Enhancement of cell wall protein SRPP expression during emergent root hair development in *Arabidopsis*

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ABSTRACT

SRPP is a protein expressed in seeds and root hairs and is significantly induced in root hairs under phosphate (Pi)-deficient conditions. Root hairs in the knockout mutant *srpp-1* display defects, i.e., suppression of cell growth and cell death. Here, we analyzed the expression profile of SRPP during cell elongation of root hairs and compared the transcript levels in several mutants with short root hairs. The mRNA level was increased in wild-type plants and decreased in mutants with short root hairs. Induction of SRPP expression by Pi starvation occurred one or two days later than induction of Pi-deficient sensitive genes, such as *PH1* and *PHF1*. These results indicate that the expression of SRPP is coordinated with root hair elongation. We hypothesize that SRPP is essential for structural robustness of the cell walls of root hairs.

Semen hairs are specialized epidermal cells with large surface areas exposed to the soil, and they exhibit tip growth.1,2,3 The physiological roles of root hairs have been investigated.4,5,6 Root hairs must maintain water and nutrient absorption capacity and integrity to protect them from injury and pathogen attack. A unique protein, the seed and root hair protective protein (SRPP), was recently identified in the cell walls of root hairs and seeds.7 The SRPP (SEED AND ROOT HAIR PROTECTIVE PROTEIN) gene was identified previously as root hair-specific 13 (RHS13) by microarray analysis.8 SRPP is highly expressed and the protein was detected in root hairs grown under phosphate (Pi)-deficient conditions. SRPP knockout mutants have short, bent root hairs and root hair death as phenotypic properties.7 SRPP consists of 165 amino-acid residues and is rich in basic residues (pI, 9.21). A protein linked to green fluorescent protein was detected in the cell wall spaces of root hairs. Although the primary sequence of SRPP is similar to those of proline-rich proteins, the protein lacks a proline-rich motif. Molecular and biochemical information on SRPP is currently limited.

Here, we focused our attention on the expression profile of the SRPP gene and examined whether enhancement of SRPP expression is triggered by Pi depletion or coupled with emergent root hair elongation. The purpose of this study was to determine whether SRPP is involved in cell differentiation or root hair elongation. We determined the transcription levels of SRPP in the roots of short-root-hair and no-root-hair mutant seedlings, as well as in wild-type plants. The mRNA levels after Pi depletion treatment were also determined. Furthermore, genes co-expressed with SRPP were analyzed in the mutants and under Pi-deficient conditions.

SRPP is expressed in root hairs and seeds. This study focused on characteristics of the SRPP gene in root hairs. We investigated the relationship between root hair length and SRPP transcription level in wild-type (strain, Columbia-0), NR23#2-1, NR23#4-12, pip5k3 pip5k4, and cpc try seedlings. The transgenic lines NR23#2-1 and NR23#4-12 express a 23-amino-acid peptide in the N-terminal region of plasma membrane–associated cation-binding protein-2 (PCaP2)9,10 under the control of the root hair-specific *EXPANSIN A7* promoter.11 NR23#2–1 has short root hairs and NR23#4–12 has no root hairs.12 The double mutant *pip5k3 pip5k4*, which has mutations in the phosphatidylinositol–(4,5)-bisphosphate–generating isoenzymes, which are localized in the plasma membrane, has very short root hairs with a normal distribution density.12 A double mutant of the transcription factors CPC and TRY (*cpc try*) was reported to have abnormal trichomes and no root hairs.13 The phenotypes of the mutants were observed constantly (Fig. 1B). The transcription levels of SRPP were less than 20% that of Col-0 in NR23#4–12 and negligible in *cpc try* (Fig. 1A). The levels in the NR23#2–1 and *pip5k3 pip5k4* roots were 70% and 50% that of Col-0, respectively, and higher than that of NR23#4–12. The results indicate a correlation between root hair length and SRPP expression level.

The number and length of root hairs increase under Pi-deficient conditions.14 When 14-day-old Col-0 seedlings grown on normal agar plates were transferred to Pi-deficient plates, the distribution density and length of root hairs increased...
significantly after 5 days (Fig. 2A, B). In this experiment, the distribution density and length of root hairs were determined in the region around 30 mm from the root tip. The results show that Col-0 roots require 5 days to induce root hairs. The SRPP protein was not detected in seedlings grown under normal conditions, but SRPP mRNA was detected before Pi depletion treatment (Fig. 3A). The transcription level of SRPP was increased after 5 days, in parallel with the increases in the distribution density and length of root hairs, under Pi-deficient conditions (Fig. 3A). No difference in transcription level was detected on the first day (4, 6, and 8 hours after transplantation; data not shown). We compared the induction of SRPP with that of other genes. Phosphate transporter 1 (PHT1) and phosphate transporter traffic facilitator 1 (PHF1) are genes that respond rapidly to Pi deficiency. The expression of PHT1 was clearly induced on day 3 after Pi starvation, and that of PHF1 was clearly induced on day 4. Therefore, PHT1 and PHF1 may be first-phase genes and SRPP may be a second-phase gene in response to Pi deficiency. The results suggest that SRPP gene expression is required for the maturation of root hairs, and not for the initial stage of root hair differentiation, under Pi-deficient conditions.

We surveyed genes co-expressed with SRPP under normal physiological conditions using the ATTED-II open database (http://atted.jp). Several genes, including peroxidase superfamily proteins, proline-rich proteins, actin depolymerizing factors, and expansins, were listed with mutual ranks lower than 16. Expansins A7 and A18, obtained in this search, are
root hair–specific genes, and their translation products function to loosen the cell wall.\textsuperscript{8,17,18} Some peroxidases localized in the cell walls are essential for root hair development.\textsuperscript{8} We selected two genes encoding pectin methylesterases, At5g04960 (pectin methylesterase inhibitor superfamily/pectin methylesterase) and At3g10710 (RHS12; pectin methylesterase). Both genes were reported to be expressed in roots and root hairs in the open databases TAIR and Arabidopsis eFP Browser. We hypothesized that the positively charged SRPP (pI, 9.21) would interact with the negatively charged group of pectin (galacturonic acid moiety) in the cell wall. The RHS12 expression levels were 30\% that of Col-0 in NR23\#4\#1, negligible in cpc try, and relatively low in NR23\#2\#1 and pip5k3 pip5k4 (Fig. 4). The expression level of At5g04960 had a similar profile to that of RHS12. The results indicate that both gene expression profiles are related to the length and presence/absence of root hairs as well as SRPP.

We investigated the responses of the two genes to Pi deficiency in Col-0. The RHS12 and At5g04960 genes were induced after 2 and 4 days, respectively, by Pi deficiency, and their relative expression levels reached four and three times, respectively, those on day 0 (Fig. 5). Therefore, these two pectin methylesterase genes tend to be expressed during root hair elongation and in response to Pi deficiency. Their transcriptional properties and cell-wall localization are similar to those of SRPP. However, the inductions of RHS12 and At5g04960 are a few days earlier than that of SRPP. This may be reflection of their physiological role as pectin methylesterases, which may be required at early stage of root hair development compared with SRPP.

In conclusion, this study revealed the following with regard to SRPP gene expression. (1) SRPP gene expression was suppressed in mutant roots with short root hairs or no root hairs. Enhancement of SRPP did not occur in the early stage of Pi-deficiency response in seedling roots, but in the later stage, after the induction of sensitive genes, such as PHT1 and PHF1. (2) Two pectin methylesterase genes (At5g04960; RHS12), which were listed as RHS genes co-expressed with SRPP in the ATTED-II database, were enhanced by Pi deficiency and suppressed in mutant lines with no or short root hairs. Consistent with a previous report,\textsuperscript{7} the present observations suggest that SRPP is involved in the

\textbf{Figure 3.} Changes in transcription levels of SRPP, PHT1, and PHF1 under Pi-deficient conditions. Col-0 seedlings grown under normal conditions (Pi, 280 \(\mu\)M) for 14 days were transplanted to Pi-deficient medium (Pi, 0 \(\mu\)M). Total RNA fractions were prepared from roots of seedlings each day after transplantation and then subjected to real-time RT-PCR to quantify the mRNA levels of SRPP (A), PHT1 (B), and PHF1 (C). Four replicates of 21–38 plants were averaged. Values are expressed as means ± SDs. Significant differences from day 0 are indicated by asterisks (\(P < 0.005\)).
ponent of the cell wall and determines its structural rigidity.19,20
charged SRPP molecules are thought to bind electrostatically to
the regulation of cell wall rigidity and plasticity. Positively
studies may provide more details on cell wall architecture and
formation of cell walls during emergent root hair development
induced by Pi deficiency, but not in root hair differentiation.

Although SRPP is known to function in the cell wall, further
studies may provide more details on cell wall architecture and
the regulation of cell wall rigidity and plasticity. Positively
charged SRPP molecules are thought to bind electrostatically to
negatively charged demethylated pectins. Pectin is a key com-
ponent of the cell wall and determines its structural rigidity.19,20
Co-expression of pectin methylsterase genes with SRPP may
be involved in the reformation of the cell wall structure. Then,
demethylated pectin interacts with SRPP and calcium ions,
forming a tertiary network of pectin fibers. Biochemical analy-
theses of the interaction between SRPP and pectin remain to be
conducted.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

Acknowledgments
We are grateful to Yoichi Nakanishi, Miki Kawachi, and Shoji Segami
(Nagoya University, Japan) for their valuable advice and to Takashi Aoyama
(Kyoto University, Japan) and Takuji Wada (Hiroshima University, Japan)
for sharing with us the double mutants pip5k3 pip5k4 and cpc try, respect-
ively. We would also like to thank the Riken Bioresource Center (Tsukuba,
Japan) for delivering seeds of the srpp-1 mutant (RATM13-5238-1).

Funding
This work was supported by JSPS KAKENHI (grant nos. 26252011 and
26113506 to M.M.) and by a Grant-in-Aid for JSPS Fellows (no. 26002201
to N.T.-T.).

Abbreviations
N23 23-amino-acid peptide in the N-terminal region of
PCaP2
NR23 no root hair line that expresses N23
PCaP2 plasma membrane–associated cation-binding protein-2
PHF1 phosphate transporter traffic facilitator 1
PHT1 Phosphate transporter 1
Pi inorganic phosphate
RHS root hair specific
SRPP seed and root hair protective protein

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Figure 5. Responses of two PME genes to Pi deficiency. Expression levels of RHS12 and At5g04960 in Col-0 in response to Pi-deficient conditions. Col-0 seedlings grown under normal conditions (Pi, 280 μM) for 14 days were transplanted to Pi-deficient medium (Pi, 0 μM). Total RNA fractions were prepared from Col-0 roots every day after transplantation to Pi-deficient medium (day 0, day of transplantation), and then subjected to real-time RT-PCR analyses. Four replicates with 21–38 roots were averaged. Results are means ± SDs. Asterisks, P < 0.005.
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