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# Cloning and Sequence Analysis of Ribosomal Protein S4 cDNA from Root of *Panax ginseng*

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## ABSTRACT

**Ribosomal protein complex with ribosomal RNA to form the subunits of the ribosome serve essential functions in protein synthesis. A full-length cDNA *(PRPS4)* encoding ribosomal protein S4 has been isolated and its nucleotide sequence determined in ginseng plant *(Panax ginseng)*. A *PRPS4* cDNA is 1105 nucleotides long and has an open reading frame of 792 bp with a deduced amino acid sequence of 264 residues (pI 10.67). The deduced amino acid sequence of *PRPS4* matched to the previously reported ribosomal protein S4 genes. Their degree of amino acid identity ranged from 68 to 92%. Phylogenetic analysis based on the amino acid residues showed that the *PRPS4* grouped with ribosomal protein S4 of S. *tuberosum* (CAA54095).**

***Key words :*** cDNA, *Panax ginseng*, ribosomal protein S4, root

## INTRODUCTION

Ribosomes are the most important subcellular structures in eukaryotic cells for protein synthesis. The ribosomal proteins, which number approximately 80 in eukaryotic cells, comprise the small and large subunits of the eukaryotic ribosome. Ribosomal proteins are synthesized in the cytoplasm and are transported to the nucleolus where they complex with ribosomal RNA to form the ribosome subunits. Particular ribosomal proteins have also been implicated as participants in cellular processes apart from protein synthesis, including DNA replication and repair, the control of transcription termination, the regulation of development, interorganellar protein transport, and cell transformation (Wool, 1993).

Ribosomal protein S4 is a small subunit ribosomal protein. In mammalian system, it has been found to be localized at the interface between the two ribosomal subunits (Uchiumi *et al.*, 1986) and can be cross-linked to eukaryotic initiation factor elF-3 (Westermann and Nygard, 1983), suggesting that S4 may function in the

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initiation process of protein synthesis. The yeast ribosomal protein S7, homologous to the mammalian ribosomal protein S4, was found to be an essential protein ( All-Robyn *et al.*, 1990; Synetos and Frantziou, 1996). The haplo-insufficency of human S4 protein is reported to cause Turner syndrome, which is characterized by short stature, gonadal degeneration and a variety of other anatomic abnormalities.

Several S4 genes have been cloned from dicotyledonous and monocotyledonous plants (Braun *et al.*, 1994; Turley *et al.*, 1995). However, little is known about the structure, subcellular transportation and function of plant S4 proteins (Gao *et al.*, 1994). In the present study we describe the cDNA cloning, and molecular characterization of *PRPS4* from root of *Panax ginseng*.

## MATERIALS AND METHODS

### Plant materials

Four-year old *Panax ginseng* plants grown at field were used. Roots were harvested and washed with flowing water and then immediately frozen in liquid nitrogen and stored at -80℃ further use.

### RNA purification and cDNA library construction

Total RNA was isolated from ginseng root tissues using aqueous phenol extraction procedure as described by Morris *et al.* (1990). The tissue was frozen and ground in liquid nitrogen prior to extraction of RNA. Poly $(A)^+$ RNA was isolated by oligo(dT) cellulose column using the mRNA purification kit (Amersham pharmacia, UK). A commercial cDNA synthesis kit was used to construct library according to the manufacture's instruction manual (Stratagene, USA). To produce single-stranded cDNA appropriate for directional cloning 5$\mu$g of poly $(A)^+$ RNA was primed with an oligo (dT) primer having a *Xho* I site at the 3' end. Double-stranded cDNA was produced using RNase H and *E. coli* DNA polymerase. After ligation of *EcoRI* oligonucleotide adapter, cDNA was digested with *Xho* I and then size-fractionated through a Sepharose CL-2B gel filtration medium. Size-selected cDNA was ligated into Uni-ZAP XR vector and was packaged in vitro using Gigapack III Gold Packaging Extract kits (Stratagene).

### Sequence analysis

The pBluescript SK(+/-) phagemids for ribosomal protein S4 cDNA were excised from the UniZAP-XR vector with helper phage and used as templates for sequence analysis. The cDNA insert was sequenced using the 5' and 3' sequencing primer by an automatic DNA sequencer (ABI prism 3700). Nucleotide and amino acid sequence analyses were performed using DNASIS program (Hitachi). Comparison of sequences to DNA and protein databases at NCBI was performed using the blast algorithm of Altschul *et al.* (1990).

### Sequence alignment and phylogenetic analysis

We used Clustal W (1.82) with default gap penealties to perform multiple alignment of ribosomal protein S4 isolated in ginseng and previously registered in other plants (Thompson *et al.*, 1994). Based on this alignment, a phylogenetic tree was constructed according to the UPGMA method.

## RESULTS AND DISCUSSION

Expressed sequence tags analysis was performed with a full length cDNA library prepared with four year-old root. The 5' ends of randomly selected cDNA inserts were sequenced using the 5' sequencing primer and then comparison of sequences to DNA and protein databases at NCBI was performed using the blast algorithm (Altschul *et al.*, 1990).

A full-length ribosomal protein S4 (*PRPS4*) homolog of analyzed EST clones was isolated and full

sequenced. A *PRPS4* gene was 1105 nucleotide long and possess an open reading frame of 792 bp with 87 bp 5'-untranslated region (5'UTR) and 223 bp 3' UTR (Fig. 1). This sequence can potentially encode a protein of 264 amino acids in length with a predicted molecular mass of 29847 Da . As expected for a basic protein, the predicted amino acid composition (pI 10.67) indicates an excess of basic 22% over acidic residues 9%. BLASTX database searches with the *PRPS4* sequence gave ribosomal protein S4 from various organism as the top 100 best matches.

The deduced S4 protein from P. ginseng was aligned

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GCACGAGGGTTTAATCAAAAGCTGAAGCAAGGAGTTTGCGGCACAGAGAGAGAGGGAGGG    60
AGACGCAGCTTATCGAACAAAGCAACCATGGCTAGAGGTTTGAAGAAGCATTTGAAGAGG   120
                          M  A  R  G  L  K  K  H  L  K  R

CTCAATGCCCCTAAGCATTGGATGCTCGATAAACTTGGTGGTGCATTTGCTCCCAAGCCT   180
L  N  A  P  K  H  W  M  L  D  K  L  G  G  A  F  A  P  K  P

TCATCTGGACCCCATAAATCCAGGGAATGCCTGCCTTTGATTCTTATTTTGCGAAACAGG   240
S  S  G  P  H  K  S  R  E  C  L  P  L  I  L  I  L  R  N  R

CTGAAGTATGCTCTAACTTACCGTGAAGTCCAATCCATCTTGATGCAACGACATGTTCTT   300
L  K  Y  A  L  T  Y  R  E  V  Q  S  I  L  M  Q  R  H  V  L

GTTGATGGGAAGGTTAGGACGGATAAGACCTATCCAGCTGGTTTCATGGATGTTGTATCA   360
V  D  G  K  V  R  T  D  K  T  Y  P  A  G  F  M  D  V  V  S

ATTCCCAAGACTAATGAGAATTTCCGTCTCCTCTATGACACAAAGGGTAGATTCCGCCTA   420
I  P  K  T  N  E  N  F  R  L  L  Y  D  T  K  G  R  F  R  L

CACTCGGTCAGGGATGAGGAGGCAAAGTTTAAGTTGTGCAAAGTTAGGTCAGTTCAGTTC   480
H  S  V  R  D  E  E  A  K  F  K  L  C  K  V  R  S  V  Q  F

GGTCAGAAAGGCATTCCGTACATCAATACCTATGATGGGCGTACTATCCGTTATCCAGAC   540
G  Q  K  G  I  P  Y  I  N  T  Y  D  G  R  T  I  R  Y  P  D

CCGCTCATTAAGGCCAATGACACCATCAAACTTGACTTGGAGGCAAACAAGATAGCTGAT   600
P  L  I  K  A  N  D  T  I  K  L  D  L  E  A  N  K  I  A  D

TTCATTAAGTTCGATGTTGGAAATGTTGTCATGGTGACTGGGGGGAGGAATACTGGGCGA   660
F  I  K  F  D  V  G  N  V  V  M  V  T  G  G  R  N  T  G  R

GTTGGAGTTATCAAGAACAGGGAGAAACATAAGGGGAGCTTCGAGACTGTTCACATTCAG   720
V  G  V  I  K  N  R  E  K  H  K  G  S  F  E  T  V  H  I  Q

GATTCCCTTGGTCACGAGTTTGCTACTCGTCTAGGCAATGTTTTTACCATCGGAAAAGGT   780
D  S  L  G  H  E  F  A  T  R  L  G  N  V  F  T  I  G  K  G

ACAAAGCCATGGGTGTCTCTTCCCAAGGGCAAGGGTATCAAATTAACCATCATTGAGGAG   840
T  K  P  W  V  S  L  P  K  G  K  G  I  K  L  T  I  I  E  E

GCCAGGAAGAGGAATGTTGCCCAGTCTGCTACAACTGCATAAACAAAAAATTTATGCATG   900
A  R  K  R  N  V  A  Q  S  A  T  T  A  *

TGTTGCTGTTAAAAGTTTTATCTTCTGGTCTTGCTTGCAGGTTGGTGCTGTGGTCTAGCT   960
AACTTAGGATTTATGCTTAAAATTATGTGTTGTTTTCATTTCAGACAATTTTGAAAGATA  1020
TTTTGTTGTTGCAAATTTGATTAAAACTCTTCTGCTGGCTGATTTTGGCAACAAATAGAC  1080
AACTTAATCAAGTCTACAATCTTTT                                      1105
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Fig. 1. Nucleotide and deduced amino acid sequence with the open reading frame from 88 to 882 bp. The positions of nucleotides are shown on the left and the positions of amino acids under the below. Asterisk shows the termination codon. The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequencing Database under the accession number AB232685.

with known S4 protein sequences from other phylogenetic kingdoms using the CLUSTALW program (Fig. 2). It shares amino acid identity ranged from 68 to 92%. A *PRPS4* protein sequence shared the highest similarity (92%) with S. *tuberosum* (CAA54095), followed by 91% with L. *esculentum* and O. *sativa* (BAD28085). Altogether, these data indicate that the putative P. *ginseng* basic ribosomal protein S4

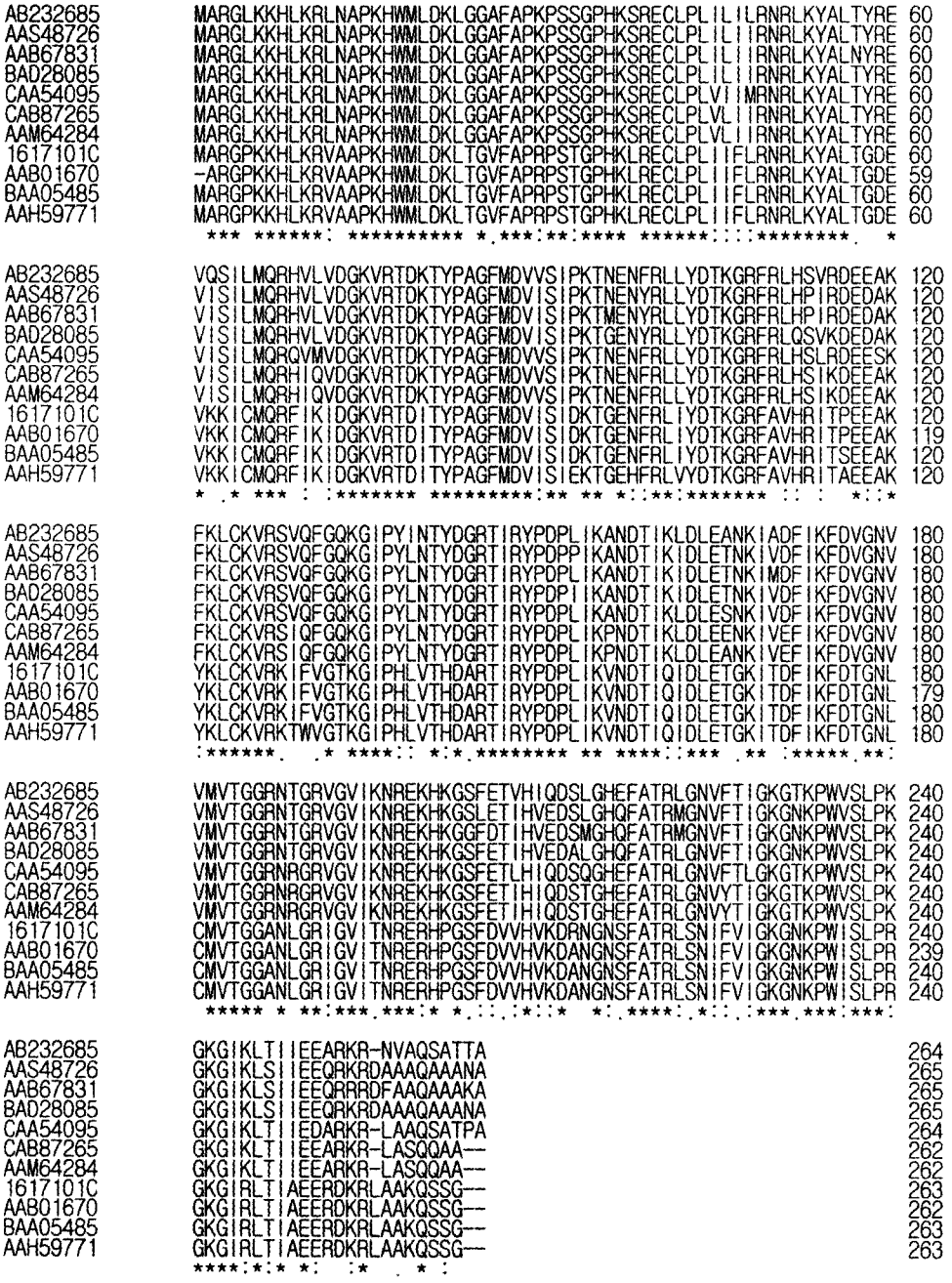


Fig. 2. Alignment of the amino acids residues of *PRPS4* (*AB232685*) with other plants; S. *tuberosum* (CAA54095), A. *thaliana* (CAB87265), Z. *mays* (AAS48726), O. *sativa* (BAD28085), A. *thaliana* (AAM64284), C. *griseus* (BAA05485), Z. *mays* (AAB67831), R. *norvegicus* (1617101C), X. *tropicalis* (AAH59771), F. *catus* (AAB01670).

(*PRPS4*) gene encodes a highly conserved protein. We compared the phylogenetic relationships of the *PRPS4* with those of the eukaryotic ribosomal protein S4. The *PRPS4* was clustered with ribosomal protein S4 of S. *tuberosum* (CAA54095) (Fig. 3).

We describe here the isolation and molecular characterization of ribosomal protein S4 in P. *ginseng*, *PRPS4*. The sequence of amino acid are highly conserved among aligned ribosomal protein S4s. Ribosomal protein S4 may function in the initiation process of protein synthesis, but their major function is elucidating.

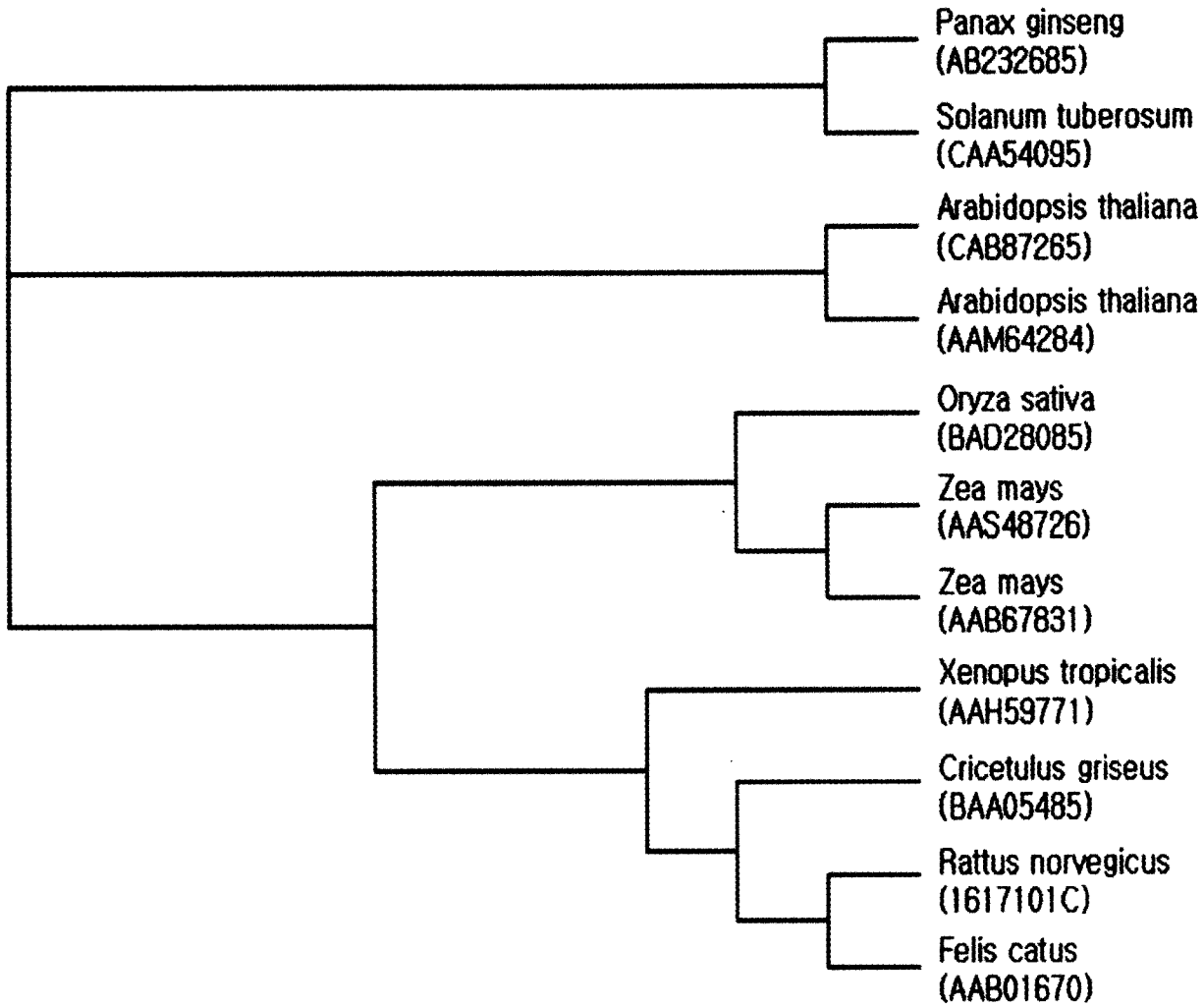


Fig. 3. Phylogenetic relationship of the ribosomal protein S4 family from P. ginseng and other plants. *Phylogenetic* analysis is based on the deduced amino acid sequences of ribosomal protein S4 genes from various plant species. The branch lengths are proportional to divergence, with the scale of 0.1 representing 10% change.

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