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Molecular Characterization of a Protein Kinase Gene in Chinese Cabbage (*Brassica campestris* subsp. *napus* var. *pekinensis*)

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Key Words:
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Random sequencing of expressed sequence tags in roots of Chinese cabbage led to isolation of a partial cDNA clone, BR77, which encoded a putative protein kinase. Using the BR77 cDNA as a probe, we isolated a full-length cDNA encoding the *Brassica campestris* protein kinase 1 (Bcpk1). The *Bcpk1* cDNA contained one open reading frame encoding a polypeptide of 439 amino acids. The putative polypeptide consisted of a short N-terminal region and a protein kinase catalytic domain. The catalytic domain of Bcpk1 showed a high homology to cAMP- and calcium-phospholipid-dependent subfamilies of serine/threonine protein kinases. Eleven major catalytic domains in protein kinases were well conserved in Bcpk1. However, Bcpk1 contained a unique nonhomologous intervening sequence between subdomains VII and VIII, which was not found in protein kinases of animals and lower eukaryotes. Genomic DNA gel blot analysis showed that *Bcpk1* genes might be present as three copies in the Chinese cabbage genome. These imply that Bcpk1 belongs to a plant-specific serine/threonine protein kinase subfamily.

Plants have many unique developmental processes such as regeneration potential of single cells, indeterminate growth, germination, seed development, and photomorphogenesis (Goldberg, 1988). Such processes in plants seem to be controlled by reversible protein phosphorylations, which are known to be predominant strategies used to control the activity of proteins in signal transduction pathways (Bowler and Chua, 1994). Protein kinases are very important key molecules because they are essential regulators in the signal transduction pathways and play a pivotal role counteracting with protein phosphatases. Phosphorylation of a target molecule by a protein kinase leads to transfer of information to a downstream cascade, and dephosphorylation by a protein phosphatase works *vice versa*. Therefore, isolation and characterization of genes involved in the signal transduction pathways during the developmental processes are basic prerequisites for the study on the developmental processes at the molecular level.

In animal and lower eukaryotic systems, it has been reported that receptor molecules spanning the cell membrane perceive external signals (reviewed by Cohen, 1992; Drayer and van Haastert, 1994). Upon ligand binding, the stimulated receptor molecule undergoes conformational changes that consequently activate downstream signal transduction pathways. The transfer of information requires phosphorylations of protein kinases along the pathways, and eventually lead to gene expression in response to external signals. However, only a few cases concerning the molecular mechanisms by which plant cells perceive and transduce extracellular signals were partially elucidated (Bowler and Chua, 1994).

Protein kinases are a large family of enzymes, and the number of unique members of the protein kinase family has risen exponentially (Hanks et al., 1988; Hunter, 1995). Classification of the protein kinases is based upon highly conserved key residues in the amino acid sequence of their "catalytic domain". The protein kinase catalytic domain ranges from 250 to 300 amino acid residues in the C-terminus of protein kinases. Protein kinases also contain different amino acid sequences on either side of the catalytic domain, and often have short amino acid sequences inserted in the catalytic domain. These additional amino acid sequences are also important in that they may enable each protein kinase to recognize specific target molecules, and/or allow the activity of the protein kinase to be tightly regulated (Hanks et al., 1988).

All protein kinases which were characterized with regard to substrate specificity fall within one of two broad families, serine/threonine-specific and tyrosine-specific. Although both families of protein kinases have similar catalytic domain structures, certain short amino acid stretches appear to characterize each class and determine their substrate specificities (Kenelly and Krebs, 1991). Typical members of the

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serine/threonine protein kinase family are the cyclic nucleotide-dependent subfamily, calcium-phospholipid-dependent subfamily, calcium-calmodulin-dependent subfamily, and SNF1 subfamily. Typical members of the tyrosine protein kinase family are the *src* subfamily, *abl* subfamily, EGF receptor subfamily, and insulin receptor subfamily.

Serine/threonine protein kinases phosphorylate serine and/or threonine of target molecules in a signal transduction pathway. Over 1,000 genes of the serine/threonine protein kinase family were isolated from various organisms. Protein kinases of the cAMP-dependent subfamily and calcium-phospholipid-dependent subfamily are the most abundant in the serine/threonine protein kinase family (Hanks and Quinn, 1991).

Genes encoding components of the cAMP signal cascade have been well characterized in animals and yeast. In *Saccharomyces cerevisiae*, cAMP positively regulates cell growth *via* the cAMP-dependent protein kinase (PKA) (Toda et al., 1987). The protein kinases also play important roles in the bovine cardiac muscle and the pituitary (Showers and Maurer, 1986). A major consensus sequence recognized by cAMP-dependent protein kinases is known to be Arg-Arg/Lys-X-Ser/Thr (Kennelly and Krebs, 1991). Protein kinase C (PKC) was originally characterized as a phospholipid-diacylglycerol-calcium-dependent protein kinase (Nishizuka, 1988). When activated, protein kinase C phosphorylates specific serine/threonine residues on target proteins that vary depending on the cell type. At least two reaction pathways of protein kinase C are known so far. First, protein kinase C activates a protein kinase cascade that leads to phosphorylation and activation of a DNA-bound gene regulatory protein (Hunter and Karin, 1992). Second, protein kinase C activation leads to phosphorylation of an inhibitor protein, thereby releasing a cytoplasmic gene regulatory protein so that it can migrate into the nucleus and stimulate the transcription of specific genes (Nishizuka, 1992).

About two thousands of protein kinase genes have been reported thus far, however, the number of protein kinase genes isolated from plants reaches only about 40. Most protein kinases isolated from plants are serine/threonine protein kinases (Mizoguchi et al., 1992; Mizoguchi et al., 1994). Furthermore, there has been no report about a plant protein kinase which phosphorylates tyrosine only. Interestingly, plants have protein kinases which phosphorylate both serine/threonine and tyrosine (Ali et al., 1994; Tregear et al., 1996), which is rarely reported in animals and lower eukaryotes (Howell et al., 1991; Banuett and Herskowitz, 1994).

The root system in plants is a main system of water absorption and also perceives many internal/external signals such as water stress and gravity. However, the underground part of plants has not been extensively studied for years, because major cellular metabolisms and development are thought to occur in overground parts. Recently, many approaches have been carried out to elucidate development and physiology of roots at the molecular level (Lu et al., 1996).

As an initial step toward understanding of mechanisms governing root development, we attempted to isolate novel genes which might be involved in root development in Chinese cabbage. Here we report on the isolation of a cDNA encoding a protein kinase of Chinese cabbage. We show that the protein kinase has a high homology to a group of cAMP-and calcium-phospholipid-dependent subfamilies of the serine/threonine protein kinase family, and that it comprises the plant serine/threonine protein kinase subfamily.

Materials and Methods

Plant material

Chinese cabbage (*Brassica campestris* subsp. *napus* var. *pekinensis* cv. Jangwon F_1 hybrid no. VCh-Hy-169) was grown under a greenhouse condition.

Preparation of nucleic acids

Total RNA and poly(A)$^+$ RNA were prepared from roots of Chinese cabbage with standard methods (Chirgwin et al., 1979; Sambrook et al., 1989). Genomic DNA was extracted from leaf tissues (Ausubel et al., 1987).

Construction of cDNA libraries

cDNAs were synthesized from root poly(A)$^+$ RNA with AMV reverse transcriptase using oligo(dT) as a primer. The cDNAs were end flushed by T4 DNA polymerase and subcloned to pUC19. The recombinant plasmids were then introduced into *Escherichia coli* cells by the $CaCl_2$-mediated transformation method (Mandel and Higa, 1970). A complete cDNA library was constructed in the λZAPII vector (Stratagene) with cDNAs synthesized from root poly(A)$^+$ RNA as described by the manufacturer.

DNA sequencing and sequence analysis

Plasmid DNA was isolated by the alkaline lysis method (Birnboim and Doly, 1979) and its nucleotide sequence was determined by the standard dideoxynucleotide chain termination method (Sanger et al., 1977). Sequence similarities were identified by the BLAST program (Altschul et al., 1990). For multiple sequence comparison, the sequences were aligned using the Clustal V program (Higgins et al., 1992). A phylogenetic tree was constructed by using the distance matrix method (Saitou and Nei, 1987), with no outgroup being selected.

Screening of a cDNA library

Probes used in all hybridization procedures were prepared by the random primer labeling method (Feinberg and Vogelstein, 1984). Unincorporated radioisotopes were removed by Sephadex G-50 column chromatography.

A cDNA library was screened to isolate a full length protein kinase clone according to the procedure described by Benton and Davis (1977). Plaques were transferred onto nylon filters. After fixing in 0.4 M NaOH, the filters were hybridized in 1M NaCl and 1% SDS for 16 h at 60℃. After hybridization, the filters were washed three times with 2×SSC and 0.1% SDS for 5 min, 5 min, and 30 min, respectively. The filters were finally washed with 0.1×SSC and 0.1% SDS for 10 min at 60℃. Recombinant phages were subjected to *in vivo* excision as described by the manufacturer.

Genomic DNA blot hybridization

Five μg of genomic DNA was digested with *Eco*RI and *Xba*I, and electrophoresed on a 0.7% agarose gel. Hybridization was carried out as described by Southern (1975). Washing of filter membrane was carried out as described above.

Results

Isolation of a cDNA encoding a protein kinase in Chinese cabbage

Random sequencing of expressed sequence tags in a cDNA library constructed in pUC19 with cDNAs synthesized from poly(A)$^+$ RNA isolated from roots of Chinese cabbage led to isolation of a partial cDNA clone, BR77, encoding a putative protein kinase. The partial cDNA was 550 bp long and showed a high similarity to a group of plant protein kinases including *Arabidopsis thaliana* protein kinase homologue Atpk64 (Mizoguchi et al., 1992), whose function was unknown.

To isolate a full-length cDNA clone for the putative protein kinase, a cDNA library constructed in the λ ZAPII vector with root poly(A)$^+$ RNA was screened with the BR77 cDNA as a probe. We obtained five positive clones from 7×10^4 recombinant phages in the cDNA library. Restriction enzyme mapping and partial nucleotide sequencing of the clones showed that all the five clones have the same nucleotide sequence of BR77 cDNA (data not shown). Four clones of them had a translation start codon (ATG) in the 5' region of the cDNAs. Of 5 positive clones selected, a cDNA clone, *Bcpk1* (*Brassica campestris* protein kinase 1), containing the longest cDNA insert of 1626 bp was subjected to nucleotide sequence determination.

The nucleotide sequence and deduced amino acid sequence of the *Bcpk1* cDNA are shown in Fig. 1. The *Bcpk1* cDNA contained one open reading frame encoding a putative protein kinase of 439 amino acids. The polypeptide consists of a short N-terminal region and a putative protein kinase catalytic domain. Amino acid stretches comprising a transmembrane domain or ligand-binding domain were not found in the N-terminal region, suggesting that Bcpk1 may not be a receptor kinase. The catalytic domain of Bcpk1 has highly conserved 11 major subdomains of the protein kinases (Hanks et al., 1988) except for Asp^{228} (The amino acid number refers to the position in the Bcpk1 protein). It replaces a glycine residue in the subdomain VII of protein kinases in animals and lower eukaryotes.

Multiple sequence alignment of Bcpk1

To figure out the structural features of Bcpk1, its deduced amino acid sequence was compared with those of protein kinases using the Clustal V program (Higgins et al., 1992). They include four plant protein kinases, pea Pspk3 (U11553, unpublished), rice RicG11A (Lawton et al., 1989), maize protein kinase Mzesmdpk (Biermann et al., 1990), and *A. thaliana* Atpk64 (Mizoguchi et al., 1992), which are most homologous to Bcpk1 among protein kinases in the GenBank data base. The *A. thaliana* ribosomal protein kinase Atpk1 (Zhang et al., 1994), which is less related to Bcpk1, and yeast cAMP-dependent protein kinase Scypl203w (Z73559, unpublished), which is a protein kinase A, were also included as references in the comparison.

As shown in Fig. 2, although the N-terminal regions of these protein kinases do not show any apparent homology, the 11 major subdomains of protein kinases are well conserved in all the protein kinases. Furthermore, Bcpk1, Pspk3, RicG11A, Mzesmdpk, and Atpk64 share some common features in the catalytic domain. They all have an aspartate residue in the subdomain VII and a long nonhomologous intervening sequence between the subdomains VII and VIII. However, although Atpk1 is of plant origin, it is structurally more similar to the yeast protein kinase in that it has a glycine residue (Gly^{277}) instead of an aspartate in the subdomain VII and does not have a long intervening sequence in the catalytic domain, suggesting that it may recognize different effector molecules from other plant protein kinases.

Phylogenetic status of Bcpk1

A phylogenetic status of Bcpk1 among similar protein kinases was investigated by constructing a phylogenetic tree with amino acid sequences of forty-eight protein kinases which are most homologous to Bcpk1 among protein kinases in the GenBank data base. Amino acid sequences of maize calcium/calmodulin

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GGCACGAGTCTCAATCATAAACACTCCTTTCGGATTCCTATTTCTCTACACGTTTCTCCA     60
ATTTTTACTTTAATTTCCGACGATGTTACTAGAATCAGACGGCGAGATGAGCTTAGAGAC    120
                          M  L  L  E  S  D  G  E  M  S  L  E  T     13
AACAAACTCGCCGATTAGCAGCGGAACCGAGAGTTGCAGCAGTTTCAGCCGGCTATCTTT    180
  T  N  S  P  I  S  S  G  T  E  S  C  S  S  F  S  R  L  S  F     33
CGACGCGCCGCCGTCAACCACCGCCATAATCCCCGAGGAAGAGAGCTGCCTCTCTCTTAA    240
  D  A  P  P  S  T  T  A  I  I  P  E  E  E  S  C  L  S  L  K     53
ACCCCACAGATCCTCCGACTTCGCTTACGCGGAGATCCTACGACGGCGGAAACACAGCCT    300
  P  H  R  S  S  D  F  A  Y  A  E  I  L  R  R  R  K  H  S  L     73
AACGTTCCGAGATTTTCGCCTTATGCGTCGTATCGGCGCCGGAGATATCGGAACCGTATA    360
  T  F  R  D  F  R  L  M  R  R  I  G  A  G  D  I  G  T  V  Y     93
CCTATGCCGTCTCGCCGGAGATCAAGAAGAGAGCCGGAGCTCGTACTTCGCGATGAAAGT    420
  L  C  R  L  A  G  D  Q  E  E  S  R  S  S  Y  F  A  M  K  V    113
TGTGGATAACGAAGCGCTTGCGATGAAGAAGAAGATGCACAGAGCTGAGATGGAGAAAAA    480
  V  D  N  E  A  L  A  M  K  K  K  M  H  R  A  E  M  E  K  K    133
GATATTGAAGATGCTCGACCATCCGTTTTTGCCGTCTCTTTACGCAGAGTTCGAAGCTTC    540
  I  L  K  M  L  D  H  P  F  L  P  S  L  Y  A  E  F  E  A  S    153
ACACTTCTCTTGCATCGTCATGGAATATTGCTCCGGCGGAGATTTGCATTCTCTCCGTCA    600
  H  F  S  C  I  V  M  E  Y  C  S  G  G  D  L  H  S  L  R  H    173
CAGACAGCCTCAACACAGATTCTCCCTCTCCTCCGCCAGATTTTACGCGGCTGAAGTTCT    660
  R  Q  P  Q  H  R  F  S  L  S  S  A  R  F  Y  A  A  E  V  L    193
AGTGGCGTTAGAATATCTACACATGTTGGGTATCATCTACAGAGATCTCAAGCCTGAAAA    720
  V  A  L  E  Y  L  H  M  L  G  I  I  Y  R  D  L  K  P  E  N    213
TATCTTAGTTAGATCGGACGGTCACATCATGCTCTCTGACTTTGACCTCTCCTTATGCTC    780
  I  L  V  R  S  D  G  H  I  M  L  S  D  F  D  L  S  L  C  S    233
CGACTCAATCGCCGCCGTTGAATCCTCCTCATCCACGCCGGAGAATTATCCCCACTCTTC    840
  D  S  I  A  A  V  E  S  S  S  S  T  P  E  N  Y  P  H  S  S    253
CCCGCGTCGACTCACTCGTCTCGCTAAGCTTTTCAACCGCGTCTTACGGTCCAAAAAGGT    900
  P  R  R  L  T  R  L  A  K  L  F  N  R  V  L  R  S  K  K  V    273
CCAGACGCTAGAACCGAACCGTCTCTTTGTGGCTGAACCGGTTACGGCCCGGTCCGGTTC    960
  Q  T  L  E  P  N  R  L  F  V  A  E  P  V  T  A  R  S  G  S    293
CTTTGTTGGTACGCATGAGTACGTTGCACCAGAAGTCGCCTCTGGTGGGTCCCATGGGAA   1020
  F  V  G  T  H  E  Y  V  A  P  E  V  A  S  G  G  S  H  G  N    313
CGCCGTTGACTGGTGGGCCTTCGGAGTGTTCCTCTACGAGATCATTTACGGCCGGACTCC   1080
  A  V  D  W  W  A  F  G  V  F  L  Y  E  I  I  Y  G  R  T  P    333
CTTCGCCGCGCCGACAAATGACGTCATCCTACGTAACATAGTGAAAAGACCATTGAGTTT   1140
  E  A  A  P  T  N  D  V  I  L  R  N  I  V  K  R  P  L  S  F    353
TCCGACCGATTCGCCGGCGACGATGTTTGAGCTTCACGCTCGGAGTTTGATCTCCGGGTT   1200
  P  T  D  S  P  A  T  M  F  E  L  H  A  R  S  L  I  S  G  L    373
GCTCAACAAGGATCCGAGTACACGGCTCGGCTCACGGCGAGGTGCGGCGGAGGTGAAAGT   1260
  L  N  K  D  P  S  T  R  L  G  S  R  R  G  A  A  E  V  K  V    393
GCACCCGTTTTTCAAAGGTCTAAATTTTGCGCTCATCCGCACAATGACTCCGCCTGAGGT   1320
  H  P  F  F  K  G  L  N  F  A  L  I  R  T  M  T  P  P  E  V    413
TCCCTCCGACGTCAGGAGACCGAAGAAGTCGGCGACGTTTGGTGGTAGAAGTAGCAAACC   1380
  P  S  D  V  R  R  P  K  K  S  A  T  F  G  G  R  S  S  K  P    433
AGCGGCGTTCGATTTCTTTTGAAAACCAGTGTTAGCGATTAGTTAGCATCACGTGTTAGT   1440
  A  A  F  D  F  F  *                                           439
CGGTTCAAGTCGGTGCCAGATTTTTAGCGACTGCCTTGTGAAGATAGCTGTTTTAGCTGT   1500
ACATATTCCCTTGATGATGTCCTTGTTTTAAAAAATCGTTTATCTGACTTTTAGTATTTA   1560
AATCAGATGAGTTCTCACGCGATTTTGTACCATAAACGGATAAATCTAAACTAATCATAT   1620
CTTGGG                                                         1626
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the *Bcpk1* cDNA. Amino acids are denoted by single-letter codons. An asterisk indicates a stop codon. The GenBank, EMBL, and DDBJ accession number for the nucleotide and predicted amino acid sequences of the *Bcpk*, cDNA is U93559.

(CaM) kinase (Lu et al., 1996) and rat tyrosine kinase (Sanchez et al., 1994) were also processed as references.

As shown in Fig. 3, all protein kinases except for rat tyrosine kinase formed a clustered group, which confirmed that they all belong to the serine/ threonine protein kinase family. The rat tyrosine kinase was located very far from the others in the tree, which suggests that the tyrosine protein kinase family shared little homology with the serine/threonine protein kinase family. The phylogenetic tree indicated that three groups are apparent in the serine/threonine protein kinase family. cAMP-dependent protein kinases (protein kinases A) and calcium-phospholipid-dependent protein kinases (protein kinases C) in animals and yeast were clustered into separate groups even though they share some common features in the conserved catalytic domain. Fifteen protein kinases including Bcpk1 formed a distinct group, which is apparently discrete from protein kinases of animals and lower eukaryotes.

It is a novel aspect that all members of the group including Bcpk1 are from plants. They all have a nonhomologous intervening sequence between the subdomains VII and VIII. Furthermore, an aspartate residue in the subdomain VII was conserved in all the protein kinases except for an *Arabidopsis* protein kinase (U79744; unpublished) which contains a tyro-

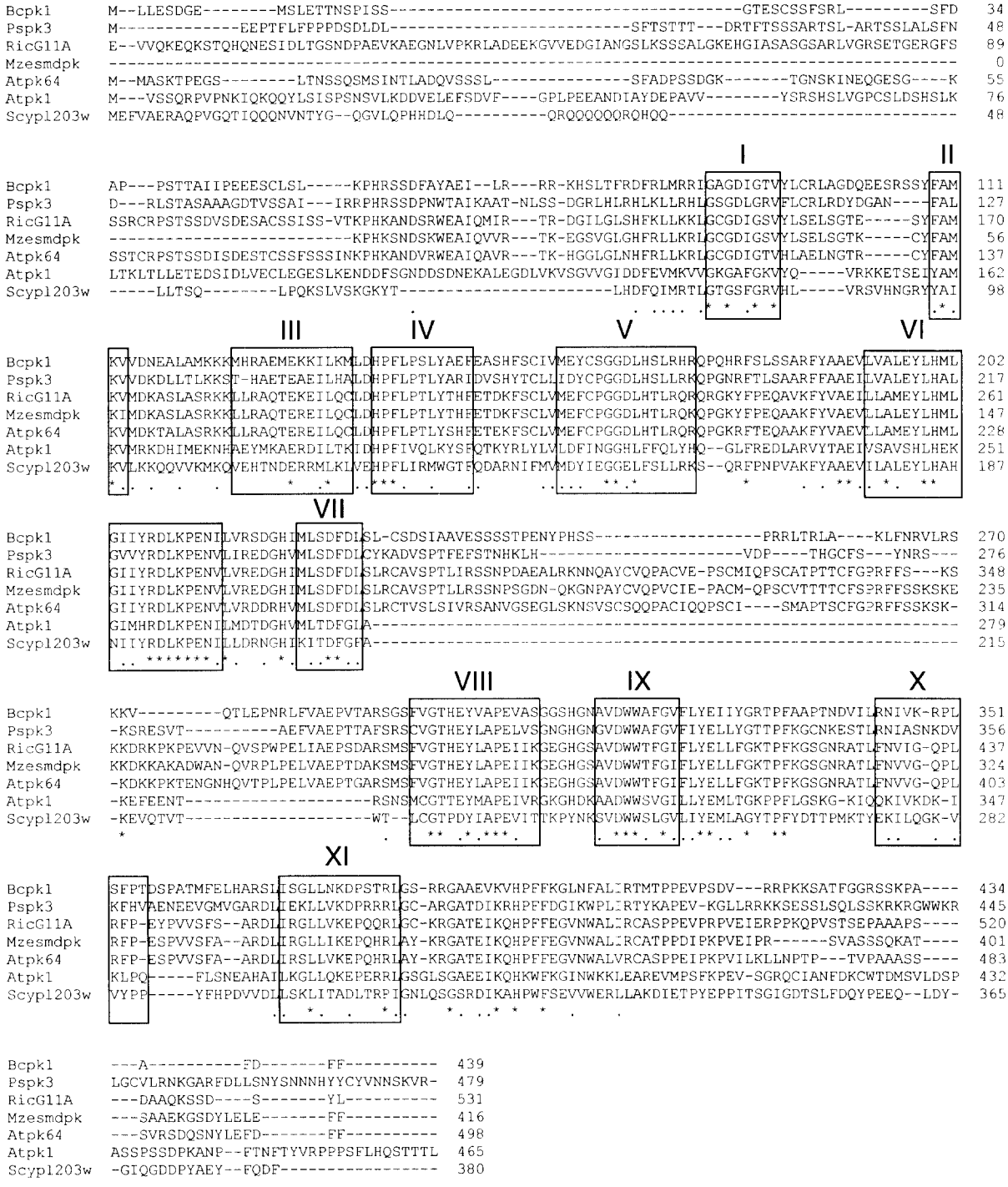

Fig. 2. Comparison of the deduced amino acid sequence of Bcpk1 with those of other protein kinases. The deduced amino acid sequence of Bcpk1 is aligned with those of pea Pspk3 (U11553; unpublished), rice RicG11A (P47997; Lawton et al., 1989), maize protein kinase Mzesmdpk (A45510; Biermann et al., 1990), *Arabidopsis thaliana* Atpk64 (S20918; Mizoguchi et al., 1992), *A. thaliana* ribosomal protein kinase Atpk1 (L29030; Zhang et al., 1994), and yeast cAMP-dependent protein kinase Scypl203w (Z73559; unpublished). Amino acids in boxes are the 11 major conserved subdomains of protein kinases (Hanks et al., 1988). Dashes indicate gaps introduced to optimize the alignment. Eleven conserved domains of protein kinases are denoted by bold Roman characters.

sine residue instead. The aspartate residue was replaced by a glycine residue in other protein kinases of animals and lower eukaryotes. Although functions of the protein kinases including Bcpk1 are still unknown, it is suggested that the fifteen protein kinases are plant-specific serine/threonine protein kinases.

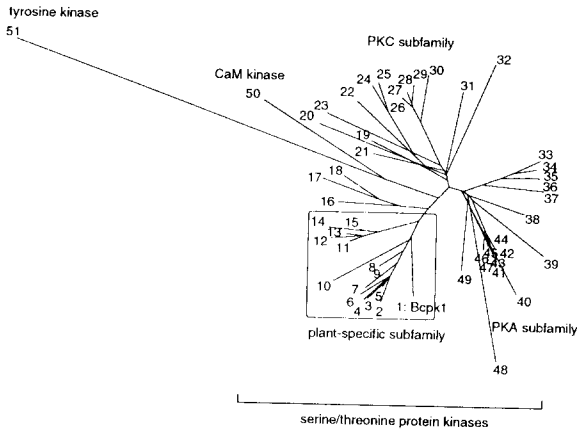

Fig. 3. Phylogenetic status of Bcpk1 in serine/threonine protein kinases. Amino acid sequences of 48 protein kinases were obtained from the GenBank data base and their phylogenetic relationship was analyzed by the distance matrix method (Saitou and Nei, 1987), with no outgroup being selected. Accession numbers are given in parenthesis. S/T PK, PK, PKA, and PKC are abbreviations for serine/threonine protein kinase, protein kinase, cAMP-dependent protein kinase, and calcium-phospholipid-dependent protein kinase, respectively. 1, Bcpk1; 2, *A. thaliana* S/T PK (S20918); 3, rice S/T PK in leaf (P47997); 4, kidney bean S/T PK (P15792); 5, maize PK (A45510); 6, potato S/T PK (X90990); 7, *Solanum berthaultii* S/T PK in leaf (X97980); 8, common ice plant PK in leaf (Z30331); 9, common ice plant PK (S42865); 10, pea PK in seedling (U11553); 11, pea PK in bud (M92989); 12, common ice plant PK (S42866); 13, pea PK in leaf (U83281); 14, spinach PK (S42868); 15, *A. thaliana* S/T PK (U79744); 16, fission yeast S/T PK (Q09831); 17, bakers yeast S/T PK (P53739); 18, bakers yeast S/T PK (P25341); 19, slime mold PKA (P28178); 20, human PKC in blood (Q04759); 21, rat PK (A48094); 22, fungi S/T PK (U05811); 23, fission yeast PKC (P36582); 24, bakers yeast S/T PK (P18961); 25, bakers yeast PK (M21307); 26, rat PKC (D49836); 27, human PKC (S36389); 28, human PK (P31749); 29, human S/T PK in lung (M77198); 30, fruit fly PK (A55888); 31, fruit fly PK (U67304); 32, fruit fly PKC (P13677); 33, bakers yeast PKA (P05986); 34, bakers yeast PKA (P06244); 35, bakers yeast PKA (A27070); 36, bakers yeast PKA (P06245); 37, fungi PK (23730); 38, fungi PKA (S41099); 39, fruit fly PKA (P16910); 40, bakers yeast S/T PK (P53894); 41, pig roundworm PKA (P49673); 42, fruit fly PKA (P12370); 43, *C. elegans* PKA (P21137); 44, pig PKA (P05383); 45, mouse PKA (P05206); 46, bovine PKA (P05131); 47, sea urchin PKA (D83380); 48, fission yeast S/T PK (P38938); 49, *Plasmodium yoelii* PKA (D45849); 50, maize CaM kinase (S82324); 51, rat tyrosine kinase (Ratptk3d).

Genomic DNA gel blot analysis of Bcpk1

To determine the copy number of the *Bcpk1* gene in Chinese cabbage, 5 μg of genomic DNA isolated from leaves was digested with two restriction enzymes and analyzed by DNA gel blot analysis. As shown in Fig. 4, the three clear bands appeared in each lane, indicating that the *Bcpk1* gene may be present as three copies in the Chinese cabbage genome. This suggests that the *Bcpk1* gene may exist as a small multigene family in the genome.

Discussion

We have isolated a cDNA encoding a putative protein kinase in roots of Chinese cabbage (Figs. 1 and 2). The putative protein kinase showed a high homology to a group of cAMP-dependent and calcium-phospholipid-dependent serine/threonine protein kinases. Phylogenetic analysis showed that Bcpk1

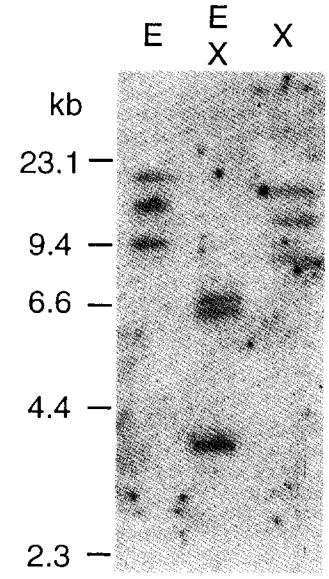

Fig. 4. Genomic DNA gel blot analysis of the *Bcpk1* gene. Five μg of leaf genomic DNA was digested with restriction enzymes and electrophoresed for 12 h on a 0.7% agarose gel. Hybridization was carried out with the standard method using the *Bcpk1* cDNA as a probe. E, *Eco*RI; X, *Xba*I.

belongs to the plant-specific serine/threonine protein kinase subfamily. The *Bcpk1* gene existed as at least three copies in the Chinese cabbage genome (Fig. 4).

Although the N-terminal region of Bcpk1 showed no apparent homology with other plant protein kinases, the 11 major subdomains conserved in protein kinases are present in the catalytic domain of Bcpk1 (Fig. 2). Furthermore, Bcpk1 exhibits two common features identified in plant serine/threonine protein kinases, having an aspartate residue in the subdomain VII and a long nonhomologous intervening sequence between the subdomains VII and VIII. The N-terminal region of Bcpk1 may represent a regulatory domain that is involved in binding of effectors as it is in animal protein kinases. Catalytic domains from protein kinases having similar modes of regulation or substrate specificities also tend to have similar primary structures (Hanks et al., 1988). With the unique amino acid sequences in the N-terminal and intervening regions, Bcpk1 may participate in a novel signal transduction cascade in roots of Chinese cabbage, which remains to be determined.

We selected forty-eight protein kinases whose amino sequences were similar to that of Bcpk1, and constructed a phylogenetic tree by using the distance matrix method (Saitou and Nei, 1987). Maize CaM kinase (Lu et al., 1996) and rat tyrosine kinase (Sanchez et al., 1994) were also processed as references. As shown in Fig. 3, the rat tyrosine kinase was located far from serine/threonine kinases in the phylogenetic tree, suggesting that there is a

great sequence dissimilarity between the two major protein kinase families. The analysis showed that plant protein kinases including Bcpk1 was similar to the protein kinases in the cAMP-dependent and calcium-phospholipid-dependent protein kinase subfamilies in the serine/threonine protein kinnase family, rather than other protein kinase subfamilies, such as the CaM kinase subfamily.

The phylogenetic tree also showed that nine protein kinases which are most similar to Bcpk1 are of plant origin (Fig. 3). They include protein kinases of pea (U11553, unpublished), rice (Lawton et al., 1989), maize (Biermann et al., 1990), and *Arabidopsis* (Mizoguchi et al., 1992) shown in Fig. 2. Although their functions are still unknown, all of them, including Bcpk1, have some features in common. They formed a separate group which is apparently discrete from other protein kinases of animals and lower eukaryotes. They all have an aspartate residue in place of a glycine residue in the subdomain VII. The reason for this substitution is not known for any other protein kinases. They also have a highly heterogeneous region between the subdomains VII and VIII, which is about 50-80 amino acids in length. The region is not found in other subfamilies of animal and plant protein kinases such as protein kinase A and protein kinase C. These observations suggest that the protein kinases including Bcpk1 comprise a novel plant-specific subfamily of serine/threonine protein kinases (Toda et al., 1987; Zhang et al., 1994).

The recent version of the data base by Hanks and Quinn (1991) classified protein kinases into 5 groups and 55 subgroups, which had been conventionally classified to the serine/threonine protein kinase family and tyrosine protein kinase family. They classified some protein kinases of plants into a subgroup, which was named as the "flowering plant PVPK1 protein kinase homolog family". The subgroup contained kidney bean PVPK1 (Lawton et al., 1989), rice RicG11A (Lawton et al., 1989), maize ZmPP (Biermann et al., 1990), *Arabidopsis* Atpk5 (Hayashida et al., 1993), *Arabidopsis* Atpk7 (Hayashida et al., 1992), and *Arabidopsis* Atpk64 (Mizoguchi et al., 1992). They are also included in the plant-specific serine/threonine protein kinase subfamily in Fig. 3.

The functions of the plant-specific protein kinase subfamily are still unknown, even though they are serine/threonine protein kinases. Further studies will be needed to unveil their effectors, and then they would be classified into a new subfamily of serine/threonine protein kinases based on the effectors.

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The recent version of the data base of Hanks and Quinn were searched through http://www.sdsc.edu/kinases/vol1.1/pk_catalytic/pk_hanks_seq_align_long.html.

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