosaic virus 35S promoter, and the resulting plasmid was named pBI121-PP1. A. thaliana root explants were infected with LBA4404(pBI121-PP1) and LBA4404(pBI121), and Km' transformant calli were regenerated to mature plants. The transformation efficiency and degree of greening of calli were almost the same for both strains. However, shoot induction from calli occurred at a significantly lower efficiency with pBI121-PP1 than with pBI121. Almost all calli derived from pBI121-PP1 represented abnormal morphology showing strikingly tufty hairy roots, and their green portions gradually disappeared without further shoot induction (data not shown). We obtained only two regenerated mature plants after extensive efforts. Therefore, overexpression of the antisense AT-PP1 gene appeared toxic to plants, and the regenerated plants might be silent upon expression of the introduced gene.
M. AOKI and A. OKA.

(A) 1 A AAT CTC CTT TCG TTT TCT ACC TGG TTT AGA TCG GAG ATG GCG GAG 46
1 M A E 3

47 AAG CCC GCG CAA GAG CAA GAG CAA GAG CAG CGG ATG GAA CCA GCT 94
4 K P A Q E Q E Q K R A M E P A V 19

95 CTC GAC GAT ATT ATT CTT CTT TGG GAG TTT CGG AAC AGC AGA CCT 142
20 L D D I I R R L V E F R N T R P 35

143 GGA TCG GGG CAA GGT CAT CTC AGT GAA GGT GAA GCT GAG CTT 190
36 S G M K Q V H L E G E I R Q L 51

191 TGT GCT GTC TCC AAA GAA AYA TTT CTT CAA GAG CCG AAT CTT GCT GAA 238
52 C A V S K E I F L Q Q P H N L 152

239 TGG TAA OCT CCC ATC AGG TGG GAT GAA GCT GCT OCT GAA GCT OCT AAT 286
68 L E A P I K I C G D I H G Q S 83

287 GAT CTA TGG AGG CTT TTT GAT TAA GGA GGA TGG GCC CTT OCT COT CAA 334
94 D L L R L F E Y C G F P P E A N 117

335 TAT TGG TCC TGG GAT GAT TAT GAT GAT GCT GAT GAA CAA GCT GAA 382
100 T Y L F L G D I V D R G K Q S L E 158

383 GCA ATA TOT CTT CTA GTC TAC AAA ATC AGG TAC CTT GAG AAC TCC 430
116 T I C L L A Y K I K Y P E F 131

431 TCG TCG TGG AGA GGG CAA GAT GAA CTA GCC GCG AGA CCT 478
132 F L E A P I S A S S S N R I Y 147

479 GGT TTC TAT GAT GAG GGG TCC AAA CCC AGG TTC AAT GCT ACA CTC TGG AAA 526
148 G F Y D E C K R R F N V R L W K 163

527 ATA TCC ACC GAT TGG TTT AAG TGT CT TCT GGC GCC TTA ATT GAT 574
164 I P T D C F W N C L P V A A L I D 179

575 GAC AGA ATA CTA TOT ATG CAT GGT GGG GAA TGT TAA GGT GAG AAT 622
180 D R I L C M H G G I S F E L K S 195

623 TGG GAC CAG ATT AGA AAT GTT GCA CCG AGG GAT GAT GGG TAC TTT GCT 670
196 L D Q I R N I A R P M D I P E S 211

671 GGT TTT GAT TAA TGT TAT GAT TAA CTA TGG GAT GCA GAT TTA AGAG 718
212 G L V C D L L W S D P S G D V G 227

719 TGG GCC AGG AAT GAT GTT GTC TAC ATT TTG GAT GAC AAG 766
228 W G H N D R G V S Y T F G A D K 243

767 GTC GCA GAG TTC TGG GAG AAA CAT GAC AAG AAC GCT CTT AGC TCT GCT GGC 814
244 V A E F L E K H D M L I C H A 259

815 CCC AGG GCT GCT GAA GAT GGG TAT GAG TTC TTT GCA GAA AGA CAA CTT 862
260 H H Q V V E D G Y E K F F A K R Q L 275

863 GTT ACA GTA TTT TCA GCC ACC AAC TAC TGG GAA GAA TGC AAG CCT 910
276 V T V F S A P N Y C G E F D N A 291

911 GCG GCA AAG ACG TCA GAA AGG AAA TGG TCT TTA AGG AGC TTA AGT TCA GCC AAT 958
292 G A M M S I S L M C S F Q I 307

959 CTA AAG CCC TCA GAA AGG AAG TGG CTT CTT AAT CTA AAG CTA TTA TCC 1006
308 L K F S E K K S P F L * 319

1007 AGT GAC CA

(300)
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Fig. 1. Nucleotide sequences and the deduced amino acid sequences of AT-PP1 (A) and AT-PP2A (B) cDNA, respectively. The amino acid sequences are given below the nucleotide sequences. The LRGNHE and RAH regions are shaded, respective potential phosphorylation sites and the corresponding kinases are underlined and cited, and a potential Asn-glycosylation site is boxed. CKII, casein kinase II; PKC, protein kinase C.
Fig. 2. Amino acid sequence comparison of Ser/Thr-specific phosphoprotein phosphatases. From upper to lower rows, rabbit PP1α,\(^a\) S. pombe dis2 (PP1),\(^b\) AT-PP1, PCR-1 translation product, human PP2Aα,\(^c\) S. pombe ppa2 (PP2A),\(^d\) AT-PP2A. The regions for PP1 PCR primers are underlined. Asterisks under the sequences represent highly conserved amino acid residues among PP1 and PP2A enzymes, and hyphens represent spacing for the best match. The LRGNHE and RAH regions and the RRTPDYFL region are indicated by shading and wavy lines, respectively.
Fig. 3. Hydropathy profile of AT-PP1. Positive and negative values indicate hydrophobic and hydrophilic characters, respectively.
Fig. 4. Northern-blot hybridization analysis of AT-PP1 mRNA. Total plant RNA (lane 1, 0.8 μg RNA; lane 2, 4.0 μg) was fractionated by gel electrophoresis, blotted to a membrane filter, and hybridized with the 32P-labeled PCR-2 segment. The position of ribosomal 28S and 18S RNAs are indicated by arrowheads.

Fig. 5. Genomic Southern-blot hybridization analysis of the A. thaliana phosphoprotein phosphatase genes. A. thaliana DNA (5 μg/lane) was digested with BamHI (lane B) or EcoRI (lane E) followed by gel electrophoresis and then by blotting. The probes used are indicated at the top. HIGH, MODE, LOW, and POOR are the high, moderate, low, and poor stringency for hybridization, respectively (see Materials and methods). The band positions of the λ HindIII fragments are indicated at the left for a size marker.
Type 1 phosphoprotein phosphatases of A. thaliana

The extremely conserved regions common to PP1c, PP2Ac, and PP2Bc are LRGNHE of residues 134–139 and RAH of residues 258–260, and are presumed to play critical roles in the phosphatase activity and/or interaction with regulatory subunits. In fact, a yeast dominant-negative mutant (dis2-11) contains a substitution by Q for R in the RAH sequence. AT-PP1 completely conserved all of these sequences, implying that AT-PP1 actually encodes an A. thaliana Ser/Thr-specific phosphoprotein phosphatase.

From motif analysis of AT-PP1 with the PROSITE program, we found SEGE (at amino acid residues 44–47) and SIDE (at amino acid residues 296–299) for potential casein kinase II targets, and SGK (at amino acid residues 37–39) and SEK (at amino acid residues 311–313) capable of being phosphorylated by protein kinase C. Since the former two sequences are preserved among PP1 member enzymes, the PP1 activity itself is likely to be regulated through phosphorylation by Ser/Thr-specific protein kinases.

Genomic Southern-blot hybridization analysis with the PP1 PCR segments indicated that five to eight PP1 genes including pseudogenes if any, are present on the A. thaliana genome, constituting a multi-gene family. Rabbit and yeast (S. cerevisiae and S. pombe) have only two PP1 loci, and Drosophila melanogaster contains three PP1 loci. Therefore, PP1 genes seem to generally comprise a multi-gene family. It is known that two S. pombe PP1 genes (dis2 and sds21) have overlapping functions: a mutation within either gene is viable, while their simultaneous disruptions lead to defect in sister chromatid separation. By analogy, multi PP1 genes of A. thaliana presumably have overlapping functions critical for proliferation of plant cells. During the preparation of this manuscript, clonings of A. thaliana PP1 genes including the same one as described here have been reported, and one of them has been shown to complement the S. pombe cold sensitive dis2-11 mutation.

Type 2A phosphoprotein phosphatases of A. thaliana

Genomic Southern-blot hybridization analysis with a B. napus partial PP2A cDNA probe revealed the existence of two to three copies of PP2A genes in the A. thaliana genome. Similarly, S. pombe has two PP2A genes (ppa1 and ppa2) highly homologous to each other, and also to the two genes of rabbit PP2A. Their double disruptions result in the lethal phenotype, while either disruption does not, as in the case of the PP1 genes. However, no overlapping has been observed between PP1 and PP2A functions.

We isolated a cDNA clone similar to B. napus and mammalian PP2A genes. Its motif analysis revealed the LRGNHE and RAH regions characteristic of Ser/Thr-specific phosphoprotein phosphatases are completely conserved as in AT-PP1, intimating that this clone (AT-PP2A) is derived from one of A. thaliana PP2A genes. AT-PP2A contains a conserved sequence of KIFTDLFDY at amino acid residues 144–152 that is a potential target of protein tyrosine kinase as predicted by the PROSITE analysis. Besides, there are a possible Asn-glycosylation site at amino acid residues 229–232 and latent target sites for both casein kinase II and protein kinase C (at amino acid residues 201–204 and 212–214, respectively). Another conserved sequence, RRTPDYFL at the C-terminal portion, though lacking in the AT-PP2A clone, contains a
probable target of protein tyrosine kinase 30) (Fig. 2). Thus, the AT-PP2A activity as well as AT-PP1 seems to be controlled by post-translational modifications including phosphorylation. Type 2B and 2C phosphoprotein phosphatases of A. thaliana

Biochemical analyses with B. napus seed extract, wheat and pea leaf extracts, and carrot suspension culture have failed to identify PP2B-like enzymatic activities.10-12) Genomic Southern-blot hybridization analysis with a rat PP2C probe indicated the presence of one PP2C gene on the A. thaliana genome (Fig. 5-C). PP2C is distinctive from other protein phosphatase families not only structurally but also characteristically: it acts as a monomer, being insensitive to okadaic acid.5) Thus PP2C member enzymes may have quite different category of functions in cells.

Tyrosine-specific phosphoprotein phosphatases of A. thaliana

Though in animal cells reversible phosphorylation/dephosphorylation on tyrosine residues has been thought to play critical roles in the control of cellular functions, plants and yeast seem to carry no protein tyrosine kinase genes similar to those of animals.13) Since the cell-cycle-controlling Cdc2 kinase (or its structural gene) whose activity is modulated by phosphorylation/dephosphorylation on its Tyr-1535) has been identified in several plant species,19,36-38) Tyr-specific kinase and Tyr-specific phosphatase for the Cdc2 kinase (yeast Weel and Cdc25 equivalents, respectively) should also be present in plant cells. We provided evidence to support this prediction that genomic Southern-blot analysis with either yeast pyp1 or cdc25 probe identified the same DNA fragment (Fig. 5-D). However, a human receptor type PTP probe did not visualize A. thaliana homologs in genomic Southern-blot hybridization analysis, nor were PCR fragments similar to animal PTP enzymes obtained. Therefore, the observed signal probed with pyp1 and cdc25 appears to correspond to the A. thaliana counterpart of cdc25. The results further support the view that A. thaliana PTP gene(s) are structurally diverged from those found in fungi and animals.

5. CONCLUSIONS

We revealed the presence of several types of A. thaliana phosphoprotein phosphatase genes similar to fungous and animal counterparts, being consistent with enzymatic activities previously identified in higher plants. The isolated clones described here provide a useful tool for functional analysis of PP1 and PP2A by over- and under-expressing the PP1 and PP2A genes in transgenic plants, through which it is expected to obtain new insight into the molecular mechanisms underlying protein phosphorylation/dephosphorylation regulation.

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