The transporters responsible for sugar uptake into non-photosynthetic sink tissues in plants, such as roots and flowers, have not been fully identified and analyzed. Plants encode around 100 putative sugar transporters within the major facilitator superfamily, yet only a few have been studied. Here we report the analysis of a sugar alcohol permease homolog (AtPLT5, At3g18830) from *Arabidopsis*. A wide range of sugars including hexoses, pentoses, tetrose, a sugar acid, and sugar alcohols but not disaccharides induced inward currents in oocytes expressing AtPLT5. AtPLT5 expression also resulted in 14C-labeled substrate uptake in oocytes, indicating that AtPLT5 encodes an ion-coupled uptake transporter.

Expression of AtPLT5 was found primarily in sink tissues: in the elongation zone of roots, in the inflorescence stem, and several floral structures, especially in the floral abscission zone. Expression was induced by mechanical wounding and insect feeding. Analysis of transport properties and expression in *Arabidopsis* indicate that AtPLT5 functions to transport a wide range of sugars into specific sink tissues in the plant.

Most plant cells are connected by plasmodesmata, forming multicellular "symplasmic domains" (1) that allow exchange of small metabolites between cells. Transporters in the plasma membrane are required for transmembrane metabolite uptake into specific cell types, such as guard cells, pollen cells, and the sieve element/companion cell (SE/CC) complex that are symplasmically isolated. In addition, symplasmic domains of sink cells, for example, in fruit (2), require metabolite uptake transporters to support growth and development (for review, see Ref. 3). Furthermore, events such as wounding, dehiscence, or programmed cell death will release metabolites into the cell wall space, and it is expected that surrounding cells have the capability to take up the released compounds. Although not yet functionally characterized, specific sugar transporter homologs are expressed during senescence (4), dehydration (5, 6), or pathogen attack (7) and may function in metabolite uptake.

*Arabidopsis* encodes a wide array of metabolite transporters, around 100 potential metabolite transporters within the major facilitator superfamily (MFS) alone. Available sequence data indicates that a similar situation exists in other plant species as well. Aside from the well characterized STP family of hexose transporters (8), very little information is available concerning transport activity of plant MFS members.

Within the MFS, *Arabidopsis* encodes three groups of transporters that are closely related to the inositol permeases ITR1 and ITR2 from *Schizosaccharomyces pombe* (9) and *Saccharomyces cerevisiae* (10) and the xylose permease from *Lactobacillus brevis* (11). One group of four genes contains AtpGlcT (At5g16150), the plasticid glucose translocator (12). A second group of four genes is most similar to the plant vascular inositol permeases (Mitr1 and Mitr2) first characterized from *Mesembryanthemum* (13). AtPLT5 (At3g18830) is a member of a third group of 6 genes that is most closely related to the celery mannitol permease AgMAT1 (14), and the sorbitol permeases PsSOT1 and PsSOT2 from cherry fruit (15) and PmPLT1 and PmPLT2 from *Plantago* (16). Based on localization data, these sugar alcohol permease homologs appear to have two main functions: 1) loading sugar alcohols into the phloem for long-distance transport (14, 16) and 2) uptake of sugar alcohols into sink cells. However, a detailed analysis of transport function has not been reported for any member of the family. AgMAT1 was expressed in yeast and demonstrated to transport mannitol (14). A number of sugar alcohols inhibited mannitol uptake as did glucose, indicating that potentially AgMAT1 also transports hexoses. Other homologs have only been tested for the ability to transport sugar alcohols (15, 16), and the substrate specificity of transporters in this family has remained in question.

Whereas celery and *Plantago* are known to transport manitol and sorbitol, respectively, in the phloem and sour cherry fruit accumulate sorbitol during ripening, *Arabidopsis* does not translocate or accumulate either sugar alcohol in significant quantities. Therefore, it is likely that the sugar alcohol permease homologs in *Arabidopsis* have different physiological roles than those proposed for homologous transporters in celery, *Plantago*, and sour cherry (14–16). Here we report the cloning of AtPLT5 from *Arabidopsis*, expression in *Xenopus* oocytes, and the analysis of substrate specificity and transport kinetics by two-electrode voltage clamping. Results show that AtPLT5 transports a wide range of hexoses, pentoses, and sugar alcohols. AtPLT5 is expressed throughout the plant, especially in sink tissues, and is induced by mechanical wounding and insect feeding. It is likely that AtPLT5 functions as a nonspecific sugar uptake transporter in developing sinks and in cells surrounding wounded tissue.

**EXPERIMENTAL PROCEDURES**

**Cloning of AtPLT5**—The entire coding region of AtPLT5 (At3g18830) was amplified by PCR using primers At3g18830-F (5'-AGGACACGGT-GCCACCCGGAAAAC-3') and At3g18830-R (5'-CTAGAATTCCTGGT-GTCTCCTTC-3') using a cDNA library from *Arabidopsis* ecotype Col-0 seedlings as template. The PCR product was cloned into pCR2.1-TOPO (Invitrogen). Sequencing confirmed that there were no base pair changes between the PCR product and the predicted sequence from

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‡ The abbreviations used are: MFS, major facilitator superfamily; MES, 4-morpholineethanesulfonic acid.
Col-0. For oocyte expression, AtPLT5 was cut out of pCR2.1-TOPO using EcoRI and subcloned into the EcoRI site of p002 (17), an expression vector containing *Xenopus* β-globin 5' and 3'-untranslated regions and a 92-bp poly(A) tail. This construct was linearized using PmaCl (Panvera), and 1 μg was used as template for cRNA synthesis using the mMessage mMachine kit (Ambion, Austin, TX).

**Oocyte Expression—*Xenopus laevis* oocytes, stages V and VI, were isolated by incubation in 10 mg/ml collagenase A (Roche Applied Science) in Barth’s medium (88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄, 2.4 mM NaHCO₃, 10 mM HEPES, pH 7.6, 100 μg/ml penicillin, and 100 μg/ml streptomycin) for 2–3 h until separated. The oocytes were then washed five times in 1 mg/ml bovine serum albumin in Barth’s medium. The oocytes were injected with 50 nl (1 ng/nl) of AtPLT5 cRNA and incubated at 15 °C in Barth’s medium supplemented with 10 μg/ml gentamycin. Electrophysiological experiments were performed 3–5 days following the RNA injection. Uptake experiments with radiolabeled substrates were performed 6 days after injection. Oocytes were incubated in Na-Ringer solution, pH 5.5, containing 14C-radiolabeled substrate (ICN) at a final concentration of 3.6 μM for 15 min at room temperature. To stop the reaction, oocytes were washed gently with 2 ml of Na-Ringer solution containing 1 mM of the corresponding unlabeled substrate. Oocytes were then lysed in 2% SDS for 30 min, the scintillation mixture was added, and the sample was counted.

**Electrophysiological Methods—**Oocytes were bathed in Na-Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, pH as indicated in the figure legends, and 10 mM NaHCO₃) with continuous perfusion at 1 ml/min. Recording pipettes, filled with 1 M KCl, with resistances between 1.5 and 3 megohms were used. Currents were measured using the two-electrode voltage clamp technique.

**FIG. 1.** *Arabidopsis* sugar alcohol permease homolog AtPLT5 transports a range of monosaccharides. A, *Xenopus* oocytes, either injected with AtPLT5 mRNA (top trace) or uninjected (bottom trace) were voltage clamped and currents were recorded. The membrane potential was held at −40 mV in Na-Ringer solution at pH 5.6. Substrates were applied at 10 mM in the same solution where indicated, and downward deflections indicate inward coupling ion current. Abbreviations: d-arab, D-arabinose; l-rham, L-rhamnose; l-arab, L-arabinose. B, inward currents were induced at all potentials tested. Glucose-induced currents were recorded under the same conditions as in A, before, during, and after application of 10 mM glucose (symbols for currents recorded before and after glucose application overlap).
with a Dagan TEV 200A amplifier (Dagan Corp., Minneapolis, MN). Currents were filtered on line at 200 Hz and digitized at 2000 Hz using pClamp 5.5.1 (Axon Instruments, Inc., Union City, CA). The holding potential was −40 mV, and voltage pulses from −137 to 37 mV were applied for 200 ms. Steady-state currents are presented as the mean current between 160 and 190 ms following the onset of voltage pulses. Substrate-dependent currents were obtained by subtracting an average of background currents before and after substrate application. For Na\(^{+}\) substitution experiments, a modified Na-Ringer solution (115 mM NaCl, 2 mM KCl, 1.8 mM CaCl\(_2\), 1 mM MgCl\(_2\), 5 mM MES/Tris, pH 5.6) was used and an equimolar concentration of choline was substituted for Na\(^{+}\).

RNA Extraction and RT-PCR Analysis—RNA was extracted from Arabidopsis tissues using the RNeasy Plant RNA extraction kit (Qiagen, Valencia, CA). For RT-PCR analysis the Qiagen One-Step RT-PCR kit was used with 100 ng of RNA per reaction. Primers for the detection of AtPLT5 expression were 5′-GACTTCTATCCTCCTTTGGTTATGATATAGG (forward) and 5′-ATGACTGTTCCTCCTCGACACCTGAACG (reverse) and for the detection of the actin control 5′-GGTACGGCCCTTTATGGCCGTTCGGTTTTCCGGTGTGGCACC (reverse). They were designed to include SfiI restriction sites (underlined). The PCR product was cloned into pCR2.1-TOPO (Invitrogen) and sequenced. The promoter fragment was cut out with SfiI and directionally cloned into the SfiI sites of the univector p713–909. The resulting construct was then recombined in vitro with the binary vector p713–905 using Cre recombinase. The resulting final construct consisted of the promoter region followed by the first 11 amino acids of AtPLT5 (MT-GATPENRTA) in-frame with GUS. Restriction digest with EcoRI was used to detect the correct recombination product. Sequencing across the junction site confirmed the correct frame.

Arabidopsis (Col-0) plants were transformed using the floral-dipping method (18). Transformants were selected by spraying seedlings with a 1:1000 diluted solution of Finale (equivalent to an active ingredient content of 0.12 g/liter glufosinate-ammonium, Farnam Companies, Phoenix, AZ). To detect GUS activity, plants were infiltrated with GUS-staining solution (1 mM 5-bromo-4-chloro-3-indolyl-glucuronide, 0.5% Triton X-100, 50 mM sodium phosphate buffer, pH 7.2) and incubated at 37 °C overnight. After staining, tissues were cleared with 70% (v/v) ethanol. For wounding experiments, attached rosette leaves were cut with scissors 24 h before infiltration with the staining solution.

**Fig. 2.** Kinetic analysis of glucose and sorbitol transport by AtPLT5 at pH 4.5 and 5.6. Substrate-dependent currents (background subtracted) were recorded in Na-Ringer solution at −117 mV. Currents were fit to the Michaelis-Menten equation and normalized to V\(_{\text{max}}\). Data are presented as mean ± S.E. (n = 3 except for glucose at pH 5.6, where n = 4). A, sorbitol transport at pH 4.5; B, sorbitol transport at pH 5.6; C, glucose transport at pH 4.5; D, glucose transport at pH 5.6.

Promoter GUS Fusion Constructs, Plant Transformation, and GUS Staining—For the analysis of the expression pattern of AtPLT5 a 2-kb promoter-GUS translational fusion was constructed. The promoter region was amplified by PCR using genomic DNA from Arabidopsis ecotype Col-0 as template. The primers used were 5′-CACTAGGGCCAAATTCGCCCCCACGAATTATGAAGAAGCCT (forward) and 5′-GGTACCAGGCCCTTATGGCCGTTCGGTTTTCCGGTGTGGCACC (reverse). They were designed to include SfiI restriction sites (underlined). The PCR product was cloned into pCR2.1-TOPO (Invitrogen) and sequenced. The promoter fragment was cut out with SfiI and directionally cloned into the SfiI sites of the univector p713–909. The resulting construct was then recombined in vitro with the binary vector p713–905 using Cre recombinase. The resulting final construct consisted of the promoter region followed by the first 11 amino acids of AtPLT5 (MT-GATPENRTA) in-frame with GUS. Restriction digest with EcoRI was used to detect the correct recombination product. Sequencing across the junction site confirmed the correct frame. Arabidopsis (Col-0) plants were transformed using the floral-dipping method (18). Transformants were selected by spraying seedlings with a 1:1000 diluted solution of Finale (equivalent to an active ingredient content of 0.12 g/liter glufosinate-ammonium, Farnam Companies, Phoenix, AZ). To detect GUS activity, plants were infiltrated with GUS-staining solution (1 mM 5-bromo-4-chloro-3-indolyl-glucuronide, 0.5% Triton X-100, 50 mM sodium phosphate buffer, pH 7.2) and incubated at 37 °C overnight. After staining, tissues were cleared with 70% (v/v) ethanol. For wounding experiments, attached rosette leaves were cut with scissors 24 h before infiltration with the staining solution.

\(^{2}\)T. Shigaki and K. D. Hirschi, unpublished data.
RESULTS

The Arabidopsis sugar alcohol permease homolog AtPLT5 was expressed in Xenopus oocytes and analyzed by voltage clamping. At a holding potential of −40 mV several pentoses (arabinose, xylose, and ribose) and hexoses (L-rhamnose and D-fucose) induced inward currents (Fig. 1A) in AtPLT5-injected oocytes. This was the first indication that AtPLT5 could potentially transport a wide range of metabolites. Under the same recording conditions (pH 5.6 and a membrane potential of −40 mV), glucose-dependent inward currents averaged 66 ± 9 nA (mean ± S.E., n = 17 oocytes) and sorbitol-dependent inward currents averaged 48 ± 6 nA (n = 15 oocytes) over the course of the study that included 26 batches of oocytes from 13 different frogs. In contrast, maltose did not induce detectable currents and no currents were induced in uninjected control oocytes for any of the substrates tested (Fig. 1A). The inward currents generated by the application of uncharged substrates to the outside of the oocytes are consistent with a proton-coupled transport mechanism in which the coupling ion (proton) movement across the membrane is measured as an inward current. Substrate-dependent currents were inwardly directed at all membrane potentials from −137 to 37 mV and for all transported substrates. An example for glucose is given in Fig. 1B. Substrate-dependent currents were not voltage regulated; they were linear with membrane potential indicating that the transporter was not activated or inactivated by membrane potential.

Kinetic analysis of sorbitol and glucose-dependent currents was performed using substrate concentrations between 50 μM and 30 mM. At both pH values tested, AtPLT5 showed a higher apparent affinity for glucose compared with sorbitol (Fig. 2). External pH values of 4.5 to 5.6 are considered to be within the physiological range for the extracellular pH of plant cells, and the affinity of AtPLT5 for sorbitol and glucose was strongly regulated within this range (compare Fig. 2, A versus B and C versus D). The K₀.₅ values of AtPLT5 for glucose and sorbitol were lower at pH 4.5 compared with pH 5.6 (Fig. 2). It was also clear that K₀.₅ values for sorbitol (Fig. 3A) and glucose (Fig. 3B) were not highly regulated by membrane potential at pH 4.5 or 5.6. At pH 5.6, K₀.₅ values for glucose were more voltage dependent than at pH 4.5 (Fig. 3B), however, this was not true for the K₀.₅ values for sorbitol that appear voltage independent at both pH values tested (Fig. 3A). Sorbitol- and glucose-induced currents through AtPLT5 were maximal at acidic external pH values (Fig. 4) although the effect of pH was not identical for the two substrates. At the most acidic pH tested (4.5), sorbitol produced higher current densities.

Specific transporters within the MFS have been demonstrated to function as sensors, for example, on binding glucose, human SGLT3 forms a Na⁺-permeable channel and conducts Na⁺ but not glucose (19). To determine whether the ion currents observed in oocytes when AtPLT5 was expressed were coupled to substrate uptake, [14C]-labeled glucose, sorbitol, sucrose, or maltose were applied externally to oocytes. Oocytes expressing AtPLT5 accumulated both [14C]-labeled glucose and sorbitol and did not accumulate [14C]-labeled sucrose or maltose (Fig. 5A). In addition, more glucose was accumulated compared with sorbitol and this is consistent with lower sorbitol-induced inward currents compared with glucose-induced currents at pH 5.6 (Fig. 4). To determine whether sugar transport through AtPLT5 was coupled to Na⁺ or H⁺, experiments were performed in Na⁺-free Ringer solution (Na⁺ replaced by choline). Glucose-induced currents in Na⁺-Ringer and choline-Ringer solutions were identical (Fig. 5B), consistent with a proton-coupled transport mechanism for AtPLT5.

To obtain information on the substrate specificity of AtPLT5, the ability of 29 potential substrates to induce currents in AtPLT5-injected oocytes was tested. Substrates were applied at 10 mM concentrations in Na⁺-Ringer solution at pH 5.6. At least 3 oocytes were recorded for each substrate and the currents were normalized to sorbitol-induced currents (Fig. 6). Several pentoses (ribose, D- and L-arabinose, and xylose) were transported at rates as high or higher than sorbitol. Several hexoses (glucose, mannose, galactose, fructose, L-rhamnose, and fructose) also served as good substrates. The tetrose erythrose was transported at a rate similar to sorbitol. The sugar alcohols erythritol, xylitol, and inositol as well as sorbitol were transported. Interestingly, glycerol also served as a substrate, whereas mannitol was not transported. None of the disaccharides tested (sucrose, cellobiose, lactose, maltose, and trehalose) were transported nor was the trisaccharide raffinose. The plant glucoside salicin was not transported nor was the glucosinolate sinigrin. α-Methylglucose was transported at a high rate indicating
that α-glucosides can serve as substrates, however, glucosamine was not transported. The sugar acid glucuronate was transported at a low rate, whereas gluconate was not transported. The results indicate that AtPLT5 transports a wide range of monosaccharides and modified sugars.

To characterize the apparent affinity of AtPLT5 for some of the transporters, sorbitol and glucose, glucose and sorbitol-dependent currents (background currents subtracted) were recorded at a membrane potential of −98 mV, at 10 mM substrate concentrations in Na-Ringer solution adjusted to pH values between 4.5 and 7.5. All currents were normalized to currents recorded with 10 mM glucose at −79 mV, pH 5.6, for each oocyte. Data are presented as means of three observations ± S.D.

**Fig. 4.** pH dependence of AtPLT5 transport activity for sorbitol and glucose. Glucose and sorbitol-dependent currents (background currents subtracted) were recorded at a membrane potential of −98 mV, at 10 mM substrate concentrations in Na-Ringer solution adjusted to pH values between 4.5 and 7.5. All currents were normalized to control for differences in expression between oocytes. Data are presented as means of three observations ± S.D.

**Fig. 5.** Analysis of uptake of 14C-labeled substrates and coupling ion specificity by Xenopus oocytes expressing AtPLT5. A. Oocytes were injected with AtPLT5 cRNA 6 days before the experiment. Controls were un.injected oocytes. Oocytes were incubated in Na-Ringer solution, pH 5.5, containing radiolabeled substrate at a final concentration of 3.6 μM for 15 min at room temperature. The values are expressed as femtomoles of substrate taken up during 15 min after subtraction of the background currents determined for un injected oocytes. Results presented are mean ± S.D. of values determined for 3–4 oocytes per substrate tested. B, inward currents induced by 10 mM glucose in AtPLT5-injected oocytes was recorded in either modified Na-Ringer or Na free choline-Ringer solution at pH 5.6. Data from one oocyte are presented, similar results were obtained from four oocytes.

**Fig. 6.** Substrate specificity of AtPLT5. Substrate-dependent currents (background subtracted) were recorded under voltage clamp conditions from Xenopus oocytes expressing AtPLT5. Substrates were applied at 10 mM in Na-Ringer solution at pH 5.6. Currents were recorded at a membrane potential of −117 mV and normalized to sorbitol-dependent currents to control for differences in expression between oocytes. Mean currents ± S.D. for n = 3 oocytes are presented except for glucose (n = 11) and sorbitol (n = 11). Average sorbitol-induced currents were 94 ± 38 nA.

To understand the function of a transporter it is necessary to know in which tissues it is expressed and whether expression is regulated or constitutive. A 2.0-kb promoter from the AtPLT5 gene was translationally fused to the GUS reporter gene and used to stably transform Arabidopsis plants (Col-0 ecotype). In seedlings, GUS expression was found in the root and in the vascular tissue of the cotyledons (Fig. 8, D and E). In older plants, expression in the root was predominantly observed in the cortex of the elongation zone (Fig. 8, B and C). This staining pattern was found both in the primary root and in lateral roots. No staining was observed at the root tip (Fig. 8B). In developmentally older parts of the root system no staining was observed. GUS expression was also found in the major vein of older, fully expanded leaves (Fig. 8A). In addition, expression was induced in leaves by mechanical wounding (Fig. 8, M and N) and by insect feeding (Fig. 8, O and P). During flowering, expression was found in a variety of tissues: the most rapidly elongating part of the floral stem (Fig. 8G), in the base and tip of anthers but not in pollen (Fig. 8L), in the style (Fig. 8H) and in the pedicel (Fig. 8, G and H). In developing fruit, expression was found at the base and tip of the siliques (Fig. 8, I and J), in particular at the floral organ abscission zone (Fig. 8K) but not in seeds (Fig. 8, I and J). In later stages of silique development the staining at the tip end decreased (Fig. 8J). GUS expression was also induced at the site of wounding in the floral stem (Fig. 8R). During germination, GUS staining was noted in the seed coat (Fig. 8P). Tissue specificity of AtPLT5 expression was confirmed by RT-PCR. Expression was found in all tissues sampled and in inflorescence stem induction because wounding was found within 30 min (Fig. 9). The expression pattern is also consistent with publicly available microarray data (cbs.umn.edu/arabidopsis).
Results of expression analyses indicate that AtPLT5 has a complex expression pattern. It is expressed in sink tissues that import sugars and other metabolites to support growth and development. In addition, AtPLT5 expression in tissue surrounding wounding sites and in the floral abscission zone indicates that it may function in recovery of sugars or metabolites in these tissues. In summary, the expression and transport activity data are consistent with a function as a broad-specificity proton-coupled sugar uptake transporter.

**DISCUSSION**

Substrate Specificity—By expression in oocytes, the sorbitol permease homolog AtPLT5 was found to transport hexoses, pentoses, a tetrose, both acyclic and cyclic polyols, and an

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**FIG. 7.** Kinetic analysis of ribose and fucose transport by AtPLT5 expressed in Xenopus oocytes. **A**, ribose-dependent currents (background subtracted) at −117 mV are plotted against ribose concentration. Recordings were made in Na-Ringer at pH 5.6 and currents for each oocyte were normalized to $V_{\text{max}}$. Line is a least-squares fit of the Michaelis-Menten equation to the data. **B**, voltage dependence of $K_{0.5}$ for ribose. $K_{0.5}$ values were measured as in A at different membrane potentials. **C**, fucose-dependent currents recorded as in A. **D**, voltage dependence of $K_{0.5}$ for fucose. In A–D data are means of three independent experiments; error bars are ±S.E.
AtPLT5 Transport Activity

Similarly, glucose strongly inhibited sorbitol uptake into yeast cells expressing PcSOT1 from sour cherry. However, this inhibition was interpreted to be an artifact of heterologous expression in yeast and a high specificity for sorbitol was attributed to PcSOT1 (15). The analysis of AtPLT5 from Arabidopsis in this study indicated that transported substrates include a wide range of sugars and sugar alcohols.

Many of the substrates transported by AtPLT5 are found in Arabidopsis: these are the sugar alcohols erythritol, glycerol, inositol, and the sugars erythrose, fructose, fucose, glucose, galactose, mannose, and rhamnose (21). All or some of these may therefore be substrates of AtPLT5 in planta. However, whereas it is possible that AtPLT5 functions in the plant to transport a wide array of sugars with moderate affinity as found for sorbitol, glucose, ribose, and fucose in this study, it cannot be excluded that AtPLT5 may have a higher affinity for a substrate not tested.

All previously characterized Arabidopsis monosaccharide uptake transporters belong to the STP family of sugar transporters that are part of the MFS. AtPLT5 is in a distinct subfamily within the MFS. It is therefore the first transporter, outside of the STP family, shown to catalyze the cellular uptake of hexoses to be described from Arabidopsis. STP-type transporters studied to date include AtSTP1 (22, 23), AtSTP2 (24), AtSTP3 (25), AtSTP4 (26), AtSTP6 (27), and AtSTP9 (28). Except for AtSTP3, all are high affinity transporters with $K_m$ for glucose between 15 and 84 $\mu M$. AtPLT5 shares a number of features with members of the STP family such as 12 predicted transmembrane spans, proton-coupled transport mechanism, and a broad substrate specificity (23). However, AtPLT5 shows interesting differences in its substrate selectivity compared with the STPs. D- and L-forms of arabinose were transported equally well by AtPLT5 (Fig. 6) indicating that IC and CI conformations are not distinguished. In contrast, AtSTP1 transports hexoses only in the CI conformation (23). For STP-type transporters, with the exception of STP6 in pollen, transport rates for fructose were typically less than 6% of the rate for glucose (22, 24, 25, 28). This is interesting because the main carbohydrate delivered to sink cells via the phloem in most plants is sucrose, which is either transported directly into sink cells or is hydrolyzed in the wall space to yield fructose and glucose. The transport rate for fructose determined for AtPLT5 was 50% of the transport rate for glucose, indicating that AtPLT5 may function in the uptake fructose to a greater extent than the STPs.

In plants a transmembrane proton gradient drives solute uptake through coupled transporters such as AtPLT5. Differences in pH optimum for glucose and sorbitol transport (Fig. 4) imply that external pH also regulates AtPLT5 and can influence substrate selectivity. The $K_{0.5}$ for glucose and sorbitol were highly pH-dependent (Fig. 3) indicating that in addition to increasing the driving force for transport, extracellular acidification would increase AtPLT5 affinity, at least for sorbitol and glucose. Interestingly, AtPLT5 $K_{0.5}$ values were only slightly voltage-dependent (Figs. 3 and 7).

Localization of AtPLT5 and Function in the Plant—The sugar alcohol permease homologs PmPLT1 and PmPLT2 from Plantago were both localized to companion cells (16). Similarly, in celery AgMaT1 was been cloned from phloem tissue (20). In both cases the expression of these transporters is consistent with a proposed role in phloem loading of sugar alcohols. In contrast, two sugar alcohol permease homologs, PcsOT1 and PcsOT2, were isolated from sour cherry fruit (15). Their expression pattern is consistent with their predicted role in sorbitol uptake into sink tissue and with the
fact that sour cherries contain large amounts of sorbitol when ripe. ATP75 expression was detected in sink tissues such as growing roots, developing silique and anthers. In addition, expression was induced in older leaves, mainly along the midvein and also in wounded leaves and inflorescence stems. The expression pattern and substrate specificity of ATP75 indicate that it may play a role in providing sink tissues with a range of carbohydrates known to be present in the phloem (29) and assumed to be transported into sink cells.

Rapidly expanding tissues, such as in the elongation zone in roots, are strong sinks for photoassimilates. The osmotic driving force necessary for phloem unloading could be generated via sucrose hydrolysis by invertase in the wall space, a process that would require subsequent hexose uptake into sink cells, or by sucrose cleavage within the sink cells. The parallel induced expression of cell wall invertase and a hexose transporter has been described for Chenopodium rubrum cultured cells (30). Cell wall invertase expression has been demonstrated in actively growing Arabidopsis roots (31). In this study we found high ATP75 promoter-driven GUS activity in the elongation zone of the root (behind the root tip). In older parts of the root system the ATP75 promoter was not active except at the site of emerging lateral roots. ATP75 expression was not found at the root tip. Using green fluorescent protein as a marker, symplasmic phloem unloading through plasmodesmata was demonstrated in Arabidopsis root tips but not in the elongation zone (32). These data are consistent with sucrose hydrolysis in the wall space and subsequent hexose uptake via ATP75 as a mechanism for phloem unloading in the root elongation zone.

ATP75 may also play a role in the reuptake of sugars from aging or damaged tissues as indicated by the induction of its expression in the floral abscission zone, in older leaves and in response to wounding. The floral abscission zone consists of distinct layers of cells located where the floral organs such as petals, sepals, and anthers are shed after fertilization has occurred (33). During the abscission process glucanases and chitinases are induced, the middle lamella is dissolved and the cells eventually separate (33, 34). ATP75 is strongly induced in the floral abscission zone after fertilization has taken place. It may therefore have a role in the re-uptake of sugars resulting from the activity of polysaccharide degrading enzymes. Senescing leaves are rich in sugars and other metabolites that are important for the plant to recover before the leaf eventually dies (33). ATP75 expression, visualized by GUS staining, was induced in cells surrounding the vascular tissue in the midvein in mature leaves. Visibly senescent leaves, on the other hand, did not show GUS activity. This suggests that ATP75 is active during early stages of senescence by recovering sugars leaking either out of the phloem or out of parenchyma cells surrounding the vascular tissue. In both cases ATP75 activity is likely to be important in supporting sugar export from the leaves without being directly involved in phloem loading.

The strong increase in GUS staining after wounding indicates that ATP75 expression is rapidly induced under these circumstances. During wounding, oligosaccharides are released from the wounding site in two ways: 1) by mechanical destruction of the cell, followed by the discharge of the contents of the cell, and 2) by induction of endogenous polygalacturonases. Oligosaccharides released from wounding sites have been suggested to have a role in signaling, but aside from that they are also an important resource for the plant that needs to be recovered (35, 36). In addition, invertases are also induced in wounded tissues, for instance, in wounded taproots of carrots (37). These enzymes have the ability to hydrolyze sucrose and other β-fructos containing oligosaccharides such as raffinose and stachyose (38). As a broad specificity transporter, ATP75 may have an important role in recovery of a variety of sugars and sugar derivatives.

Based on transport studies and the expression data we propose that sugar alcohol permease homologs in Arabidopsis are broad specificity transporters that most likely: 1) transport a range of substrates into sink cells, and 2) assist in scavenging sugars from senescing or dying tissue. There are five other members of this family in Arabidopsis that may have similarly broad substrate specificity. Publicly available expression data (cbis.umn.edu/arabidopsis) indicates that these other homologs may also play a role in import into sink tissues such as pollen (At2g16120, At2g16130, At2g18480, At2g20780), seeds (At2g18480, At4g36670), and seedlings (At4g36670). Whereas the apparent redundancy of hexose transporters may seem surprising, a similar situation occurs in the unicellular S. cerevisiae, which contains at least 18 genes encoding hexose transporters (39). Additional work will be necessary to determine the precise function of all of the putative sugar transporters in Arabidopsis.

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