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Identification of two phosphatidylinositol/phosphatidylcholine transfer protein genes that are predominately transcribed in the flowers of *Arabidopsis thaliana*

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Summary

The Sec14 protein (Sec14p) and its homologs are involved in the transfer of phosphatidylinositol/phosphatidylcholine phospholipids in eukaryotic cells. In the completely sequenced genome of *Arabidopsis thaliana*, multiple genes encoding putative Sec14p homologs have been predicted based on bioinformatic analysis. Here we report the identification of two yeast *Sec14*-like genes (designated as *AtSFH3* and *AtSFH12*, respectively) that are predominately transcribed in *Arabidopsis* flowers. The deduced amino acid sequences of AtSfh3p and AtSfh12p exhibited high similarity to that of Sec14p. Ectopic expression of AtSfh3p or AtSfh12p corrected the high temperature sensitive phenotype caused by Sec14p functional deficiency in *Saccharomyces cerevisiae*, indicating that the two plant homologs are functional in the intracellular environment. *AtSFH3* transcripts were detected in flowers, stems and immature siliques but not roots and leaves, with a relatively higher transcript level in the flowers. In contrast, *AtSFH12* transcripts were only detectable in the flowers. Based on histochemical staining of β-glucuronidase (GUS) activities in the transgenic *Arabidopsis* plants harboring promoter::GUS constructs, *AtSFH3* transcription was first detected in the stigma papillae of the flowers at stage 11, and then in the pollen grains before and after fertilization. On the other hand,

Abbreviations: AtSFH, *Arabidopsis* Sec14 homolog; GFP, green fluorescence protein; GUS, β-glucuronidase; PCR, polymerase chain reaction; PITP, phosphatidylinositol/phosphatidylcholine transfer protein; Pld1p, phospholipase D1; PtdCho, phosphatidylcholine; PtdIns, phosphatidylinositide; RT-PCR, reverse transcription-polymerase chain reaction; Sec14p, Sec14 protein

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AtSFH12 transcription was only found in the mature and germinating pollen grains. The information from this study may provide useful clue for further analysis of the function of plant Sec14p homologs in the development of the male gametic cells and/or the fertilization process in higher plants.
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Introduction

Phosphatidylinositol/phosphatidylcholine transfer proteins (PITPs) are ubiquitous proteins that bind and exchange one molecule of phosphatidylinositide (PtdIns) or phosphatidylcholine (PtdCho) and facilitate the transfer of these phospholipids among the different membrane compartments of eukaryotic cells (Cleves et al., 1991a; Wirtz, 1991). The available results suggest that PITPs can be divided into two classes, one composed of the proteins from mammalian, amphibian and insect cells and the other of the proteins from fungal, mammalian and plant cells (Bankaitis et al., 1989, 1990; Dickeson et al., 1989; Lopez et al., 1994; Chinen et al., 1996; Merkulova et al., 1999; Kempná et al., 2003). Although the two classes of proteins share no significant amino acid sequence similarity with each other, yeast and metazoan PITPs execute very similar functions in PtdIns and PtdCho transfer and secretory vesicle formation within the cellular environment (Bankaitis et al., 1989; Dickeson et al., 1989).

To date, three types of soluble PITPs have been found in mammalian cells, PITPα, PITPβ and RdgBp (Dickeson et al., 1989; Tanaka and Hosaka, 1994; Milligan et al., 1997). In *Saccharomyces cerevisiae*, Sec14 protein (Sec14p) is the major PITP that is essential for protein secretion from the trans-Golgi network (Bankaitis et al., 1989, 1990). Functional deficiency of Sec14p leads to the arrest of cell growth at elevated temperature (37 °C). But this growth defect is suppressed by the *cki1* mutation in the CDP-choline pathway of PtdCho biosynthesis (Cleves et al., 1991b). Further analysis has shown that the *cki1* suppression depends on the existence of a functional phospholipase D1 (Pld1p) that mediates PtdCho turnover in yeast cells (Rudge et al., 2004). Consequently, the *sec14^{ts} cki1* double mutant, but not the *sec14^{ts}cki pld1* triple mutant, is viable at the restrictive temperature (37 °C, Xie et al., 1998). *S. cerevisiae* cells also express five Sec14p homologs (named as Sfhs 1 to 5, Li et al., 2000a). Despite significant amino acid sequence identities, Sec14 and SFH proteins may differ in some functional properties. For example, ectopic expression of Sec14p can rescue the growth of *sec14^{ts}* and *sec14^{ts}cki pld1* mutants with similar efficiency, whereas that of Sfh4p can only restore the growth of the *sec14^{ts}* mutant (Schnabl et al., 2003). Unlike Sec14p that may transfer both PtdIns and PtdCho, Sfh2-4 proteins may transfer only PtdIns (Li et al., 2000a). Additionally, the six proteins also exhibit differences in subcellular localization and several other properties (Schnabl et al., 2003). Taken together, the results gathered so far appear to indicate that Sec14p and its homologs are an important family of proteins that may be essential for the normal growth of *S. cerevisiae* cells.

Plant proteins similar to Sec14p have been identified. They include soybean Sec14p homologs (Ssh1p, Ssh2p), LjPLPs (*Lotus japonicus* PITP-like proteins) and *Arabidopsis thaliana* Sec14 homologs (AtSFHs) (Jouannic et al., 1998, Kearns et al., 1998; Kapranov et al., 2001; Monks et al., 2001; Böhme et al., 2004; Peterman et al., 2004; Vincent et al., 2005). Ssh1p is a novel phosphoinositide binding protein (Kearns et al., 1998). Phosphorylation of Ssh1p occurs rapidly under hyperosmotic stress, which may protect the plant from being injured (Kearns et al., 1998; Monks et al., 2001). LjPLPs have both the Sec14p-related lipid binding domain and the Nlj16-like nodulin domain in their primary structure (Kapranov et al., 2001). The genes encoding LjPLPs are transcriptionally controlled and may function in the signaling pathways that regulate membrane biogenesis during nodule development (Kapranov et al., 2001). Using the completed genome sequencing data, 31 Sec14p homologs have been identified in *Arabidopsis*, among which six members have been named as PATLs 1 to 6 and 14 members as AtSFHs 1–14 (Peterman et al., 2004; Vincent et al., 2005). Jouannic et al. (1998) reported the first molecular and functional analysis of an *Arabidopsis* PITP member. Subsequently, the PATL1 (Patllin1) protein was found to associate with the cell plate and may play an important role in the membrane trafficking events involved in cell plate expansion or maturation (Peterman et al., 2004). AtSFHs 1–14 exhibit high similarity to LjPLPs (Vincent et al., 2005). Molecular genetic and cell biological investigations have shown that the COW1/AtSfh1p protein is required for polarized membrane growth during the development

of *Arabidopsis* root hairs (Böhme et al., 2004; Vincent et al., 2005).

Compared to the knowledge on mammalian and yeast PITPs, the understanding of the molecular biology and biochemical properties of plant PITPs is still limited. The existence of more than 30 PITP members in *Arabidopsis* suggests that these proteins may be important to many aspects of the growth, development and reproduction of plants. However, the presence of multiple PITP proteins in a given plant species may make it difficult to dissect the function of individual members because of potential functional redundancy. Although the knock-out of COW1/AtSfh1p has been found to confer abnormal root hair development in *Arabidopsis* (Böhme et al., 2004; Vincent et al., 2005), no abnormal morphological phenotypes were scored in our initial screening of an additional set of 43 T-DNA insertional mutants of 25 *Arabidopsis* PITP members under standard growth condition (unpublished data). Therefore, the purpose of this work was to identify the *PITP* genes that are predominantly expressed in given *Arabidopsis* vegetative or reproductive organs, which should facilitate the selection of unique members for more advanced functional analysis.

Table 1. Yeast strains used in this work

Name	Genotype	Source
NY430	*MATa ura3-52, sec14-3^{ts}*	Novick et al. (1980)
PGY84	*MATα leu2, trp1, lys2, ura3, his3, sec14-1^{ts}*	Schnabl et al. (2003)

Materials and methods

Plant and yeast materials, oligonucleotide primers and standard molecular methods

The Col-0 ecotype of *A. thaliana* was used throughout this study. General condition for *Arabidopsis* growth was described previously (Li et al., 2002). For the functional complementation experiments, two yeast strains (NY430, PGY84, Table 1) were employed. The *sec14^{ts}* mutant alleles in NY430 (*sec14-3^{ts}*) and PGY84 (*sec14-1^{ts}*) block cell growth at the restrictive temperature (37 °C, Novick et al., 1980; Schnabl et al., 2003). Yeast growth media were prepared according to the methods described by Invitrogen (http://www.invitrogen.com/content/sfs/manuals/pyes2_man.pdf). The oligonucleotide primers used in this study were listed in Table 2. For the molecular experiments involving DNA or RNA manipulations, the methods described by Sambrook and Russell (2001) were followed. Restriction enzyme digestion analysis and DNA sequencing were always carried out to confirm the correctness of the cloning experiments.

Table 2. Oligonucleotide primers used in this work

Name	Sequence (5′-3′)[a]	Specificity	Use
AS3F	AGAGCGGCCGCATGACGGATACAATGTC	*AtSFH3*	cDNA cloning
AS3R	TCAGGATCCTCAGAATCCAAAGAAC		
AS12F	CCTTACCTGCCTAATTCCAAC	*AtSFH12*	cDNA cloning
AS12R	CTTCATGGTTCTTCTCTCACC		
Y-AtSFH3F	AGAGGATCCATGACGGATACAATGTC	*AtSFH3*	Preparation of yeast expression construct
Y-AtSFH3R	TCACTCGAGTCAGAATCCAAAGAAC		
Y-AtSFH12F	ATTAAGCTTATGACTTTGATCCAAGATGC	*AtSFH12*	Preparation of yeast expression construct
Y-AtSFH12R	ATTGAATTCTCACCAGTAG TTGAACAGC		
AtSFH3F	CGAGCTTCTCCCTTCCAAAC	*AtSFH3*	Semi-quantitative RT-PCR
AtSFH3R	CAAGTGCAGTTACCACCCAAG		
AtSFH12F	AAGATGTGCATGACGCAGAG	*AtSFH12*	Semiquantitative RT-PCR
AtSFH12R	TGCTGTAGTCTTCGGGTCAAG		
P-AtSFH3F	AATGAGCTCAGCTGGAAGCTTGGGGTCATTC	*AtSFH3*	Amplification of promoter element
P-AtSFH3R	CAACCATGGGGTTAAAGTCTGGATTTAAG		
P-AtSFH12F	AATGAGCTC AACACCGAAACTCTTTTTTC	*AtSFH12*	Amplification of promoter element
P-AtSFH12R	ATTCCATGG GATTTTGCCA GATAGTTGG		
TuF	AGAACACTGTTGTAAGGCTAAAC	Tubulin	Semi-quantitative RT-PCR
TuR	GAGCTTTACTGTCTCGAACATGG		

[a]The underlined nucleotides form *Bam*HI (GGATCC), *Eco*RI (GAATTC), *Hind*III (AAGCTT), *Nco*I (CCATGG), *Not*I (GCGGCCGC), *Sac*I (GAGCTC), and *Xho*I (CTCGAG) restriction sites, respectively.

Cloning and sequencing of cDNAs

Total RNA samples were prepared from *Arabidopsis* plants using RNeasy plant mini kit (Qiagen, Germany). After further treatment with the RNAase-Free-DNAase set (Qiagen, Germany), RNA was converted to cDNA using M-MLV reverse transcriptase (Promega). To amplify the cDNAs for the complete coding regions of *AtSFH3* (*At2g21540*) and *AtSFH12* (*At4g36490*) by reverse transcription-polymerase chain reaction (RT-PCR), oligonucleotide primers (Table 2) were synthesized according to the genome sequence information of *Arabidopsis* (http://www.arabidopsis.org/). The amplified cDNA fragments were cloned using the pGEM-T easy vector (Promega). Multiple independent plasmid clones were sequenced commercially (Takara, Japan), and the resulted information was used to construct the nucleotide sequences for the coding regions of *AtSFH3* and *AtSFH12*, respectively.

Complementation of *S. cerevisiae sec14^{ts}* mutants by *AtSFH* cDNAs

AtSFH3 and *AtSFH12* cDNAs were individually inserted into the yeast expression vector pYES2 (Invitrogen) under the control of the *GAL1* promoter using the restriction sites *Bam*HI/*Xho*I and *Hind* III/*EcoR*I, respectively. The derived constructs were referred to as pAtSFH3 and pAtSFH12, respectively. Both constructs and the vector pYES2 were each transformed into the two yeast strains listed in Table 1 using a simplified yeast transformation method (Gietz and Woods, 2002). The recombinant yeast strains were selected by growing on synthetic medium without uracil, and were further verified using PCR amplification of *AtSFH3* or *AtSFH12* coding regions as described previously (Ling et al., 1995). For the functional complementation tests, the recombinant strains were first grown on the liquid medium supplemented with 2% galactose and 1% raffinose until A_{600} reached 1.0. Serial dilutions were then prepared and spotted onto two sets of identical plates containing the solid medium (with 2% galactose and 1% raffinose). After spotting, the two sets of plates were incubated for 3 days at 28 and 37 °C, respectively, to assay if the expression of *AtSFH3* or *AtSFH12* cDNAs would correct the high temperature sensitivity of the mutant yeast strains.

Evaluation of *AtSFH* expression patterns in *Arabidopsis* organs

Root, stem, leaf, flower and immature silique samples were collected from 6 to 8 weeks old *Arabidopsis* plants. Total RNA samples were prepared and converted to cDNAs as described above. After normalization of the cDNA samples, the evaluation of the transcript levels of *AtSFH3* and *AtSFH12* was conducted using gene specific oligonucleotide primers (Table 2) as reported in a previous study (Li et al., 2002).

Development of transgenic lines expressing *AtSFH3* or *AtSFH12* promoter::*β*-glucuronidase (GUS) constructs

DNA fragments, immediately upstream of the predicted translation start codon of *AtSFH3* or *AtSFH12*, were amplified by genomic PCR with the oligonucleotide primers listed in Table 2. The promoter fragments (1523 bp for *AtSFH3*, 977 bp for *AtSFH12*) were then used to replace the 35S promoter in the pJIT166 vector, which contains the complete coding region of the bacterial GUS (Guerineau et al., 1992), using the restriction sites *Sac*I/*Nco*I. This created two constructs in which the GUS coding region is downstream of the promoter fragments of *AtSFH3* or *AtSFH12*, respectively. The promoter::GUS reporter cassettes were each excised using double-enzyme digestions with *Sac*I and *Xho*I, followed by cloning into the T-DNA vector pBINPLUS (van Engelen et al., 1995). The resultant T-DNA constructs were used to transform *Arabidopsis* using the floral dip method (Clough and Bent, 1998). Homozygous transgenic lines, bearing the promoter::GUS reporter for *AtSFH3* and *AtSFH12*, respectively, were identified (using kanamycin resistance and PCR amplification of the GUS coding region) in the T3 generation.

Histochemical staining of GUS activity

The promoter::GUS reporter transgenic plants were grown under normal conditions to the flowering stage. They were then processed for histochemical staining of GUS activity as described previously (Gallagher, 1992). The treated materials were examined under a dissection microscope (Olympus SZX12, Japan) to locate GUS signals. The staining results were recorded using a digital camera equipped with the dissecting microscope.

Results

Isolation of cDNA clones corresponding to the complete coding regions of *AtSFH3* and *AtSFH12*

In RT-PCR experiments, the cDNA fragments corresponding to the complete coding regions of

AtSFH3 and *AtSFH12* were most readily amplified from the cDNA sample derived from flowers. The nucleotide sequences determined for *AtSFH3* and *AtSFH12* coding regions in this work were identical to those deposited in the GenBank database (AK176420 and AY057587 for *AtSFH3*, AY050419 and BT000834 for *AtSFH12*). Like the well characterized COW1/AtSfh1p protein, the deduced amino acid sequences of AtSfh3p and AtSfh12p contained the Sec14p-related lipid binding domain and the Nlj16-like nodulin domain as previously described by Vincent et al. (2005). Amino acid sequence comparisons revealed that COW1/AtSfh1p, AtSfh3p and AtSfh12p were more than 60% identical.

Complementation of yeast *sec14^{ts}* mutant alleles by expression of *AtSFH3* and *AtSFH12* cDNAs

In past studies, complementation of *S. cerevisiae* *sec14^{ts}* mutants has frequently been used as a reliable strategy for revealing the functionality of *Sec14*-like genes from higher plants. However, during the complementation tests, some authors employed the *sec14-1^{ts}* mutant (Kearns et al., 1998; Kapranov et al., 2001; Böhme et al., 2004; Vincent et al., 2005), while the others used the *sec14-3^{ts}* mutant (Jouannic et al., 1998). To facilitate the comparison of our complementation data with those published previously, both *sec14* alleles were employed for testing the functionality of *AtSFH3* and *AtSFH12* in the present study, even though the two *sec14^{ts}* alleles may be identical (Li et al., 2000b). From Fig. 1, it is clear that, under the control of the *GAL1* promoter, ectopic expression of *AtSFH3* or *AtSFH12* cDNAs restored the growth to the NY430 strain possessing the *sec14-3^{ts}* allele at the restrictive temperature (37 °C). A similar result was obtained when the PGY84 strain (carrying the *sec14-1^{ts}* allele) was employed for the functional complementation tests (data not shown).

Detection of *AtSFH3* and *AtSFH12* transcripts in *Arabidopsis* organs

Using gene specific oligonucleotide primers and semi-quantitative RT-PCR, the transcriptional patterns of *AtSFH3* and *AtSFH12* in five *Arabidopsis* organs (roots, stems, leaves, flowers, immature siliques) were examined. *AtSFH3* transcription was detectable in stems, flowers and immature siliques, with a relatively higher transcript level found in the flowers (Fig. 2). In contrast, the transcription of *AtSFH12* was only detectable in the flowers but not the other four organs (Fig. 2). These transcriptional patterns were reproducible with variable numbers of PCR amplification cycles (Fig. 2), and in independent semi-quantitative RT-PCR experiments (data not shown).

Comparative analysis of in situ transcriptional patterns of *AtSFH3* and *AtSFH12* during *Arabidopsis* flower development

In addition to the semi-quantitative RT-PCR experiment described above, homozygous transgenic

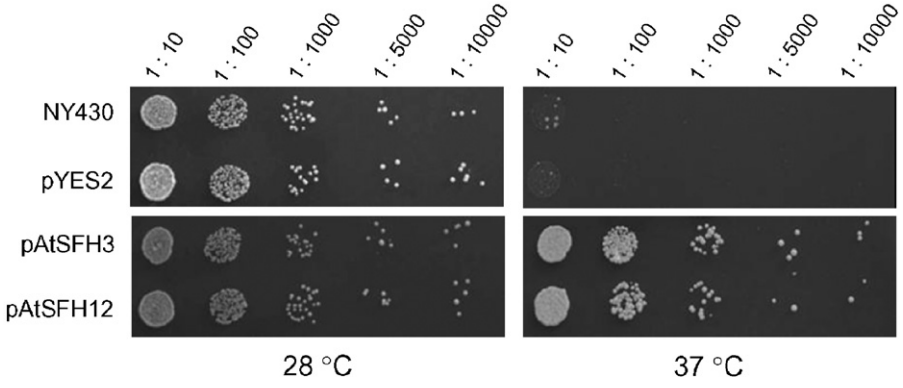

Figure 1. Complementation of *S. cerevisiae* *sec14^{ts}* mutant alleles by the cDNAs of *AtSFH3* or *AtSFH12*. The Sec14p defective mutant strain NY430 (with *sec14-3^{ts}* allele) and the recombinant strains containing the plasmids pYES2 (as control), pAtSFH3 (for expressing *AtSFH3* cDNA in NY430 background) or pAtSFH12 (for expressing *AtSFH12* cDNA in NY430 background) were serially diluted and spotted onto two sets of identical plates. They were then incubated for 3 days at 28 and 37 °C, respectively. The growth of the cells harboring pAtSFH3 or pAtSFH12 at 37 °C indicated functional complementation of the *sec14-3^{ts}* allele by the cDNAs of *AtSFH3* or *AtSFH12*. Using a similar strategy, the cDNAs of *AtSFH3* and *AtSFH12* were also found to complement the *sec14-1^{ts}* allele in another Sec14p defective mutant strain PGY84 (data not shown).

Arabidopsis plants containing promoter::GUS reporter were employed to compare, in more detail, the transcriptional patterns of *AtSFH3* and *AtSFH12* during flower development in *Arabidopsis*. For the *AtSFH3* promoter::GUS plants, the GUS signals were first detected in the stigmatic papillae at stage 11, followed by the pollen grains inside the anthers at stage 12 and the germinating pollen grains on top of the stigmatic papillae and the stylar tissue at stage 13 (Fig. 3). On the other hand, in the *AtSFH12* promoter::GUS plants, no GUS signal was found in the stigmatic papillae at stages 11, 12 or 13 (Fig. 3). Instead, positive GUS signals were only found in the pollen grains inside the anthers (at stage 12) and the germinating pollen grains on top of the stigmatic papillae (at stage 13) (Fig. 3).

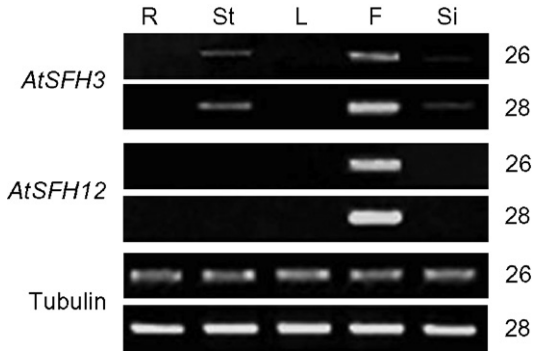

Figure 2. Relative transcript levels of *AtSFH3* and *AtSFH12* in the vegetative and reproductive organs of *Arabidopsis* assessed using semi-quantitative RT-PCR. The cDNA samples for roots (R), stems (St), leaves (L), flowers (F) and immature siliques (Si) were normalized by amplifying tubulin transcripts. The relative transcript levels of *AtSFH3* and *AtSFH12* in the different organs were then evaluated using the normalized cDNA samples with gene specific oligonucleotide primers (Table 2). The RT-PCR experiments were conducted with varying numbers of thermocycles (26, 28) to confirm the linearity of the amplification and the reproducibility of the transcriptional patterns revealed.

Discussion

In the present study, we have used several complementary approaches to study the potential functionality and transcriptional patterns of *AtSFH3* and *AtSFH12*, two *Arabidopsis* genes whose deduced protein products show high similarity to the Sec14 protein of *S. cerevisiae*. Because of its well characterized phenotype, complementation of the yeast *sec14*^{ts} mutants has been successfully used in the initial characterization of the genes that are homologous to *Sec14* (Jouannic et al., 1998; Kearns et al., 1998; Kapranov et al., 2001; Böhme et al., 2004; Vincent et al., 2005). So far,

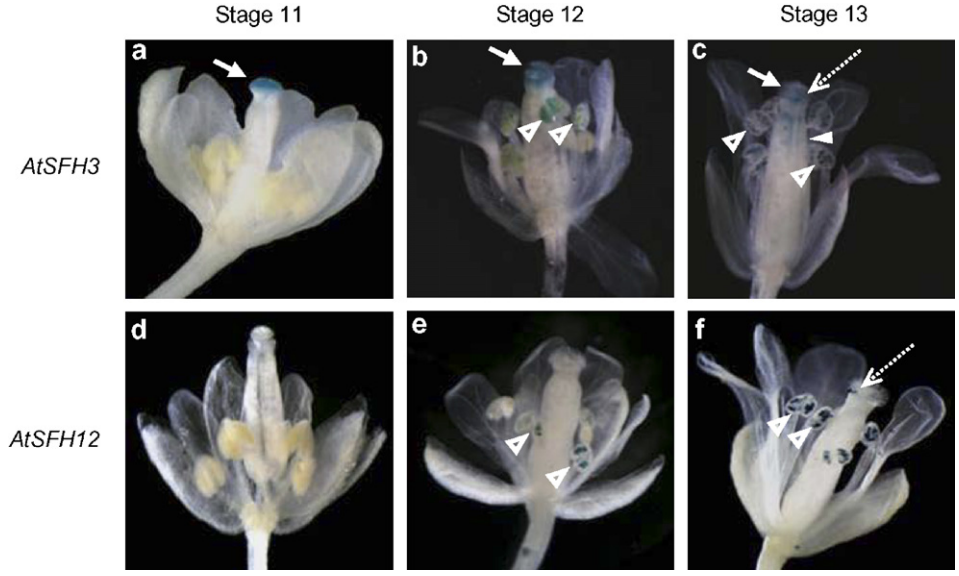

Figure 3. Comparison of in situ transcriptional patterns of *AtSFH3* and *AtSFH12* during *Arabidopsis* flower development by histochemical staining of transgenic plants harboring promoter::GUS reporter constructs. The blue precipitates represent positive GUS staining. Judging from the GUS signals, *AtSFH3* transcription may occur in the stigma papillae (arrows in (a)–(c)), the pollen grains inside the anthers (empty arrowheads in (b)), the germinating pollen grains on top of the stigma papillae (dashed arrow in (c)), and the stylar tissues (filled arrowhead in (c)). In contrast, *AtSFH12* transcription may take place in only the pollen grains inside the anthers (empty arrowheads in (e)) and the germinating pollen grains on top of the stigma papillae (dashed arrow in (f)). Furthermore, *AtSFH3* transcription may start earlier than that of *AtSFH12* during *Arabidopsis* flower development (compare (a) and (d)). The flowering stages shown in this figure were determined according to the study by Smyth et al. (1990).

nine *Arabidopsis* PITP genes (including *AtSFH12*) have been shown to complement *sec14*^{ts} mutant (Jouannic et al., 1998; Böhme et al., 2004; Vincent et al., 2005). In this work, we have for the first time reported the complementation of the *sec14-1*^{ts} and *sec14-3*^{ts} mutants by *AtSFH3*. In addition, we also confirmed the capacity of *AtSFH12* to complement *sec14*^{ts} mutant as observed before (Vincent et al., 2005). This suggests that the protein products of *AtSFH3* and *AtSFH12* are functional in an intracellular environment. However, unlike the wild type *Sec14* that can restore the growth of the PGY57 strain that is deficient for both Sec14p and Pld1p activities (Schnabl et al., 2003), we recently found that neither *AtSFH3* nor *AtSFH12* could rescue the growth of the PGY57 cells at 37 °C (unpublished data). The ectopic expression of AtSFH12p lipid-binding domain also failed to rescue the *sec14*^{ts} mutant in the Pld1p (SPO14) deficient genetic background (Vincent et al., 2005). These data indicate that the protein products of *AtSFH3* and *AtSFH12* may not be identical to yeast Sec14p in biochemical and/or cell biological properties.

In all the previous studies on plant Sec14p-like proteins that contain both lipid-binding domain and Nlj16-like domain in their primary structure, ectopic expression of the lipid-binding domain alone, but not the full length protein, has been found to be optimal for complementing *sec14*^{ts} mutants (Kapranov et al., 2001; Böhme et al., 2004; Vincent et al., 2005). Because the Nlj16 domain of LjPLP-IV has been found to target green fluorescence protein (GFP) to the plasma membrane (Kapranov et al., 2001), it is possible that the Nlj16-like domain may affect the subcellular localization of plant Sec14p-like proteins in yeast cells, which may interfere with the ability of the full length protein to complement *sec14*^{ts} mutants. In the present study, we found that full length AtSfh3p and AtSfh12p complemented *sec14*^{ts} mutants when ectopically expressed using the pYES2 vector. A similar finding has been made recently in a new set of complementation experiments involving the expression of full length AtSfh3p and AtSfh12p using an alternative yeast expression vector (p181AINE, unpublished data). The reason(s) underlying above difference between our complement study and those published previously is not clear at present. The Nlj16-like domains among individual plant Sec14p-like proteins can differ considerably in size and amino acid sequence identity (unpublished data). There is a possibility that difference in the functionality of different Nlj16-like domains, which may be caused by divergence in amino acid sequence, would affect the behavior of plant Sec14p-like proteins in yeast cells and thus their capacity to complement *sec14*^{ts} mutants. Further experiments are under way to test this hypothesis.

Based on their relative transcript levels in the vegetative and reproductive organs, we deduce that the expression of *AtSFH3* and *AtSFH12* may be subject to spatial regulation in *Arabidopsis* plants. The expression of *AtSFH12* may be restricted to the flower organ because its transcripts were only detectable in the flowers. The expression of *AtSFH3* may be regulated in a more complex manner because its transcription was detected in three organs (stems, flowers, immature siliques) with the highest relative transcript level detected in the flowers. The transcriptional patterns revealed for *AtSFH3* and *AtSFH12* by this work are consistent with the microarray analysis of *Arabidopsis* gene expression profiles deposited in the public database (http://www.cbs.umn.edu/arabidopsis/) or described by Ma et al. (2005). In addition to the investigations of the relative transcript levels of *AtSFH3* and *AtSFH12* using semi-quantitative RT-PCR, comparative analysis of the transgenic plants expressing promoter::GUS reporter provided more details on the transcription of the two members during flower development in *Arabidopsis*. *AtSFH3* differed from *AtSFH12* by being transcribed in more tissue types (stigmatic papillae, style, mature and germinating pollen grains). The transcription of *AtSFH12* was again found highly regulated with its transcripts being only detectable in the mature and germinating pollen grains. To our knowledge, this is the first time that a plant PITP member specifically transcribed in the mature and geminating pollen grains is reported.

The highly specific transcriptional patterns of *AtSFH3* and *AtSFH12* during flower development raise the question if the two members would play a role during the fertilization process of *Arabidopsis*. It is well known that the fertilization process of higher plants, including the germination of pollen grains on the stigma, guidance of pollen tubes in the style and delivery of the sperm nuclei to the ovule, requires polarized membrane trafficking (Edlund et al., 2004). Considering that the products of *AtSFH3* and *AtSFH12* and their close homolog COW1/AtSfh1p have all been shown to share similar biochemical properties with Sec14p (Böhme et al., 2004; Vincent et al., 2005; this work), and the fact that COW1/AtSfh1p has been found essential for polarized membrane growth in *Arabidopsis* root hairs, it is probable that *AtSFH3* and *AtSFH12* may be involved in certain aspects of protein secretion and polarized membrane trafficking during the fertilization process. The finding of *AtSFH3* and *AtSFH12* to be predominantly transcribed in the

flowers by this work indicates that, in addition to playing significant roles in cell plate expansion and root hair growth (Böhme et al., 2004; Peterman et al., 2004; Vincent et al., 2005), some PITP members may also be important in the reproductive biology of higher plants. To examine this hypothesis more systematically, we are now in the process of characterizing *Arabidopsis* mutants with altered expression levels of *AtSFH3* or *AtSFH12*, and studying the biochemical properties of the two members using purified proteins.

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