Plants Express a Lipid Transfer Protein with High Similarity to Mammalian Sterol Carrier Protein-2*

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This is the first report describing the cloning and characterization of sterol carrier protein-2 (SCP-2) from plants. Arabidopsis thaliana SCP-2 (AtSCP-2) consists of 123 amino acids with a molecular mass of 13.6 kDa. AtSCP-2 shows 35% identity and 56% similarity to the human SCP-2-like domain present in the human D-bifunctional protein (DBP) and 30% identity and 54% similarity to the human SCP-2 encoded by SCP-X. The presented structural models of apo-AtSCP-2 and the ligand-bound conformation of AtSCP-2 reveal remarkable similarity with two of the structurally known SCP-2s, the SCP-2-like domain of human DBP and the rabbit SCP-2, correspondingly. The AtSCP-2 models in both forms have a similar hydrophobic ligand-binding tunnel, which is extremely suitable for lipid binding. AtSCP-2 showed in vitro transfer activity of BODIPY-phosphatidylcholine (BODIPY-PC) from donor membranes to acceptor membranes. The transfer of BODIPY-PC was almost completely inhibited after addition of 1-palmitoyl 2-oleoyl phosphatidylcholine or ergosterol. Dimyristoyl phosphatidic acid, stigmasterol, steryl glucoside, and cholesterol showed a moderate to marginal ability to lower the BODIPY-PC transfer rate, and the single chain palmitic acid and stearoyl-coenzyme A did not affect transfer at all. Expression analysis showed that AtSCP-2 mRNA is accumulating in most plant tissues. Plasmids carrying fusion genes between green fluorescent protein and AtSCP-2 were transfected with particle bombardment to onion epidermal cells. The results from analyzing the transformants indicate that AtSCP-2 is localized to peroxisomes.

The mammalian sterol carrier protein-2 (SCP-2)* is an intracellular, small, basic protein that in vitro enhances the transfer of lipids between membranes. The core of the SCP-2 protein forms a five-stranded β-sheet flanked by five α-helices. A C-terminal segment, together with part of the β-sheet and four α-helices form a hydrophobic tunnel constituting the binding site for cholesterol, phospholipids, fatty acids, and fatty acyl coenzyme A (1–3). The human 13.2-kDa SCP-2 is encoded by the gene SCP-X, which apart from SCP-2 encodes thiolase. SCP-X has two separate promoters controlling the transcription of two transcripts of 3.2 and 1.8 kb, respectively (4). Translation of the longer messenger RNA yields the 58-kDa protein SCP-X, which consists of thiolase carrying SCP-2 in the C-terminus. The shorter transcript is translated into pre-SCP-2 of 15 kDa. The mature SCP-2 is formed by cleavage of either SCP-X or pre-SCP-2. SCP-2 carries a peroxisomal targeting signal in the C-terminal, and is predominantly localized in the peroxisomes although a substantial proportion is present in other cellular locations, such as the mitochondria, cytoplasm, and the endoplasmic reticulum (reviewed in Ref. 5).

SCP-2 was originally described as a sterol-binding protein, and one of the hypotheses regarding its function is that SCP-2 is involved in cytosolic non-vesicular transfer of cholesterol (5). There is some experimental support for this hypothesis, such as results showing that the intracellular transport of cholesterol to the plasma membrane slows down when fibroblasts are treated with SCP-2 antisense oligonucleotides (6). Moreover, there was an increase in the rates of cholesterol transfer to the plasma membrane when SCP-2 was overexpressed in rat hepatoma cells (7).

A second hypothesis suggests that SCP-2 plays a role in peroxisomal oxidation of fatty acids, where it might facilitate the presentation of the substrates and/or stabilizing the enzymes involved in catalyzing the reaction cycles (8). This hypothesis gains support from experiments showing interactions in the peroxisomes between SCP-2 and enzymes involved in β-oxidation such as acyl-CoA oxidase, 3-ketoacyl-CoA thiolase, and the bifunctional enzyme (9). Furthermore, gene targeting in mice (10) revealed that complete deficiency of SCP-2 resulted in an impaired catabolism of methyl branched-chain fatty acyl-CoAs as shown by a 10-fold accumulation of phytanic acid in SCP-2(−/−) mice.

In mammals, a SCP-2 domain is also present in the D-bifunctional protein (DBP). DBP is formed as an 80-kDa precursor with domains for acyl-CoA-dehydrogenase, 2-enoyl-CoA-hydratase, and SCP-2 (11). Processing occurs after transport to the peroxisomes at the junction between acyl-CoA dehydrogenase and 2-enoyl-CoA hydratase. The 80-kDa fusion protein as well as the SCP-2-like domain were shown to have lipid transfer activities similar to SCP-2 (12). The function of the SCP-2-like domain in DBP remains unclear. The domain may be required to ensure the peroxisomal localization of the whole
protein. Although, the structural and functional conservation may indicate that the SCP-2-like domain has additional functions, such as transfer of substrates for DBP from the peroxisomal membrane to the catalytically active site in the peroxisomal matrix (12).

A SCP-2 domain is also present in the Caenorhabditis elegans behavioral gene unc-24. The unc-24 gene encodes a bipartite protein with a domain similar to part of two ion channel regulators (the erythrocyte integral membrane protein stomatin and the C. elegans neuronal protein MEC-2) juxtaposed to a domain similar to SCP-2 (13). Unc-24 mutants are known to have difficulty in moving forward. UNC-24 is a membrane protein and it has been suggested that the SCP-2 domain is involved in the transfer of lipids between adjacent membranes. The human homologue of unc-24 is known as SLP-1, which also is a bipartite protein with a stomatin-like domain as well as a SCP-2 domain (14).

The fused SCP-X protein can be traced back to Drosophila melanogaster suggesting that SCP-XSCP-2 fusions are widespread in vertebrates and insects. DBP enzymes with SCP-2-like domains have been detected in vertebrates, insects, nematodes, fungi (15), and the slime mold Dictyostelium (16). Peroxisomal SCP-2-like proteins are also present in some budding yeasts, such as Candida tropicalis (17) and Candida maltosa (18). In these yeasts a separate gene without any fusion partner encodes SCP-2. Separate genes encoding putative SCP-2-like proteins have also been identified in bacteria (19, 20) and archaea (21–23). Curiously, it has never been reported that plants contain any SCP-2-like protein. In this report we provide evidence that also plants express a peroxisomal SCP-2 protein with lipid transfer activity.

**EXPERIMENTAL PROCEDURES**

**Plant Materials and Bacterial Strains—**We used Arabidopsis thaliana Columbia (Col-0). Seeds were surface sterilized (washed in 70% ethanol for 2 min and in 15% chlorine and 0.5% SDS for 10 min followed by at least four washes in sterile-distilled water) and sown on 1× Murashige and Skoog medium (MS) (24). Before cultivation, seed dormancy was broken by 3 to 4 days of cold treatment (4°C). Plants grown under nonsterile conditions were planted on soil mixed with vermiculite (21). The plants were cultivated under controlled environmental chambers at 20 to 22°C under long day (18 h of light and 6 h of darkness) conditions. Onions, Allium cepa L., used for detection of glutathione S-transferase (GST) and AtSCP-2. The GST-AtSCP-2 was induced with the addition of 0.6–0.7 expression of the GST-AtSCP-2 was induced with the addition of 100 μg/ml ampicillin in 37°C. In the morning the cells were diluted 40 times in 200 ml of LB containing 100 μg/ml ampicillin. When A600 reached 0.6–0.7 expression of the GST-AtSCP-2 was induced with the addition of 0.6 mm isopropyl 1-thio-β-D-galactopyranoside. Cells were then grown for an additional 3 h.

For preparation of cell extracts and purification of GST-AtSCP-2 we followed the procedures described in the handbook for the GST Gene Fusion System (Amersham Biosciences). Bacterial cells were pelleted by centrifugation and resuspended in 10 ml of 1× phosphate-buffered saline. Cells were lysed by sonication for 12 × 15 s with a Labsonic U sonicator (B. Braun, Germany). The lysate was centrifuged in 4°C for 20 min at 10,000 × g. The supernatant was desalted by dialysis against 20 mM Tris-HCl and the protein was in-gel digested with restriction enzymes EcoRI and NotI, and subcloned into the EcoRI-NotI site of the vector pGEX-6X-2 (Amersham Biosciences) to obtain a gene fusion between glutathione S-transferase (GST) and AtSCP-2. The GST-AtSCP-2 was inserted in the obtained plasmid pGEX-SCPAt was confirmed by DNA sequencing. Plasmid pGEX-SCPAt was transformed into E. coli BL21 for cell expression. The cells were grown overnight in 5 ml of LB containing 100 μg/ml ampicillin in 20°C.

For adsorbing the GST-AtSCP-2 fusion protein, 200 μl of 50% slurry of glutathione-Sepharose 4B (Amersham Biosciences) was added to 10 ml of the bacterial extract. The mixture was incubated for 30 min in room temperature and then centrifuged at 500 × g for 5 min to sediment the matrix. The matrix was washed three times with 1× phosphate-buffered saline. To release GST-AtSCP-2 from the GST-tag, 5 μg of Factor Xa (Amersham Biosciences) and 100 μl of Factor Xa cleavage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM CaCl2) were added to the glutathione-Sepharose pellet. The cleavage reaction was incubated for 16 h at room temperature. The suspension was then centrifuged at 500 × g for 5 min. The supernatant was removed to a fresh tube and saved. To increase the yield of the GST-AtSCP-2 protein the glutathione-Sepharose suspension was washed with 100 μl of 1× phosphate-buffered saline. The protein concentration in the supernatants was 2 μg/μl, indicating that the yield of the purified recombinant AtSCP-2 was ~2 mg/liter of bacterial culture. Glyceraldehyde was added to a final concentration of 15%, before placing the supernatants in −20 °C.

Five μg of the purified protein fraction was analyzed by one-dimensional gel electrophoresis on NuPAGE 4–12% BisTris gels (Invitrogen) for 1 h at 200 V. Running buffer was MOPS, 7 M urea, pH 7.3. The gels were stained with Coomassie Brilliant Blue and the protein band of interest was excised using a sharp knife, cut into smaller parts, and then washed in water. The protein was in-gel digested with trypsin, extracted, and analyzed by mass spectrometry. For the mass analysis we used an instrument equipped with quadrupole and time-of-flight mass detectors (Micromass Q-Tof, Micromass Ltd.) with a nanospray ion source using the procedure described previously.

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2 K. Hofmann and M. Baron, unpublished data.
Lipid Transfer Protein from A. thaliana

Preparation of Antibodies against SCP-2—The synthetic peptide SCP1 with the amino acid sequence AQKFTPEIFPKP was designed based on the amino acid sequences of the plant SCP-2 sequences. A segment corresponding to the C terminus was chosen for immunization because of its high conservation and its presumed exposed position. AgriSera (Vänersborg, Sweden) performed peptide synthesis, coupling to bovine serum albumin, immunization of a rabbit, bleedings, serum preparations, and affinity purification of the plant SCP-2 antibodies.

Isolation of RNA and Reverse Transcriptase-PCR—RNA was isolated from A. thaliana tissues with the RNeasy Plant Mini kit (Qiagen). Plant tissues were disrupted in buffer RLT, which contains guanidium isothiocyanate. Reverse transcriptase (RT)-PCR was done with the first strand synthesis and the Expand High Fidelity enzyme blend for the PCR part. Approximately 100 ng of plant total RNA was used for each RT-PCR.

RESULTS

Identification of an A. thaliana Protein Similar to Sterol Carrier Protein-2—Previously, we reported the initial characterization of the endosperm proteome of Euphorbia lagascae and its changes during seed germination (34). In that study, we used two-dimensional gels to visualize proteins accumulating in the endosperm of E. lagascae seeds during germination. One of the E. lagascae proteins showed similarity to a putative protein, At5g42890, with unidentified function from A. thaliana. When we used the A. thaliana sequence to search the public sequence data bases, such as GenBankTM, we identified that it showed significant similarity to DBP from mammals.

A closer look at the results from the BLAST searches revealed that the similarities were restricted to the SCP-2-like domain of DBP. The A. thaliana At5g42890 protein shows 35% identity and 56% similarity to the human SCP-2-like domain present in DBP and 30% identity and 54% similarity to the human SCP-2 encoded by SCP-X. From now on, we will refer to At5g42890 as A. thaliana SCP-2 (AtSCP-2). AtSCP-2 consists of 123 amino acids, has a theoretical pi of 9.2, and a molecular mass of 13.6 kDa. The molecular mass of human SCP-2 is 13 kDa and pi has been determined to 8.7 (44). AtSCP-2 contains a peroxisomal targeting signal, SKL, in the C terminus, which suggests that the peroxisomes are the main cellular location for the protein. The gene encoding AtSCP-2 is located on chromosome 5 in the A. thaliana genome. The gene is a separate gene that is not fused to any other gene. We have not identified any other genes encoding SCP-2 domains or SCP-2-like proteins in the completely sequenced A. thaliana genome.

Identification of cDNAs Encoding SCP-2-like Proteins from Other Plants—We used the A. thaliana sequence to search data bases for expressed sequence tags that encoded SCP-2-like proteins from other plants. We succeeded in identifying such sequence tags from the moss Physcomitrella patens, from conifers, monocotyledons, and many other dicotyledons suggesting that a SCP-2-like protein is expressed in most plants. The multiple sequence alignment in Fig. 1 shows the similarity of the SCP-2 sequences from plants as well as between sequences from plants and humans. The main difference between SCP-2 from dicotyledons and other plants is that the dicotyledons contain glycinie residues in positions 67 and 68, using the numbers according to the A. thaliana SCP-2, whereas only one glycinie is present at this spot in SCP-2-like proteins from bryophytes, conifers, and monocots. These residues are in a region that correspond to a turn between β-strands III and IV in the mammalian protein (1, 2).
To understand more about the evolutionary relationships between SCP-2 domains, we searched the protein, nucleotide, and expressed sequence tag databases for SCP-2 domains from archaea, eubacteria, and eukaryota. The amino acid sequences of the SCP-2 domains were aligned and a phylogenetic tree was constructed using the neighbor-joining method (45). As expected, plant SCP-2 sequences form a specific cluster (Fig. 2). Within the plant cluster, groups are formed by the sequences from dicotyledons, monocotyledons, and gymnosperms. We can also note that the SCP-2 domains from SCP-X/SCP-2, DBP, and UNC-24/SLP-1 form separate groups. Probably, within each of these groups the SCP-2 domains have evolved from a common origin. The eukaryotic SCP-2 domains encoded by separate, unfused genes are not in a specific branch. Rather, it seems that unfused SCP-2 genes have been formed several times during the evolution of eukaryotic organisms.

Models of AtSCP-2 in the Apo and Ligand-bound Conformation—The models of AtSCP-2 in apo and ligand-bound forms were constructed using as the templates the rabbit SCP-2 structure (Protein Data Bank code 1C44 (1)) and the SCP-2-like domain of human DBP (Protein Data Bank code 1IKT (2)), respectively. The sequence alignment was manually refined prior to model building at the Gly58-Gly67 position of AtSCP-2 to align Val60 with Val61 of rabbit SCP-2 and Val59 of the SCP-2-like domain of human DBP, because this valine seems to be structurally important based on the structure-based sequence alignment and visual inspection of the SCP-2 structures. Based on the sequence alignment used for modeling, AtSCP-2 shares 30.6 and 32.5% sequence identity with the rabbit and human SCP-2 template sequences, respectively, and reliable models could thus be generated. The mosquito SCP-2 sequence has significantly lower, only 20%, sequence identity to AtSCP-2 and, therefore, was used only for structural comparison.
SCP-2-like domain of human DBE. Both in the SCP-2-like domain of human DBP and mosquito SCP-2 structures, which presumably represent the ligand-bound conformation, the C-terminal part is folded into a long H9251 helix (helix E) whereas in the rabbit apo-SCP-2 the C-terminal end of the H9251 helix has an irregular coil structure instead (Fig. 3). It is currently unclear whether these two conformations truly represent the SCP-2 conformation with and without ligand because the SCP-2 structures in both of the conformations are not known from the same organism. The loop between H9252 strands I and II of the central H9252-sheet is another structurally variable area. In addition, the sequence identity between AtSCP-2 and the templates is low at this loop and, thus, the models are less reliable at this loop (Fig. 3).

The structural models of AtSCP-2 have a central five-stranded H9252-sheet that is covered on one side by five H9251 helices (A–E). Both of the models also have a large hydrophobic cavity that is similar to the one found in the template SCP-2 structures. This ligand-binding tunnel has an entrance between helices D and E and H9252-strand V and an exit that is composed of

FIG. 2. Phylogenetic analysis of SCP-2 amino acid sequences by neighbor joining. Numbers indicate the percentage of 1000 bootstrap resamplings that support the inferred topology. All sequences from Bacteria, Archaea, and Viridiplantae are from unfused separate SCP-2 genes. Sequences from metazoa, fungi, and mycetozoa are labeled SCP-2, SCP-2/SCP-X, DBP, or SLP-1/UNC-24 to distinguish in which type of gene the domain is present. SCP-2 domains from unfused, separate genes are denoted SCP-2, SCP-2 domains from SCP-X gene fusions are denoted SCP-X/SCP-2, SCP-2 domains from peroxisomal bifunctional proteins are denoted PBE, SCP-2 domains sequences from stomatin-like fusions are marked SLP-1 or UNC-24. Included in the analysis are the sequences described in the legend to Fig. 1 as well as Oryctolagus cuniculus AAC15422.1, Ferrophlasma acidarmanus ZP_00001631.1, Thermoplasma volcanium NP_110914.1, Aeropyrum pernix NP_148292.1, Sulfolobus solfataricus NP_343850.1, Desulfitobacterium hafniense ZP_00099389.1, Pseudomonas aeruginosa PA01NP_250521.1, Pseudomonas syringae pv. syringae B728a ZP_00123832.1, Rhodobacter sphaeroides ZP_00055781.1, C. elegans NP_496161.1, Bos taurus P07857, Thermoplasma acidophilum NP_393770.1, D. melanogaster NP_651578.1, D. melanogaster NP_572917.1, D. melanogaster NP_650765.1, Culex pipiens quinquefasciatus AAO43438.1, Aedes aegypti AA034708.1, Yarrowia lipolytica CAD24067.1, Azotobacter vinelandii ZP_00088844.1, Anopheles quadrimaculatus AAN16393.1, Anopheles gambiae XP_312778.1, Glomus mosseae CAB55552.1, Candida tropicalis BAA51512.1, Neoroserpa cerasi CAD21491.1, Drosophila melanogaster (DMD).
The cavity is lined with hydrophobic residues, Ile11, Met12, Met14, Met15, Tyr35, Ile37, Val53, Phe74, Phe76, Phe81, Val84, Ala85, Pro91, Phe95, Met100, Ile102, Leu106, Ala109, Phe112, and Phe117 in the AtSCP-2 model, in the same way as in the template structures. Of these residues Pro91 and Phe95 are conserved in both of the template structures. In addition, residues Val53, Ile102, and Ala109 are conserved in the rabbit SCP-2 structure and residues Phe81 and Val84 are conserved in the SCP-2-like domain of human DBP. Whereas only Gln92 is conserved in the rabbit SCP-2 structure, whereas Gln110 is conserved in the SCP-2-like domain of human DBP, which has a methionine in the position corresponding to Gln110. In the SCP-2-like domain of human DBP Gln108 (Gln110 in AtSCP-2) forms a hydrogen bond with Triton X-100. The residue corresponding to Thr113 is a glutamine in both of the template structures. Thr113 is located in the middle of helix E. At the end of helix E an additional residue, Ser121, points toward the cavity at the exit of the ligand-binding tunnel in the ligand-bound conformation of AtSCP-2 (Fig. 3). In the template

![Fig. 3. The -fold and the hydrophobic cavities of the AtSCP-2 models and the known SCP-2 structures. A, the model of apo-AtSCP-2 based on B, the rabbit SCP-2 structure. C, the model of the ligand-bound form of AtSCP-2, based on D, the structure of the SCP-2-like domain of human DBE. The overall -fold of the two AtSCP-2 models and their templates is a five-stranded -sheet (dark magenta) that is covered on one side by five -helices (purple). The C-terminal end (green) has a different -fold in the apo and ligand-bound forms of SCP-2. It is folded into a long -helix in the putative ligand-bound form of AtSCP-2 and SCP-2, whereas the most C-terminal part of this helix has a coil structure in the apo form of AtSCP-2 and SCP-2. The amino acids that line the hydrophobic ligand-binding cavities are shown in grey. The figures were prepared using Molscript 2.1.2 (52), Raster3D 2.7b (53), and GIMP 1.3.29 (available at www.gimp.org).](image1)

![Fig. 4. Heterologous expression of AtSCP-2 in E. coli. In A is a SDS-PAGE analysis, and B, a Western blot of purified AtSCP-2 expressed in E. coli. Lanes with AtSCP-2 are indicated with S. The size markers for gel electrophoresis is shown in the lanes indicated with M. The numbers on the side of the marker lane correspond to the size in kDa of the molecular markers. To visualize the proteins in A, gels were stained with Coomassie Blue. The blot in B was treated with rabbit antiserum against plant SCP-2. Samples were prepared as described under “Experimental Procedures.”](image2)

![Fig. 5. AtSCP-2-mediated lipid transfer of purified E. coli recombinant protein. Monitoring of inter vesicular fluorescently labeled lipid transfer from neutral bovine brain sphingomyelin/cholesterol mixed (6:1) donor as a function of time using 0.049 mM protein. The overlapping traces for BODIPY-GlcCer and NBD-Cer represents no transfer activity, or very slow spontaneous transfer suggesting that they are not substrates from AtSCP-2.](image3)
structures there is an alanine in this position. In general, the ligand-binding cavity is smaller in the apo-AtSCP-2 model than in the structural model of the ligand-bound conformation of AtSCP-2. This is mainly because of the different fold at the C-terminal end of the models.

Lipid Transfer Capability of AtSCP-2—By searching the data bases we identified several full-length cDNA clones encoding *A. thaliana* SCP-2. We obtained one such clone, U11030, from the Arabidopsis Biological Resource Center at the Ohio State University. This cDNA was derived from mRNA isolated from light grown, 7-day-old seedlings of *Arabidopsis*. The AtSCP-2 cDNA was subcloned behind GST in the prokaryotic expression vector pGEX-5X-2 yielding plasmid pGEX-SCPAt. This plasmid was transformed into *E. coli* BL21 and the expression of a GST-AtSCP-2 fusion protein was induced by the addition of isopropyl 1-thio-β-D-galactopyranoside to the growth media. After lysis of the bacterial cells, the fusion protein was adsorbed to glutathione-Sepharose. Factor Xa was added to cleave the fusion protein at a site between the GST tag and AtSCP-2. AtSCP-2 was then eluted from the glutathione-Sepharose. When the eluted fraction was analyzed by SDS-PAGE electrophoresis, a polypeptide with a molecular mass of ~13 kDa was detected (Fig. 4A). To verify that this polypeptide corresponded to AtSCP-2 we cut the band from the gel, and added trypsin to digest the protein into smaller peptides. The trypsin digest was analyzed with electrospray mass spectrometry in a mass spectrometer equipped with quadrupole and time-of-flight mass detectors. The obtained peptide masses and peptide sequences matched the theoretical peptide masses and sequences of AtSCP-2 (data not shown). The 13-kDa protein was also recognized in immunoblottings using an affinity purified peptide antisera against plant SCP-2 (Fig. 4B).

To examine the intermembrane lipid transfer capability of the recombinant AtSCP-2 we used a FRET approach developed previously for monitoring protein-mediated glycolipid transfer between vesicle membranes (25). We assayed the transfer activity using the following fluorescent lipids, BODIPY-PC, BODIPY-GlcCer, NBD-ceramide, and AV-GalCer. Using the FRET assay we show that the recombinant AtSCP-2 is capable of transferring phosphatidylcholine, whereas the enzyme is inactive on glucosylceramide and ceramide (Fig. 5) (Table I). In the lipid transfer assays, glycolipid transfer protein was used as control for the assay integrity.

To further examine the ability of AtSCP-2 to mediate transfer of unlabeled lipids we used a competition assay (Fig. 6). In
this assay setup the BODIPY-PC and the added unlabeled lipids compete as substrates for AtSCP-2. We analyzed the ability of POPC, DMPA, bovine brain ceramide, cholesterol, palmitic acid, and stearoyl-coenzyme A and a wide range of plant lipids (Table II). The transfer rate of BODIPY-PC after addition of liposomes was almost completely inhibited for POPC and ergosterol. DMPA, stigmasterol, and steryl glucoside showed a 35–50% ability to lower the BODIPY-PC transfer rate. The rest of the tested compound only showed a marginal competing effect, and the single chain palmitic acid and stearoyl-coenzyme A did not affect the transfer at all. We confirm that the addition of ceramide did not slow down the transfer rate of BODIPY-PC, thereby validating the accuracy of the transfer assay because NBD-ceramide was neither transferred as a labeled compound (compare Figs. 5 and 6).

**Cellular Localization of AtSCP-2**—The C terminus of AtSCP-2 contains a PTS1 peroxisomal targeting sequence. A peroxisomal location has also been shown for the mammalian enzymes. To test whether the plant enzyme also is targeted to the peroxisome, we constructed gene fusions between the soluble modified red-shifted green fluorescent protein (smRS-GFP) and AtSCP-2. In plasmid pER6, the fusion is constructed such that the fusion protein consists of AtSCP-2 in the N terminus and smRS-GFP in the C terminus (AtSCP-2-GFP), whereas the fusion protein encoded by the plasmid pER7 contains smRS-GFP in the N terminus and AtSCP-2 in the C terminus (GFP-AtSCP-2) (Fig. 7). The plasmids pER6, pER7, and psmRS-GFP, which encodes smRS-GFP without fusion partner, were transformed by particle bombardment to onion cells. The transformed tissues were analyzed using fluorescence microscopy. When the onion epidermis cells were transformed with plasmids psmRS-GFP and pER6, soluble GFP were detected in the nucleus and cytosol (Fig. 7). In the pER6 encoded fusion, AtSCP-2-GFP, the putative PTS1 from AtSCP-2 is not exposed in the C terminus. However, when onion cells were transformed with pER7, which encodes GFP-AtSCP-2, carrying the PTS1 in its original position in the C terminus, fluorescence was obtained from distinct organelles. The size and the irregular shape of the fluorescent organelles, the presence of a PTS1 signal, and the need to have AtSCP-2 PTS1 exposed in the C terminus of the fusion protein let us conclude that AtSCP-2 localizes to the peroxisomes via the use of the PTS1 signal sequence.

**Expression Pattern of AtSCP-2 in A. thaliana**—Total RNA was isolated from different tissues of mature A. thaliana plants, such as inflorescence, leaves, stems, roots, siliques, and flower buds. We also isolated RNA from 3-, 7-, and 14-day-old seedlings. The expression pattern of AtSCP-2 RNA was ana-

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**Table II**

| Lipid              | Decrease in BODIPY-PC transfer |
|--------------------|-------------------------------|
| Palmitic acid      | 0                             |
| Stearyl-coenzyme A | 0                             |
| Ceramide           | 0.1                           |
| Cholesterol        | 0.1                           |
| β-Sitosterol       | 0.2                           |
| Desmosterol        | 0.25                          |
| Steryl glucoside   | 0.34                          |
| Stigmasterol       | 0.36                          |
| DMPA               | 0.46                          |
| Ergosterol         | 0.91                          |
| POPC               | 0.93                          |

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**FIG. 7.** Cellular location of AtSCP-2. Gene fusions between AtSCP-2 and smRS-GFP were constructed in plasmids pER6 and pER7. The plasmids were bombarded into onion epidermal cells. Transfomers were analyzed using a fluorescent microscope. A schematic diagram of the fusion proteins encoded by each plasmid is shown on the left. The plasmid psmRS-GFP encodes smRS-GFP without fusion partner.

**FIG. 8.** RT-PCR analysis of AtSCP-2 in A. thaliana tissues. Total RNA isolated from leaves (lane B), inflorescence (lane C), stems (lane D), roots (lane E), flower buds (lane F), siliques (lane G), 3-day-old seedlings (lane H), 7-day-old seedlings (lane I), and 14-day-old seedlings (lane J) were analyzed for the expression of AtSCP-2 and ubiquitin-conjugating enzyme E2, 21 kDa (UBL). Lane K shows the amplification of a control sample lacking RNA. The numbers to the left, refer to sizes in bp of the corresponding bands of the DNA size marker (lane A).

We have demonstrated that a protein with similarity to mammalian SCP-2 is expressed in plants. We have also constructed three-dimensional models of the AtSCP-2 structure in the two conformations, in which the SCP-2 fold is currently known to exist, in the apoconformation and in the conformation that mimics the ligand-bound conformation. The structural model of the apoconformation is based on the x-ray structure of rabbit SCP-2 (1) and the ligand-bound conformation on the x-ray structure of the SCP-2-like domain of human DBP (2). The structural models of AtSCP-2 have a central hydrophobic cavity that is extremely similar to the ligand-binding cavities in the templates. Furthermore, some of the residues are conserved between AtSCP-2 and the known mammalian SCP-2...
Lipid Transfer Protein from A. thaliana

structures. Hydrophobic residues line the interior of the ligand-binding cavity and the polar residues are located at the entrance and exit of the cavity like in the known structures. Thus, the structural models strongly suggest that AtSCP-2 is able to bind lipids with a polar head group and that it has similar but not necessarily identical substrate preferences as the mammalian SCP-2. In agreement with the structural models, we have shown that AtSCP-2 catalyze in vitro transfer of lipids between membranes. BODIPY-PC was shown to be the preferred transfer substrate of those tested so far. Addition of ergosterol efficiently inhibited the in vitro transfer of BODIPY-PC, whereas the other tested sterols interfered poorly with the AtSCP-2-stimulated lipid transfer. The chemical structure of ergosterol differs from that of the other tested sterols in having a double bond at position C7 in the second steroid ring. But because of this additional double bond ergosterol adopts a different conformation and might, therefore, be a better substrate for AtSCP-2. Ergosterol is the major sterol component of certain protozoa, fungi, and insects, and is not detected in plant tissues. Thus, our results may lead to speculations that the preference for ergosterol indicates that AtSCP-2 is involved in the defense of the plant against foreign organisms.

The peroxisomal location of AtSCP-2 indicates that the protein might play a role in peroxisomal oxidation of fatty acids. The expression and activity of A. thaliana proteins involved in β-oxidation such as acyl-CoA oxidase, multifunctional protein, and thiolase increase during germination to a peak 2 days after imbibition (47). However, according to our results AtSCP-2 is expressed in most tissues of Arabidopsis. The expression pattern of AtSCP-2 is, thus, not suggestive of that the main role of the protein is in the peroxisomes during seed germination. Although, support for a role of plant SCP-2 during germination the protein is in the peroxisomes during seed germination. and thiolase increase during germination to a peak 2 days after

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Lipid Transfer Protein from A. thaliana

53553

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