Evolution of plant sucrose uptake transporters

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INTRODUCTION

In angiosperms, sucrose uptake transporters (SUTs) have important functions especially in vascular tissue. Here we explore the evolutionary origins of SUTs by analysis of angiosperm SUTs and homologous transporters in a vascular early land plant, Selaginella moellendorffii, and a non-vascular plant, the bryophyte Physcomitrella patens, the charophyte algae Chlorokybus atmosphyticus, several red algae and fission yeast, Schizosaccharomyces pombe. Plant SUTs cluster into three types by phylogenetic analysis. Previous studies using angiosperms had shown that types I and II are localized to the plasma membrane while type III SUTs are associated with vacuolar membrane. SUT homologs were not found in the chlorophyte algae Chlamydomonas reinhardii and Volvox carteri. However, the characean algae Chlorokybus atmosphyticus contains a SUT homolog (CaSUT1) and phylogenetic analysis indicated that it is basal to all other streptophyte SUTs analyzed. SUTs are present in both red algae and S. pombe but they are less related to plant SUTs than CaSUT1. Both Selaginella and Physcomitrella encode type II and III SUTs suggesting that both plasma membrane and vacuolar sucrose transporter activities were present in early land plants. It is likely that SUT transporters are important for scavenging sucrose from the environment and intracellular compartments in charophyte and non-vascular plants. Type I SUTs were only found in eudicots and we conclude that they evolved from type III SUTs, possibly through loss of a vascular targeting sequence. Eudicots utilize type I SUTs for phloem (vascular tissue) loading while monocots use type II SUTs for phloem loading. We show that HvSUT1 from barley, a type II SUT, reverted the growth defect of the Arabidopsis atsuc2 (type I) mutant. This indicates that type I and II SUTs evolved similar (and interchangeable) phloem loading transporter capabilities independently.

Keywords: sucrose transporter, SUT, phylogeny, evolution

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monocot and eudicot divergence. Type III SUTs were first cloned from *Arabidopsis*, potato and tomato and characterized as H^+^-coupled symporters (Weise et al., 2000). Type III SUTs are localized at the vacuolar membrane (Endler et al., 2006; Reinders et al., 2008) and function in sucrose-uptake into the cytoplasm (Reinders et al., 2008; Schulz et al., 2011).

Advances in genome sequencing allow us for the first time to investigate the origins of angiosperm SUTs. Complete genome sequence is available for representative bryophyte (*Physcomitrella patens*), lycophyte (*Selaginella moellendorfii*), and chlorophytes (*Chlamydomonas reinhardtii* and *Volvox carterii*). In addition, partial sequence is available for the red algae *Galdieria sulphuraria* and *Cyanidioschyzon merolae* and EST sequence is available for several charophyte algae (Timme and Delwiche, 2010). The main questions that we can address by phylogenetic analysis are whether type I SUTs were derived from type II or type III SUTs and whether both type II and III SUTs were represented in the earliest land plants and algae.

**MATERIALS AND METHODS**

**SUT PROTEIN SEQUENCES**

All SUT protein sequences were obtained from the following species in which genome sequence is available: the eudicot *Arabidopsis thaliana*, the monocot rice (*Oryza sativa*), the lycophyte *Selaginella moellendorfii*, and the bryophyte *Physcomitrella patens* using BLAST searches on the Phytozome website 1. The same database was searched for SUT protein sequences from the chlorophytes *Chlamydomonas reinhardtii* and *Volvox carterii*. Dr. Charles F. Delwiche and Mr. James Thierer, University of Maryland, provided support by searching their algal sequence database (Timme and Delwiche, 2010). The main questions that we can address by phylogenetic analysis are whether type I SUTs were derived from type II or type III SUTs and whether both type II and III SUTs were represented in the earliest land plants and algae.

**PHYLGENETIC ANALYSIS**

Multiple protein sequence alignments were generated with Clustal X (Larkin et al., 2007). The variable length N- and C-terminal regions of the alignment were removed. Percent protein sequence identity is presented, based on the trimmed alignment, as average for each cluster (±SD). Sequences with greater than 90% overall sequence identity were not included in the phylogenetic analysis. Phylogenetic analysis was performed through the iPlant Collaborative website 4. Maximum likelihood analysis was done using PhyML 3.0 with 100 bootstrap replicates (Guindon and Gascuel, 2003; Guindon et al., 2010). Trees were visualized using the FigTree program 5.

1 [http://phytozome.net](http://phytozome.net)
2 [http://genomics.msu.edu/cgi-bin/galderia/blast.cgi](http://genomics.msu.edu/cgi-bin/galderia/blast.cgi)
3 [http://merolae.biol.s.u-tokyo.ac.jp/](http://merolae.biol.s.u-tokyo.ac.jp/)
4 [http://www.ipplantcollaborative.org/](http://www.ipplantcollaborative.org/)
5 [http://tree.bio.ed.ac.uk/software/figtree/](http://tree.bio.ed.ac.uk/software/figtree/)

**COMPLEMENTATION OF THE ARABIDOPSIS atsuc2-1 MUTANT**

Constructs for plant transformation contained the AtSUC2 (*At1g22710*) promoter, coding region of either AtSUC2 or HvSUT1 (CA2)1231.1 cDNAs and the AtSUC2 3’UTR. The AtSUC2 promoter (2 kb) was amplified using the primers 5’gaggaacttctatagaaaagttgtaccagatttcggtaaatt and 5’gaggaacttctatagaaaagttgtaccagatttcggtaaatt and cloned into the pDONR P4-P1R vector (Invitrogen) using BP clonase II. The AtSUC2 ORF was amplified using 5’caccggtttcaaatagggcgcggcgcggggcgcggcgcggggcgcggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggggcgcggg gcgg
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FIGURE 1 | Phylogenetic analysis of plant sucrose transporters and homologs. Protein alignment was done using Clustal X. Sequences with greater than 90% identity were not used in construction of the tree (they are shown in Table 1). The variable length N- and C-terminal regions were trimmed from the alignment. The maximum likelihood tree was generated using PhyML 3.0. Numbers indicate percent of 100 bootstrap analyses. Asterisk indicates the single charophyte SUT sequence, CaSUT1, from Chlorokybus atmosphyticus.
Table 1 | Sucrose transporter homologs.

| Type | Organism                  | Common name          | Gene                  | Prot ID      | Length (aa) | Reference                      |
|------|---------------------------|----------------------|-----------------------|--------------|-------------|--------------------------------|
| I    | Alonsoa meridionalis      | Thale cress          | AmSUT1                | AAF04295     | 502         | Knop et al. (2001)             |
| I    | Arabidopsis thaliana      | Thale cress          | AtSUC1 (At1g71880)   | CAAS3147     | 513         | Sauer and Stolz (1994)         |
| I    | Arabidopsis thaliana      | Thale cress          | AtSUC2 (At1g22710)   | CAAS3150     | 512         | Sauer and Stolz (1994)         |
| I    | Arabidopsis thaliana      | Thale cress          | AtSUC5 (At1g71890)   | AAGS2226     | 512         | Theologis et al. (2000)        |
| I    | Arabidopsis thaliana      | Thale cress          | AtSUC8 (At1g14670)   | AAC69375     | 492         | Lin et al. (1999)              |
| I    | Arabidopsis thaliana      | Thale cress          | AtSUC9 (At5g06170)   | BAB09682     | 491         | Tabata et al. (2000)           |
| I    | Arasinia barclaiana       | Twining snapdragon   | AsSUT1                | AAF04294     | 510         | Knop et al. (2001)             |
| I    | Beta vulgaris             | Sugar beet           | BvSUT1                | CAAS3730     | 523         | Vaughn et al. (2002)           |
| I    | Brassica oleracea         | Broccoli             | BoSUC1                | AALS071      | 513         | Gapper et al. (2005)           |
| I    | Citrus sinensis           | Sweet orange         | CsSUT1                | AAM29150     | 528         | Li et al. (2003)               |
| I    | Daucus carota             | Carrot               | DcSUT2                | CAA7369      | 515         | Shkaya and Sturm (1998)        |
| I    | Euphorbia esula           | Leafy spurge         | EsSUT1                | AAF65765     | 530         |                                |
| I    | Hevea brasiliensis        | Para rubber tree     | HbSUT3/ HbSUT1A       | ABK609190    | 535         | Tang et al. (2010)             |
| II   | Juglans regia             | English walnut       | JrSUT1                | AUA11810     | 516         | Decourteix et al. (2006)       |
| II   | Solanum lycopersicum      | Tomato               | LeSUT1                | CAAS7726     | 512         | Barker et al. (2000)           |
| II   | Medicago truncatula       | Barrel medic         | MtSUT1                | TC175182     | 525         | http://compbio.dfci.harvard.edu/tgi/ |
| IIA  | Nicotiana tabacum         | Common tobacco       | NiSUT3                | AAD34610     | 521         | Lemoine et al. (1999)          |
| IIA  | Phaseolus vulgaris        | Common bean          | PsSUF1                | ABB30165     | 509         | Zhou et al. (2007)             |
| IIA  | Pisum sativum             | Pea                  | PsSUF1                | AAD41024     | 524         | Tegeder et al. (1999)          |
| IIA  | Pisum sativum             | Pea                  | PsSUF1                | ABB30163     | 511         | Zhou et al. (2007)             |
| IIA  | Plantago major            | Common plantain      | PmSUC1                | CAAS9115     | 503         | Gahrt et al. (1996)            |
| IIA  | Plantago major            | Common plantain      | PmSUC2                | CAAS3390     | 510         | Gahrt et al. (1996)            |
| IIA  | Populus trichocarpa       | Black poplar         | PtaSUT1/ PtaSUT1.2    | 18221401     | 535         | Tuskan et al. (2006)           |
| IIA  | Ricianus communis         | Castor bean          | RsSCR1                | CAAS3436     | 533         | Weig and Komor (1996)          |
| IIA  | Spinacia oleracea         | Spinach              | SoSUT1                | CAAS7604     | 526         | Riesmeier et al. (1992)        |
| IIA  | Vitis vinifera            | Grape                | VvSUC27               | AAF68331     | 505         | Davies et al. (1999)           |
| IIA  | Arabidopsis thaliana      | Thale cress          | AtSUT2/AtSUC3(At2g02880) | CAS92307 | 595         | Meyer et al. (2000), Schulze et al. (2000) |
| IIA  | Eucomma ulmoides          | Gutta-percha tree    | EuSUT2                | AAX49396     | 604         | Pang et al. (2008)             |
| IIA  | Hevea brasiliensis        | Para rubber tree     | HbSUT2C/ HbSUT2A      | CAM13449     | 539         | Duscoito-Coucaud et al. (2009) |
| IIA  | Oryza sativa japonica     | Rice                 | OsSUT4 (Os02g58080)  | BAC67164     | 595         | Aoki et al. (2003)             |
| IIA  | Physcomitrella patens     | Tomato               | LeSUT2                | AAG12987     | 605         | Davies et al. (1999)           |
| IIA  | Physcomitrella patens     | Tomato               | LeSUT2                | AAG12987     | 605         | Davies et al. (1999)           |
| IIA  | Populus trichocarpa       | Black poplar         | PtaSUT2A              | 18241888     | 602         | Tuskan et al. (2006)           |
| IIA  | Selaginella moellendorffii| Tomato               | SmSUT2                | 15412113     | 521         | Banks et al. (2011)            |
| IIA  | Solanum lycopersicum      | Tomato               | LeSUT2                | AAG12987     | 605         | Barker et al. (2000)           |
(Continued)
| Type | Organism | Common name | Gene | Prot ID | Length (aa) | Reference |
|------|----------|-------------|------|---------|-------------|-----------|
| IIB  | Bambusa oldhamii (Dendrocalamopsis oldhamii) | Bamboo | BooSUT1 | AAY43226 | 525 | |
| IIB  | Hordeum vulgare | Barley | HvSUT1 | CAB75882 | 523 | Weschke et al. (2000), Sivitz et al. (2005) |
| IIB  | Oryza sativa japonica | Rice | OsSUT1 (Os03g07480) | BAA24071 | 537 | Hirose et al. (1997) |
| IIB  | Oryza sativa japonica | Rice | OsSUT3 (Os10g26740) | BAB68368 | 506 | Aoki et al. (2003) |
| IIB  | Oryza sativa japonica | Rice | OsSUT5 (Os02g36700) | BAC67165 | 535 | Aoki et al. (2003) |
| IIB  | Saccharum hybrid cultivar | Sugarcane | ShSUT1 | AAV41028 | 517 | Rae et al. (2005) |
| IIB  | Zea mays | Corn | ZmSUT1 | BAA83501 | 521 | Aoki et al. (1999) |
| III  | Arabidopsis thaliana | Thale cress | AtSUT4 (At1g09960) | AAL59915 | 510 | Weise et al. (2000) |
| III  | Datisca glomerata | Durango root | DgSUT4 | CAG70682 | 498 | Schubert et al. (2010) |
| III  | Daucus carota | Carrot | DcSUT1a | CAA76367 | 501 | Shakya and Sturm (1998) |
| III  | Hevea brasiliensis | Para rubber tree | HbSUT4A | ABK60191 | 498 | Tang et al. (2010) |
| III  | Hordeum vulgare | Barley | HvSUT2 | CAB75881 | 506 | Weschke et al. (2000) |
| III  | Lotus japonicus | LjSUT4 | CAD61275 | 511 | Flemetakis et al. (2003) |
| III  | Malus x domestica | Apple | MdSUT1 | AAR17700 | 499 | Fan et al. (2009) |
| III  | Medicago truncatula | Barrel medic | MtSUT4 | 17466537 | 504 | |
| III  | Oryza sativa japonica | Rice | OsSUT2 | BAC67163 | 501 | Aoki et al. (2003) |
| III  | Physcomitrella patens | | PsSUT4A | 18040351 | 532 | Rensing et al. (2008) |
| III  | Physcomitrella patens | | PsSUT4B | 18037160 | 500 | Rensing et al. (2008) |
| III  | Physcomitrella patens | | PsSUT4C | 18053343 | 524 | Rensing et al. (2008) |
| III  | Pisum sativum | Pea | PsSUF4 | ABB30162 | 507 | Zhou et al. (2007) |
| III  | Ricinus communis | Castor bean | RcSUC4 | AAU21439 | 509 | |
| III  | Selaginella moellendorffii | | SmSUT4A | 15419655 | 514 | Banks et al. (2011) |
| III  | Selaginella moellendorffii | | SmSUT4B | 15407332 | 492 | Banks et al. (2011) |
| III  | Selaginella moellendorffii | | SmSUT4C | 15417411 | 493 | Banks et al. (2011) |
| III  | Selaginella moellendorffii | | SmSUT4D | 15402611 | 531 | Banks et al. (2011) |
| III  | Solanum lycopersicum (Lycopersicon esculentum) | Tomato | LeSUT4 | AAG09270 | 501 | Weise et al. (2000) |
| III  | Vitis vinifera | Grape | VvSUC11 | AAF08329 | 501 | Davies et al. (1999) |
| III  | Zea mays | Corn | ZmSUT4 | AAT35810 | 501 | |
| Chlorokybus | Soil alga | | CaSUT1 | | | |
| atmospheric | Cyanidioschyzon merolae | | CmSUT1 | CMO328C | 502 | Matsuzaki et al. (2004) |
| Galderia sulphuraria | | | GsSUT1 | Gs18190 | 471 | Weber et al. (2004), Barbier et al. (2005) |
| Galderia sulphuraria | | | GsSUT2 | Gs34550 | 546 | Weber et al. (2004), Barbier et al. (2005) |
| Galderia sulphuraria | | | GsSUT3 | Gs56657 | 430 | Weber et al. (2004), Barbier et al. (2005) |
| Galderia sulphuraria | | | GsSUT4 | Gs29860 | 526 | Weber et al. (2004), Barbier et al. (2005) |
| Galderia sulphuraria | | | GsSUT5 | Gs08920 | 638 | Weber et al. (2004), Barbier et al. (2005) |
| Schizosaccharomyces pombe | Fission yeast | | SpSUT1 | NPS61487 | 553 | Reinders and Ward (2001) |

*sequence from DFCI (http://compbio.dfci.harvard.edu/cgi-bin/tgi/tgi/gimain.pl?gudb=medicago).

1 sequence from Phytozome v7.0 (http://www.phytozome.net/).

*not included in the phylogenetic analysis (< 90% identical to another SUT).

1 sequence from Cyanidioschyzon merolae genome project (http://merolae.biol.s.u-tokyo.ac.jp/).

1 sequence from Galderia sulphuraria genome project (http://genomics.msu.edu/galderia/index.html).
In Arabidopsis thaliana, type I SUTs display specialization in both expression and transport function. AtSUC2 is necessary for loading sucrose into the phloem (Gottwald et al., 2000). It has a $K_{m}$ (affinity) for sucrose of 1.4 mM (Chandran et al., 2003) and a wide substrate specificity for α and β glucosides that is shared with other type I SUTs (Figure 2; Chandran et al., 2003). AtSUC1 transport activity is very similar to AtSUC2 but its expression pattern is quite different. AtSUC1 is expressed in trichomes, pollen and roots (Sivitz et al., 2007). AtSUC1 is necessary for normal pollen function (Sivitz et al., 2008). Expression of AtSUC1 in the phloem, under control of the AtSUC2 promoter, has been shown to revert the growth defects of atsuc2 mutants (Wippel and Sauer, 2011). There are also examples of type I SUTs with modified transport activity. AtSUC9 has a much higher affinity for sucrose compared to other type I SUTs (66 μM; Sivitz et al., 2007) while the substrate specificity is typical of other type I SUTs (Figure 2; Sivitz et al., 2007).

**TYPE II SUTs**

Type II SUT sequences were identified in eudicots, monocots, non-vascular land plants (Physcomitrella), and vascular non-seed land plants (Selaginella). A total of 16 SUT sequences clustered in the type II group with an average of 62% (±9%) identity. The type II group was divided into two subgroups IIA and IIB. These two subgroups were identified previously (Braun and Slewinski, 2009). There is also a structural difference between type IIA and IIB SUTs. Type IIA proteins have a longer central cytoplasmic loop compared to type IIB SUTs. This is reflected in the average length of proteins in type IIA of 587 amino acids (aa) compared to 523 aa in type IIB (Table 1). Each angiosperm genome appears to have one gene in the IIA subgroup. Sequences from Physcomitrella (two) and Selaginella (one) are also included in the IIA subgroup. PpSUT2A and B from Physcomitrella and SmSUT2 contain longer central loops with conserved sequence characteristic of angiosperm type IIA transporters. Overall, this indicates that a type IIA transporter with a longer central loop was an ancestral form of the type II SUTs found in angiosperms.

The type IIB subgroup is monocot specific, rice encodes three type IIB transporters. This group contains the monocot phloem loading SUTs. ZmSUT1 has been shown to be expressed in vascular tissue and to function in phloem loading (Slewinski et al., 2009). Similar to the amplification of type I SUTs in Arabidopsis, type IIB SUTs appear to have been amplified in rice. Transport activities of OsSUT1 and OsSUT5 were analyzed by expression in oocytes and electrophysiology. OsSUT5 was found to have a higher affinity for sucrose (2.3 mM) compared to OsSUT1 (7.5 mM) and the activity of OsSUT5 was found to be less pH dependent (Sun et al., 2010).

It is interesting to note that monocots and eudicots utilize different SUTs to load sucrose into the phloem. Differences in substrate specificity between type I SUTs such as AtSUC2 that transport sucrose into the phloem in eudicots and type II SUTs such as HvSUT1 that performs the same function in monocots have been identified (Chandran et al., 2003; Sivitz et al., 2005, 2007; Reinders et al., 2006, 2008; Sun et al., 2008). Figure 2 shows a summary of substrate specificity results for five sucrose transporters. AtSUC2 and AtSUC9 are both type I sucrose transporters and although AtSUC9 has approximately a 20-fold lower $K_{0.5}$ for sucrose (Sivitz et al., 2007) compared to AtSUC2, they have almost (depending on the transporter affinity and substrate solubility). All currents were normalized to sucrose-dependent currents and are presented as mean ± SE with at least three oocytes per mean. Indicates substrate not tested. Modified with permission from Chandran et al. (2003), Sivitz et al. (2005, 2007), Reinders et al. (2006, 2008).
identical substrate specificities. These type I SUTs transport the plant \(\beta\)-glucosides salicin, arbutin, esculin, fraxin, and helicin. Notably, arbutin, esculin, and fraxin are not transported by the type II transporters ShSUT1 and HvSUT1 (Figure 2). Synthetic \(\beta\) phenyl glucosides are also transported by type I and not by type II SUTs (Figure 2).

The differences in substrate specificity between type I and type II SUTs might suggest that the specificity of phloem loading in eudicots is different from that in monocots. It is possible that type I SUTs load other glucosides, in addition to sucrose, into the phloem. To begin to address this question we used either AtSUC2 or HvSUT1 to complement the Arabidopsis atsuc2-1 mutant (Gottwald et al., 2000). The homozygous atsuc2-1 mutant has greatly reduced growth and accumulates starch in source leaves due to its reduced ability for phloem loading (Figure 3A). By comparison, growth of the atsuc2-1 heterozygous plants is indistinguishable from wild-type (Figures 3A,B). As expected, the atsuc2-1 mutant growth phenotype was complemented by expression of the AtSUC2 gene. Expression of the HvSUT1 coding region driven by the AtSUC2 promoter also resulted in growth that was indistinguishable from wild-type (Figure 3B). The type II SUT HvSUT1 appears to revert the growth reduction caused by the loss of AtSUC2 in Arabidopsis. This indicates that differences in substrate specificity between type I and II SUTs might not reflect a significant difference in physiological function, although this result is preliminary. Further work is necessary to determine if HvSUT1 fully complements under different growth and stress conditions.

Finally, the grouping of moss type II SUTs can give us a few more clues about the evolution and function of these ancestral type II SUTs. The type II moss and spikemoss sequences cluster with type IIA and contain longer central loops. Both Physcomitrella and Selaginella lack type I and type IIB SUTs. If early vascular plants such as Selaginella have SUTs that function in phloem loading, those transporters are likely to be type IIA such as SmSUT2 and are different from those used by monocots and eudicots. Also, type IIA SUTs in angiosperms do not compensate for loss of the main phloem loading SUT as evidenced by mutant phenotypes of atsuc2 (Gottwald et al., 2000) and zmsut1 (Slewinski et al., 2009) mutants.

**TYPE III SUTs**

The first type III SUTs were isolated from Arabidopsis, tomato, potato, and barley and named AtSUT4, LeSUT4, StSUT4, and HvSUT2, respectively (Weise et al., 2000; Weischke et al., 2000). AtSUT4 from Arabidopsis and HvSUT2 from barley (Endler et al., 2006), LjSUT4 from Lotus japonicus (Reinders et al., 2008), and OsSUT2 from rice (Eom et al., 2011) were demonstrated to localize to the vacuole membrane. Twenty type III SUT sequences were included in this study (Table 1) and these have an average of 65% (±8%) identity. Each angiosperm genome appears to contain a single type III SUT gene. Both Selaginella and Physcomitrella contain multiple type III SUT genes. No type III SUT homologs have been identified in green algae.

Transport activity has been characterized in detail for type III SUT LjSUT4 (Reinders et al., 2008). The substrate specificity of LjSUT4 is intermediate between type I and II SUTs (Figure 2). Like other type III SUTs (Weise et al., 2000; Weischke et al., 2000) LjSUT4 functions as a \(H^+\)-coupled sucrose-uptake transporter. This indicates that its physiological function in the vacuolar membrane is sucrose-uptake into the cytoplasm from the vacuolar lumen. This activity for AtSUT4 has been demonstrated in Arabidopsis vacuoles (Schulz et al., 2011).

**SUTs in Chlorokybus atmosphyticus, Galdieria sulphuraria, Cyanidioschyzon merolae, and Schizosaccharomyces pombe**

No SUT sequences were found in chlorophytes Chlamydomonas reinhardtii and Volvox carteri. Charophyte green algae are considered to represent ancestors of land plants. A single SUT sequence was found in the charophyte Chlorokybus atmosphyticus (CaSUT1). It did not cluster with type I, II, or III SUTs from land plants but appears to be basal to these clades (Figure 1). Since a complete genome sequence of a charophyte is not yet available it remains to be determined whether additional SUTs are present in charophyte genomes. The central loop of CaSUT1 is not extended as in type IIA SUTs. Also, the N-terminal sequence for CaSUT1 is not available so we could not determine if the putative vacuole targeting sequence is present (see Discussion).

Galdieria sulphuraria and Cyanidioschyzon merolae are closely related, unicellular red microalgae. While G. sulphuraria can grow...
on 27 different sugars and sugar alcohols (Gross and Schnarrenberger, 1995), C. merolae can not grow heterotrophically (Matsumaki et al., 2004). Five SUT homologs were identified in G. sulphuraria (GsuSUT1-5) and one, CmSUT1, was identified in the C. merolae genome (Figure 1: Table 1). This is consistent with the larger number of genes encoding transporters and enzymes involved in carbohydrate metabolism identified in G. sulphuraria compared to C. merolae (Barbier et al., 2005).

**DISCUSSION**

**THE ORIGIN OF PLANT SUTs IN CHAROPTHYTE ALGAE**

SUTs function as H\(^{+}\)-coupled cellular sucrose uptake transporters. In angiosperms, type I and II SUTs are localized to the plasma membrane while type III SUTs are localized to the vacuole membrane. They are important for the long-distance transport of sucrose in apoplastic phloem loaders (requiring transmembrane transport). Another important function for SUTs in angiosperms is in sucrose-uptake into sinks that are symplastically isolated such as seeds and pollen. The availability of bryophyte (non-vascular), lycophyte (early vascular), and algal genome sequences allows us to begin to analyze the origins of SUTs in land plants. The presence of CaSUT1 in the charophyte alga Chlorokybus atmosphyticus as well as the absence of SUTs in chlorophyte algae (Chlamydomonas reinhardtii and Volvox carterii) is consistent with the hypothesis that charophyte algae are ancestral to land plants (McCourt et al., 2004).

The physiological function of SUT homologs in Chlorokybus, which exists as small clusters of cells and in the unicellular red alga Gallideria and Cyanidioschyzon is currently unknown but will depend on their membrane localization. They are likely to function as H\(^{+}\)-coupled symporters for glucoside uptake into the cytosol whether they are localized to the plasma membrane or an internal membrane. Interestingly, Cyanidioschyzon lacks a central vacuole (Barbier et al., 2005), so it is more likely that CmSUT1 is a plasma membrane transporter. Bryophytes lack true vascular tissue yet Physcomitrella contains both type IIA and type III SUTs. In angiosperms, type IIA SUTs are localized to the plasma membrane (Barker et al., 2000; Meyer et al., 2000) while type III SUTs are vacuolar (Endler et al., 2006; Reinders et al., 2008). Therefore, it is likely that Physcomitrella contains both plasma membrane and vacuolar SUTs but this will need to be determined experimentally. Long-distance transport of photosynthate in mosses involves leptoid cells and the mechanism appears to be symplasmic, involving plasmodesmata not transmembrane transport (Raven, 2003). Therefore, if SUTs are localized to the plasma membrane in bryophytes their function is not in phloem loading but may be involved in recovery of sucrose that is released to the apoplast. Although leptoid cells evolved independently of phloem, many groups of angiosperms that utilize a similar passive mechanism for phloem loading (Rennie and Turgeon, 2009) also encode SUTs. The function of type III SUTs in bryophytes is likely to be the same as in angiosperms. Sucrose is transiently stored in the vacuole in angiosperms and type III SUTs function in the vacuole membrane to return sucrose from the vacuole lumen to the cytoplasm (Reinders et al., 2008; Schulz et al., 2011). The more recent development of type I SUTs in eudicots and type IIB SUTs in monocots is likely to be linked to the evolution of active phloem loading requiring energy and transmembrane transport.

**PUTATIVE VACUOLAR TARGETING MOTIF IN TYPE III SUTs**

Recently, a dileucine-like motif (LXXXL) in the N-terminal cytoplasmic domain of the Arabidopsis monosaccharide transporter ESL1 was shown to be necessary for localization of the transporter to the vacuole membrane (Yamada et al., 2010). Dileucine-like motifs are recognized by a clathrin-associated, heterotetrameric adaptor protein (AP-3) complex and function in sorting of vacuole membrane proteins in yeast (Vowels and Payne, 1998). Similar dileucine motifs contain an acidic residue spaced several residues prior to the leucine pair with a consensus of DXXXL or DE[LXXL][L1] (Braulke and Bonifacio, 2009). The AP-3 complex has been shown to be necessary for normal vacuole function in Arabidopsis (Zwievelka et al., 2011). An LXXL motif is found in the cytoplasmic N-terminus of type III SUTs (Figure 4) but is lacking in type I and II SUTs. All of the angiosperm type III SUTs contain a perfect LXXL motif with the exception of AtSUT4 that has the sequence KRVLL (Figure 4). AtSUT4 has been demonstrated to localize to the vacuole membrane (Endler et al., 2006) so it is likely that the first leucine of the motif is not strictly required. Recently, localization of AtSUT4 to the vacuole membrane in Arabidopsis was shown to be dependent on AP-3 (Wolfenstetter et al., 2012). None of the Physcomitrella or Selaginella type III SUTs contain a...
complete LXXLL motif and it is unknown whether they localize to the vacuole membrane.

**The Origin of Type I SUTs**

Type I SUTs are localized to the plasma membrane in eudicots. Based on phylogeny (Figure 1) and substrate specificity (Figure 2) they are more similar to type III SUTs than to type II SUTs. Since type III SUTs are present in bryophytes and lycophytes, we suggest that type I SUTs are derived from vacuolar-type III SUTs. This would likely involve mutation of the vacuolar targeting information resulting in localization to the plasma membrane, the default targeting pathway for membrane proteins in plants. We hypothesize that the LXXLL motif found in type III SUTs serves as the vacuolar targeting domain but this needs to be tested directly.

**Conclusion**

Angiosperm SUTs clustered into three groups, type I, II, and III. Type I SUTs, only found in eudicots, appear to have evolved from vacuolar-type III SUTs which were found in all land plants from bryophytes to angiosperms. Type II SUTs were divided into an ancestral form, type IA, that exist in all land plants and have an extended central loop. Type IIB SUTs only exist in monocots and include the plasmol loading transporters in those species. Here we identify an algal SUT (CaSUT1) from the charophyte *Chlorokybus* *atmosphericus*. Based on phylogenetic analysis, CaSUT1 appears basal to three types of land plant SUTs and this is consistent with the hypothesis that charophytes are ancestral to land plants.

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