Sucrose Transporters in *Selaginella moellendorffi*

Rebecca J. Baker and John M. Ward

Plant Biology Department, University of Minnesota Twin Cities

250 Biological Sciences Center, 1445 Gortner Ave., St. Paul, MN 55108, USA

INTRODUCTION

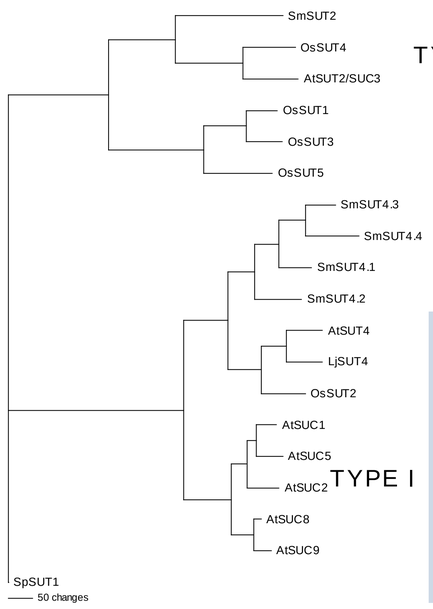
Plant vasculature is composed of two distinct types of tissue, the xylem and the phloem. Xylem transports water and solutes up from roots and phloem transports metabolites from photosynthetic mesophyll tissue in leaves to the roots and other sink tissues (Rennie and Turgeon, 2009). Vascular tissue is thought to have provided an evolutionary advantage to early land plants allowing rapid water and metabolite distribution and allowing for larger plants that competed more successfully for available sunlight. Some of the earliest vascular plants were the Lycophytes and extant examples include Selaginella. Selaginella is a heterosporous, herbaceous plant, commonly called "spikemoss" that can be traced to about 333-350 Myr ago. Selaginella is recognized as an important intermediate between vascular and non-vascular plant species and it is the first Lycophyte for which we have a genomic sequence (Banks, 2009.) *Selaginella moellendorffi* has a genome size of ~110Mbp, thus making this species of a practical choice for sequencing. Now that genome sequence is available for *Selaginella moellendorffi* we can begin to analyze the evolution of vascular tissue at the genetic level.

Selaginella moellendorffi (http://genome.jgi-psf.org/Selmo1/Selmo1.home.html).

Phloem is the highly specialized tissue for long distance transport of nutrients and carbohydrates from photosynthetic leaves to the sink tissues in roots and stalk. Sink tissues depend on this supply of metabolites. Sucrose is produced in the mesophyll cells and then moved to the phloem to be transported into non-photosynthetic tissues. The conducting units of the phloem are called sieve elements (Gottwald et al., 2000.) There are three phloem loading mechanisms identified in higher plants (Rennie and Turgeon, 2009): active, passive and polymer trapping. Passive and polymer trapping rely heavily on concentration gradients to move solutes from the mesophyll into the companion cells and into the sieve element cells (Rennie and Turgeon, 2009.) Most crop plants load sucrose into the phloem using an active mechanism that requires sucrose transporters (SUTs). Mutations in those sucrose transporter genes results in severe growth defects (Gottwald et al., 2000; Slewinski et al., 2009). Sequence analysis revealed that there are three types of SUTs called type I, II and III. Type I and II SUTs are localized to the plasma membrane and type III SUTs are localized to the vacuolar membrane. All SUTs studied to date are proton coupled sucrose uptake transporters that use the energy of a transmembrane proton gradient to accumulate sucrose in the cytoplasm.

There are five predicted SUTs in *Selaginella moellendorffi* sequence. SUTs contain highly conserved domains and that a better understanding of the evolution of sucrose transporters and phloem tissue may be gained from research on *S. moellendorffi* transporters. In this research project we attempted to clone the five predicted *S. moellendorffi* sucrose transporters and sequence them for comparison with the previously predicted sequences.

Phylogenetic Sucrose Transporter Tree



Phylogenetic tree of sucrose transporters (SUTs) in plants. Full length amino acid sequences were aligned using Clustal X and a neighbor joining tree was made using PAUP. (Sm- Selaginella, At- Arabidopsis, Os- rice, SpSUT1 from fission yeast (Reinders and Ward, 2001) was used as an outgroup to root the tree. The classification of sucrose transporters into type I, II, and III follows Aoki et al., 2003.

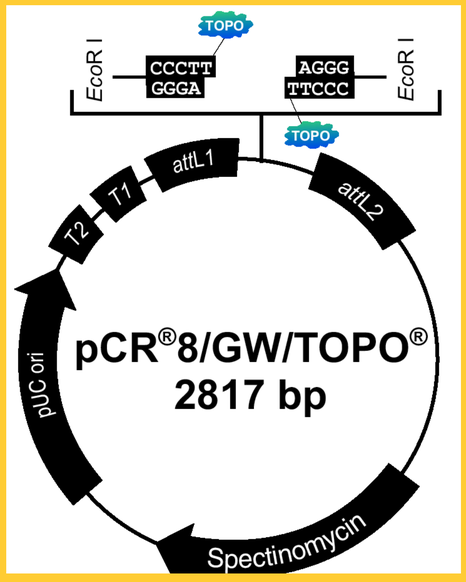
Methods

Primers were designed to amplify five SUT cDNAs using sequences from the Department of Energy Joint Genomic Institute predicted cDNA splicing gene sequences: SmSUT2, SmSUT4.1, SmSUT4.2, SmSUT4.3, SmSUT4.4. (http://genome.jgi-psf.org/Selmo1/Selmo1.home.html) Live *Selaginella moellendorffi* was clipped, crushed and prepared for RNA extraction (*Selaginella moellendorffi* stock #1165 from Glasshouse Works Stewart, OH 45778-0097). RNA was extracted using QIAGEN RNeasy Plant Mini Kit. cDNA was created using Reverse Transcription PCR 50C for 30min followed by 25 cycles of OCR with an annealing temperature of 55C (QIAGEN One Step RTPCR KIT.)

Selaginella klausiana, growing in the CBS greenhouse at the University of Minnesota.

PCR products had approximately the expected size ~1500bp and were cloned into the PCR8vector (Invitrogen). E.coli (Top10) was transformed and cells were spread on LB spectinomycin plates and incubated overnight. From these plates 74 colonies were selected and transferred into individual LB spec liquid tubes, once again incubated at 37C overnight. Plasmids were isolated using a "boiling prep" procedure. These plasmids were evaluated with an EcoR1 restriction digest. QIAGEN Sequencing Mini Prep Kit was used to isolate cDNA and cDNA was sequenced by University of Minnesota's Sequencing Center. Results were compared to the JGI predicted gene sequences (http://genome.jgi-psf.org/Selmo1/Selmo1.home.html).

PCR8 Vector

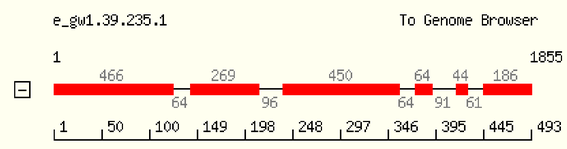


PCR8Vector (Invitrogen) Contains EcoR1 sites and Spectinomycin resistance which allowed experimenters to identify transformants on LB spectinomycin plates. The desired insert was roughly 1500bp and the plasmid was 2817bp. This difference in size helped in reading gel electrophoresis assays.

Results

A number of transformants (74) were obtained and 11 contained plasmids with inserts of the expected size. These were sequenced and only one represented a sucrose transporter cDNA. The others were mostly sucrose transporter genes (contaminating genomic DNA). The cDNA obtained is encoded by SmSUT4.3, a gene containing 6 exons, shown in the model below.

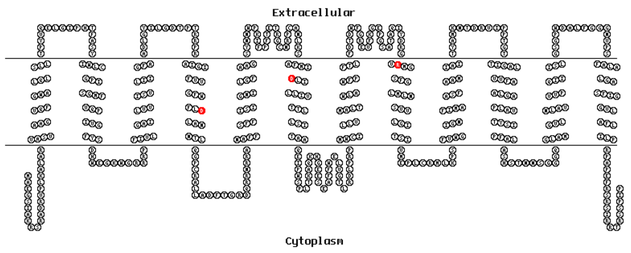
SmSUT4.3 Gene Model



Predicted by JGI (http://genome.jgi-psf.org/Selmo1/Selmo1.home.html).

SmSUT4.3 Transmembrane Model

The predicted SmSUT4.3 protein contains 492 amino acids, and has homology to type III SUTs from higher plants. Its closest homolog in Arabidopsis is AtSUT4 which is 51% identical. Like all plant SUTs, SmSUT4.3 is predicted to have 12 transmembrane spans. Other type III SUTs are localized at the vacuole membrane. Additional experiments will be necessary to determine the localization of SmSUT4.3 in Selaginella.



Transmembrane span prediction was done using the HMMTOP program: "G.E Tusnády and I. Simon (2001) The HMMTOP transmembrane topology prediction server" Bioinformatics 17, 849-850.
The membrane topology model was drawn using TOPO2 (http://www.sacs.ucsf.edu/TOPO-run/wtopo.pl). Charged groups are shown in red.

Concluding Remarks

Phylogenetic analysis indicates that SmSUT4.3 is a type III SUT. It is interesting that Selaginella has 4 type III SUTs while higher plants typically have only one. With the successful cloning of SmSUT4.3 we can now study its function. This is typically done by heterologous expression to analyze the transport properties such as substrate affinity and specificity. We can also analyze SUT4.3 gene expression and protein localization.

REFERENCES

Banks, J.A (2009). Selaginella and 400 million years of separation. Annu Rev Plant Biol 60,223-238.

Gottwald, J.R., Krysan, P.J., Young, J. C., Evert, R.F., and Sussman, M.R., (2000). Genetic evidence for the in planta role of phoem-specific plasma membrane sucrose transporters. Proc Natl Acad Sci U S A 97, 13979-13984.

Rennie, E.A., and Turgeon, R. (2009). A comprehensive picture of phloem loading strategies. Proc Natl Acad Sci U S A 106, 14162-14167.

Slewinski, T.L., Meeley, R., and Braun, D.M. (2009). Sucrose transporter1 functions in phloem loading in maize leaves. J Exp Bot 60, 881-892.