Journal of Plant Biology, June 2003, 46(2) : 130-133 **REPORT**

Identification of a Flower-Specific cDNA, *RsPCP1*, Encoding Putative Pollen Coat Protein from Radish

Yung Geun Yoo, Sang-Choon Lee, and Seong-Ryong Kim*
Department of Life Science, Sogang University, Seoul 121-742, Korea

To better understand the molecular control of floral development, we identified a flower-specific cDNA, *RsPCP1*, from Korean radish (*Raphanus sativus*). Based on nucleotide sequence analysis, this clone contains an open reading frame of 65 amino acids and shares 91% identity with a pollen coat protein from cabbage (*Brassica oleracea*). Southern analysis revealed that *RsPCP1* is present as a single-copy gene or a member of a small gene family in the radish genome. Because *RsPCP1* mRNA was present exclusively in mature floral buds but not in young floral buds or in vegetative tissues, we propose that this gene is anther-specific.

Keywords: flower-specific, pollen coat protein, radish

Flowers have been the subject of basic research as well as genetic-engineering studies that include male sterility (Mariani et al., 1990), because reproduction is a major part of the plant life cycle. During microsporogenesis, the pollen mother cell in the anther becomes the male gametophyte (Goldberg et al., 1993). While many genes are expressed in various tissues of the anther during these processes (Kamalay and Goldberg, 1980), only a small fraction of them can be considered anther-specific (Willing and Mascarenhas, 1984; Willing et al., 1988). Because they are important to understanding of floral development and agricultural applications, many anther-specific or anther-preferential genes have been characterized from various plant species (Mascarenhas, 1990; Foster et al., 1992; Kim et al., 1993; Kim and An, 1996; Rubinelli et al., 1998; Jeon et al., 1999; Walden et al., 1999; Steiner-Lange et al., 2003; Yui et al., 2003).

Pollen coat proteins (PCPs) play an important role in plant reproduction (Heslop-Harrison, 2000). One example of a well-characterized PCP in *Brassica* and *Arabidopsis* are the oleosins. These amphipathic molecules act as a "wick" for taking up water in order to initiate pollen germination on the stigma. In fact, 16 oleosin genes have been grouped into three classes based on their sequence specificity and tissue-specific expression. These have now been characterized in detail in *Arabidopsis* (Kim et al., 2002). In maize, another predominant PCP, xylanase, has been found to hydrolase xylan on the stigma, thereby generating a gap that facilitates pollen-tube growth (Wu et al., 2002). Some minor proteins, such as a 7-kD nonglycosylated polypeptide, may also be involved in determining self-incompatibility (Doughty et al., 1993; Kachroo et al., 2002). PCPs are synthesized, accumulated in the tapetum cells, then discharged onto the maturing pollen.

By analyzing mutants or transgenic plants, these genes can be functionally characterized to provide insight into pollen development as well as strategies for generating genic male sterility. Here, we present the DNA sequence, genomic organization, and expression pattern of a mature floral bud-specific cDNA that encodes a putative PCP in Korean radish (*Raphanus sativus*).

DNA Sequence Analysis of *RsPCP1*

During characterization of radish floral bud cDNA clones, we obtained a clone with higher homology to pollen coat proteins. This 520-bp clone contains a short 195-bp coding region, a 46-bp leader sequence, a 265-bp 3'-untranslated region, and a poly(A) tail (Fig. 1A). Two putative polyadenylation signals, AATAAA, are found at 19 and 32 bp upstream of the poly(A) tail. A coding region of 65 amino acids encodes a protein with a predicted size of 6.7 kD. The deduced protein has 91% identity with the PCP, BOPC15 from *Brassica oleracea* as well as high homology with other putative PCPs (Fig. 1B). Therefore, we have named it *Rs*PCP1 (*Raphanus sativus* Pollen Coat Protein 1). This protein also shares 62% and 47% identities with the *Fagus sylvatica* ABA-inducible LEA protein, FsLEA1 (Lorenzo et al., 1999) and the *Brassica napus* cold-inducible protein, BN28 (Orr et al., 1992), respectively.

*Corresponding author; fax +82-2-704-3601
e-mail sungkim@sogang.ac.kr

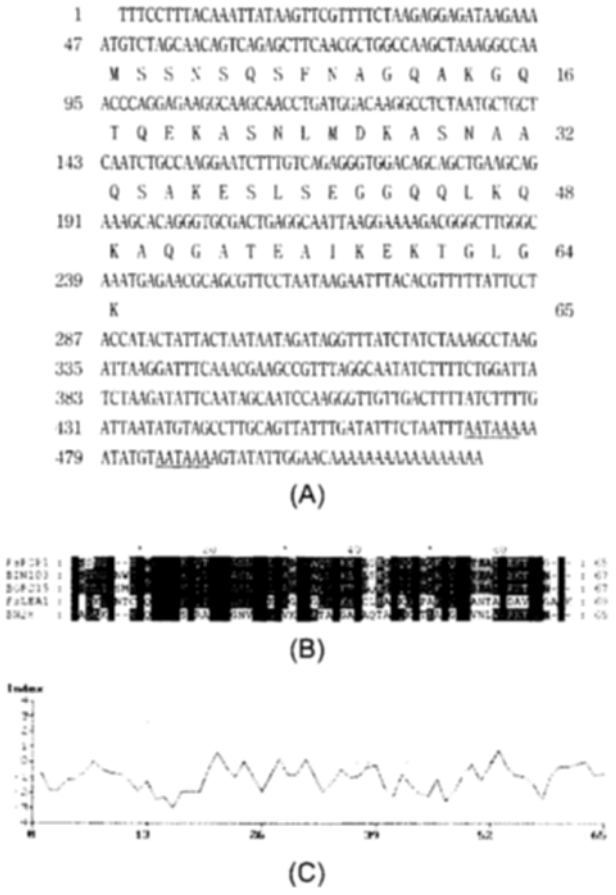

Figure 1. Sequence analysis of *RsPCP1*. (A) Nucleotides and the deduced amino acid sequence of *RsPCP1*. Positions of the nucleotides are to the left; those of the amino acids are to the right. (B) Comparison of the deduced amino acid sequence of *Rs*PCP1 with that of *Brassica rapa* PCP, BIN103 (AAC98699), *B. oleracea* PCP, BOPC15 (CAA63531), *Fagus sylvatica* ABA-inducible LEA protein, FsLEA1 (CAA10234), and *B. napus* cold-inducible protein, BN28 (1905417A). (C) Hydropathy profile of the deduced RsPCP1 amino acid sequence according to Kyte and Doolitle (1982). Hydrophobic domains are above the line and the hydrophilic domains below it.

Hydropathy analysis has revealed that *Rs*PCP1 is greatly hydrophilic (Fig. 1C). Pollen cell walls begin to develop in the anther while the tetrads of the microspore are still enclosed in the callose. In the final period of pollen maturation, coating substances derived from the tapetum are applied to the pollen. If *RsPCP1* encodes such a PCP, this *Rs*PCP1 protein should be secreted from the tissue of production. However, we cannot yet address the function of *RsPCP1* or the destination of the *Rs*PCP1 protein based on its sequence information.

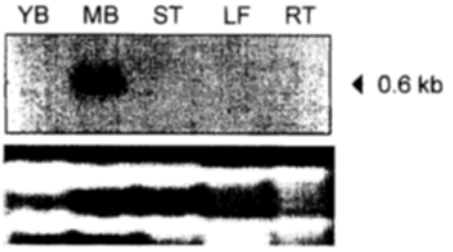

Figure 2. Northern blot analysis of *RsPCP1*. Ten μg of total RNA prepared from young floral buds (YB), mature floral buds (MB), stem (ST), leaves (LF), and roots (RT) were separated on an agarose gel, blotted onto a nylon membrane, and hybridized with the full-length *RsPCP1* cDNA. EtBr-stained rRNA bands indicate that equal amounts of RNA were loaded.

Mature Floral Bud-Specific Expression of *RsPCP1*

The expression level of *RsPCP1* in radish was studied by RNA blot analysis. Total RNA was isolated by the method of Davis et al. (1986). *RsPCP1* cDNA hybridized to mRNAs of about 0.6 kb in the mature floral buds (size >5 mm) but not in the young floral buds (<3 mm) or in vegetative organs (Fig. 2). The presence of *RsPCP1* in the mature buds indicates that this gene is expressed at the late stage of floral development. These results agree with those reported by Stanchev et al. (1996), who demonstrated that *PCP1* is highly expressed in the late stages (bud sizes of 5 to 7 mm and 7 to 10 mm) of anther development in *B. oleracea*. Their in situ analysis revealed that the gene is expressed at the cytosol of the trinucleate pollen grains but not in the tapetum. Although we did not conduct a detailed analysis of expression in the mature floral buds, based on the sequence homology and expression data of PCPs, we suggest that *RsPCP1* is also anther-specific. Further in situ analysis or RNA blot analysis using microspores would help in our understanding of the expression profile of *RsPCP1* during flower development in radish.

Southern Blot Analysis of *RsPCP1*

We performed Southern blot analyses to determine the number of homologous genes in the radish genome. Five μg of genomic DNA from leaves were digested with BamHI, EcoRI, and HindIII. Hybridization was done with an *RsPCP1* cDNA probe. One or two hybridizing bands were observed in each lane (Fig. 3), indicating that the *RsPCP1* gene may be present as either a single copy or a small gene family in the radish genome. This result differs from that reported for the *PCP1*

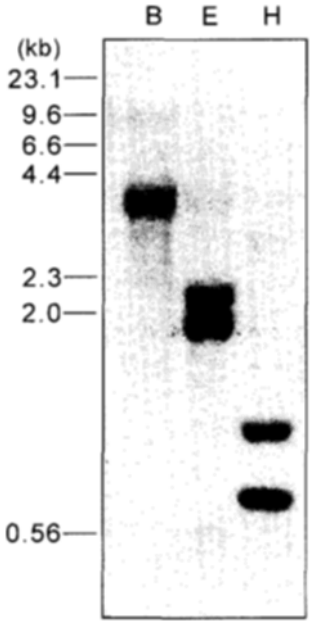

Figure 3. Southern blot analysis of *RsPCP1*. Five μg of radish leaf genomic DNA were digested with BamHI (B), EcoRI (E), and HindIII (H), electrophoresed, blotted onto Hybond-N, and hybridized with the full-length *RsPCP1* cDNA. Positions and sizes in kb of HindIII-digested lambda DNA fragments are indicated.

gene, which proved to be a member of a large family in the cabbage genome (Stanchev et al., 1996).

In conclusion, we have molecularly characterized a putative anther-specific cDNA clone, *RsPCP1*, from radish. *RsPCP1* transcript is exclusively found during the late stage of floral development and shares high homology with a PCP from cabbage. We propose that *RsPCP1* is anther-specific. Radish is an important vegetable crop in Korea. Molecular breeding and the study of anther-specific gene expression are vital to the understanding of floral development and male sterility in plants (Mariani et al., 1990). *RsPCP1* may be a good candidate for crop improvement via the engineering of that male-sterile trait. Therefore, a transgenic approach would include the study of antisense suppression or ectopic overexpression of *RsPCP1* under the CaMV 35S promoter in order to determine the in vivo function of that *Rs*PCP1 protein.

ACKNOWLEDGEMENTS

We thank Dr. Gynheung An for providing the radish cDNA library, and Ho-Jung Kwak and Byung-Chul Shin for technical assistance. This work was supported in part by grants from the Crop Functional Genomics Center, 21st Century Frontier Program (CG1113), the Ministry of Science and Technology, the Biogreen 21 Program, Rural Development Administration, and the Sogang University Research Grants in 2003.

Received April 30, 2003; accepted June 4, 2003.

LITERATURE CITED

Davis LG, Dibner MD, Batty JF (1986) Basic Methods in Molecular Biology. Elsevier, New York

Doughty J, Hedderson F, McCubbin A, Dickinson H (1993) Interaction between a coating-borne peptide of the *Brassica* pollen grain and stigmatic S (Self-Incompatibility)-locus-specific glycoproteins. Proc Nat Acad Sci USA 90: 467-471

Foster GD, Robinson SW, Blundell RP, Roberts MR, Hodge R, Draper J, Scott RJ (1992) A *Brassica napus* mRNA encoding a protein homologous to phospholipid transfer proteins is expressed specifically in the tapetum and developing microspores. Plant Sci 84: 187-192

Goldberg RB, Beals TP, Sanders PM (1993) Anther development: Basic principles and practical applications. Plant Cell 5: 1217-1229

Heslop-Harrison Y (2000) Control gates and micro-ecology: The pollen-stigma interaction in perspective. Ann Bot 85: 5-13

Jeon JS, Chung YY, Lee S, Yi GH, Oh BG, An G (1999) Isolation and characterization of an anther-specific gene, RA8, from rice (*Oryza sativa* L.). Plant Mol Biol 39: 35-44

Kachroo A, Nasrallah ME, Nasrallah JB (2002) Self-incompatibility in the Brassicaceae: receptor-ligand signaling and cell-to-cell communication. Plant Cell 14: S227-S238

Kamalay JC, Goldberg RB (1980) Regulation of structural gene expression in tobacco. Cell 19: 934-946

Kim HU, Hsieh K, Ratnayake C, Huang AH (2002) A novel group of oleosins is present inside the pollen of Arabidopsis. J Biol Chem 277: 22677-22684

Kim SR, An G (1996) Isolation and characterization of a pollen-specific cDNA clone from Easter lily. J Plant Biol 39: 197-202

Kim SR, Kim Y, An G (1993) Molecular cloning and characterization of anther-preferential cDNA encoding a putative actin-depolymerizing factor. Plant Mol Biol 21: 39-45

Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein metabolism. J Mol Biol 157: 105-132

Lorenzo O, Calvo A, Nicolas C, Nicolas G, Rodriguez D (1999) Isolation, sequence analysis and expression of a LEA protein of *Fagus sylvatica* L. seeds (Accession No AJ 130888) (PGR99-017). Plant Physiol 119: 805

Mariani C, de Beuckeleer M, Truettner J, Leemans J, Goldberg RB (1990) Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature 347: 737-741

Mascarenhas JP (1990) Gene activity during pollen develop-

ment. Ann Rev Plant Physiol Plant Mol Biol **41**: 317-338

Orr W, Iu B, White TC, Robert LS, Singh J (1992) Complementary DNA sequence of a low temperature-induced *Brassica napus* gene with homology to the *Arabidopsis thaliana* kin1 gene. Plant Physiol **98**: 1532-1534

Rubinelli P, Hu Y, Ma H (1998) Identification, sequence analysis and expression studies of novel anther-specific genes of *Arabidopsis thaliana*. Plant Mol Biol 37: 607-619

Stanchev BS, Doughty J, Scutt CP, Dickinson H Croy RR (1996) Cloning of PCP1, a member of a family of pollen coat protein (PCP) genes from *Brassica oleracea* encoding novel cysteine-rich proteins involved in pollen-stigma interactions. Plant J **10**: 303-313

Steiner-Lange S, Unte US, Eckstein L, Yang C, Wilson ZA, Schmelzer E, Dekker K, Saedler H (2003) Disruption of *Arabidopsis thaliana* MYB26 results in male sterility due to non-dehiscent anthers. Plant J 34: 519-528

Walden AR, Walter C, Gardner RC (1999) Genes expressed in *Pinus radiata* male cones include homologs to anther-specific and pathogenesis response genes. Plant Physiol 121: 1103-1116

Willing RP, Bashe D, Mascarenhas JP (1988) An analysis of the quantity and diversity of messenger RNAs from pollen and shoots of *Zea mays*. Theor Appl Genet 75: 751-753

Willing RP, Mascarenhas JP (1984) Analysis of the complexity and diversity of mRNAs from pollen and shoots of *Tradescantia*. Plant Physiol 75: 865-868

Wu SS, Suen DF, Chang HC, Huang AH (2002) Maize tapetum xylanase is synthesized as a precursor, processed and activated by a serine protease, and deposited on the pollen. J Biol Chem 277: 49055-49064

Yui R, Iketani S, Mikami T, Kubo T (2003) Antisense inhibition of mitochondrial pyruvate dehydrogenase E1alpha subunit in anther tapetum causes male sterility. Plant J 34: 57-66