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# TRANSPORT MECHANISMS FOR ORGANIC FORMS OF CARBON AND NITROGEN BETWEEN SOURCE AND SINK

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■ **Abstract** Sugars and amino acids are generated in plants by assimilation from inorganic forms. Assimilated forms cross multiple membranes on their way from production sites to storage or use locations. Specific transport systems are responsible for vacuolar uptake and release, for efflux from the cells, and for uptake into the vasculature. Detailed phylogenetic analyses suggest that only proton-coupled cotransporters involved in phloem loading have been identified to date, whereas systems for vacuolar transport and efflux still await identification. Novel imaging approaches may provide the means to characterize the cellular events and elucidate whole plant control of assimilate partitioning and allocation.

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## INTRODUCTION

As highly evolved multicellular organisms, the sporophytes of plants have distributed different tasks between organs specializing in specific functions. Whereas roots specialize primarily in the uptake of mineral nutrients and water, leaves are mainly responsible for the assimilation of $CO_2$, inorganic nitrogen, and other nutrients. To exchange nutrients between the organs, two distribution conduits were developed, the xylem for transport of nutrients from roots to shoots, and the phloem for transport from leaves to roots, apex, and reproductive organs.

If we assume that plant cells are connected by plasmodesmata that are permeable for small ions and metabolites, such compounds may diffuse from cell to cell. However, transport of small solutes through plasmodesmata has not been demonstrated experimentally. Dye coupling studies indicate that not all cells are connected by open plasmodesmata (72, 109), thus limiting solute movement at certain cellular interfaces and defining domains that require apoplasmic transport involving transport proteins. As detailed in the following sections, loading and unloading nutrients to and from the two conduits requires a myriad of polytopic plasma membrane transport proteins for the many substances translocated throughout the plant (50).

Sugars and amino acids are centrally embedded in primary metabolism. Sugars serve as the primary energy source to generate ATP (adenosine triphosphate) and redox energy. Therefore, they are taken up by all cells for transient or long-term

storage in the form of starch, as structural components (i.e., cell walls), as carbon skeletons for biosynthesis of most other metabolites, and as molecules involved in signal transduction. In animals, glucose is the most important energy source and transport form. In contrast, plants mainly use the disaccharide sucrose and, in some species, derivates such as raffinose, stachyose, and verbascose for transport. Using sucrose has several advantages for long-distance transport, and is recognized as an archaic phenomenon because cyanobacteria are already capable of synthesizing and degrading sucrose (133), and nonvascular plants such as mosses contain close homologs of sucrose transporters (SUTs). In higher plants, sucrose is loaded from sites of biosynthesis, i.e., from source tissues, into the phloem by membrane carriers and transported to the sites of use and storage, the sink tissues. According to Münch's mass flow hypothesis (104), sucrose, the major osmotically active constituent in the phloem of most plants, also provides the driving force for translocating all other compounds in the phloem sap.

In most plants, organic nitrogen is preferentially transported in the form of amino acids, which serve as hubs for many functions including nitrogen metabolism, protein synthesis, and nerve transmission, and as precursors for many important cellular constituents including nucleobases. Most cells can take up a wide spectrum of different amino acids using broad selectivity carriers. Long-distance transport occurs not only in the phloem but also in the xylem, generating a quasi circulatory system for organic nitrogen transport.

Since the first description of mass flow driving phloem by Münch (104), we have obtained a detailed understanding of many of the steps involved in loading and unloading of sugars and amino acids into and out of the phloem. In contrast, less is known about xylem transport of amino acids. The physiology of sugar and amino acid transport has been reviewed extensively in recent years (27, 37, 38, 51, 87, 91, 134, 169, 176). In short, milestones were (*a*) identification of $H^+$ cotransport as the mechanism for sucrose loading (13, 60, 82); (*b*) development of a technique for isolating plasma membrane vesicles, which was used to characterize sucrose and amino acid uptake by tracer studies (24, 26, 89, 93, 94); (*c*) use of biochemical approaches in attempts to identify the transport proteins and genes involved, leading to the identification of a sucrose binding protein, a 42-kDa protein (probably representing a SUT), and the plasma membrane $H^+$-ATPase (57, 125, 137); and (*d*) cloning of the genes encoding major components of phloem loading. *$H^+$-ATPase* genes were identified via homologies to the yeast *$H^+$-ATPase* and via degenerated oligonucleotides derived from a partial plant *$H^+$-ATPase* sequence (67). Glucose $H^+$-cotransporters were identified by an elegant molecular approach, i.e., genes induced during the shift of *Chlorella* from photoautotrophic conditions to glucose media (136). Potassium channels and sucrose and amino acid transporter genes were cloned by suppression cloning in yeast mutants (3, 54, 55, 71, 123, 144). Subsequently, the biochemical properties of the carriers were determined using tracer studies and electrophysiological tools in heterologous hosts (5, 15–17, 52, 179). Availability of the genes facilitated analysis of expression patterns and allowed determination of the physiological role using transgenic and mutant approaches.

With the *Arabidopsis* genome sequence, we learned that glucose, sucrose, and amino acid transporters belong to large gene families, leading us to question whether paralogs serve as organellar transport or solute effluxers. With the rice genome fully sequenced, and with sequences for transporter genes from a wide variety of plant species, it is now possible to carry out comparative genomic studies with the goal of defining a basic set of genes essential for each intra- and intercellular step required to achieve translocation between source and sink, local exchange and cycling of amino acids between phloem and xylem. To facilitate functional analysis, bioinformatic approaches are used to define the full complement of membrane proteins in higher plants (142, 156, 168). Knock-out and antisense strategies have already shown that at least one of the SUTs found (SUT1/SUC2) is essential for long-distance transport, and that one of the amino acid transporters (AAP1) plays a role in long-distance transport of amino acids (25, 63, 80, 124). However, owing to the number of transporters in the different families, it is necessary to analyze the properties of each individual carrier, to determine their expression patterns under various conditions, and to determine the function in the plant by characterizing transgenic and knock-out mutants.

Taken together, significant progress has been made regarding assimilate transport, but we still have only a partial picture. We are missing the genes responsible for important transport systems for sugar efflux into the apoplasm, for xylem loading, and for vacuolar transport. We also do not understand how long-distance transport is coordinated. In this review, we summarize the current status regarding transport systems for sucrose, monosaccharides, and amino acids, and we identify knowledge gaps to establish the routes to pursue.

## TRANSPORTERS FOR SUCROSE

Sucrose phosphate synthase, the key enzyme for sucrose synthesis, is localized in the mesophyll of leaves. Sucrose synthesized in the mesophyll is exported to sinks via the phloem and must move across several cell layers before it reaches the phloem (Figure 1). Although no direct evidence has been presented, sucrose is assumed to move symplasmically via plasmodesmata to the loading site inside the phloem. Efflux into the apoplasm occurs by an unknown mechanism and is probably directly juxtaposed to the sites where import occurs into the sieve element–companion cell (SE-CC) complex to prevent movement of sucrose into the apoplasm with the transpiration stream. Uptake from the apoplasm into the phloem is mediated by cellular importers, the $H^+$-sucrose cotransporters. The genes for SUTs were first identified by suppression cloning in a prototrophic yeast mutant dependent on the provision of a functional sucrose uptake system (122, 123). *SUT1* was then used to isolate paralogs from solanaceous species and homologs from many different angiosperms. The genome of the nonvascular moss *Physcomitrella patens* contains several genes highly homologous to the SUTs (S. Lalonde & W.B. Frommer, unpublished results).

SUTs are members of the glycoside-pentoside-hexuronide (GPH):cation symporter family, which belongs to the major facilitator superfamily (MFS). The

crystal structure of its prototype, the bacterial lactose permease, was used to predict a model of the SUTs (1, 43, 76, 132). SUTs have a similar structure, with 12 predicted transmembrane domains, and are assumed to form a single pore for sucrose, with N- and C-termini and 5 even-numbered loops located in the cytosol (Figure 2) (153). The structure probably originated from an ancient duplication and fusion of a gene encoding a protein with six transmembrane domains, indicating that originally six-spanners formed homodimers around a single pore. Thus, the central loop serves merely as a linker connecting the fused domains. SUT1 and SUC1 have been characterized as $H^+$-cotransporters with a 1:1 proton/sucrose transport ratio electrophysiologically after heterologous expression in *Xenopus* oocytes (17, 178).



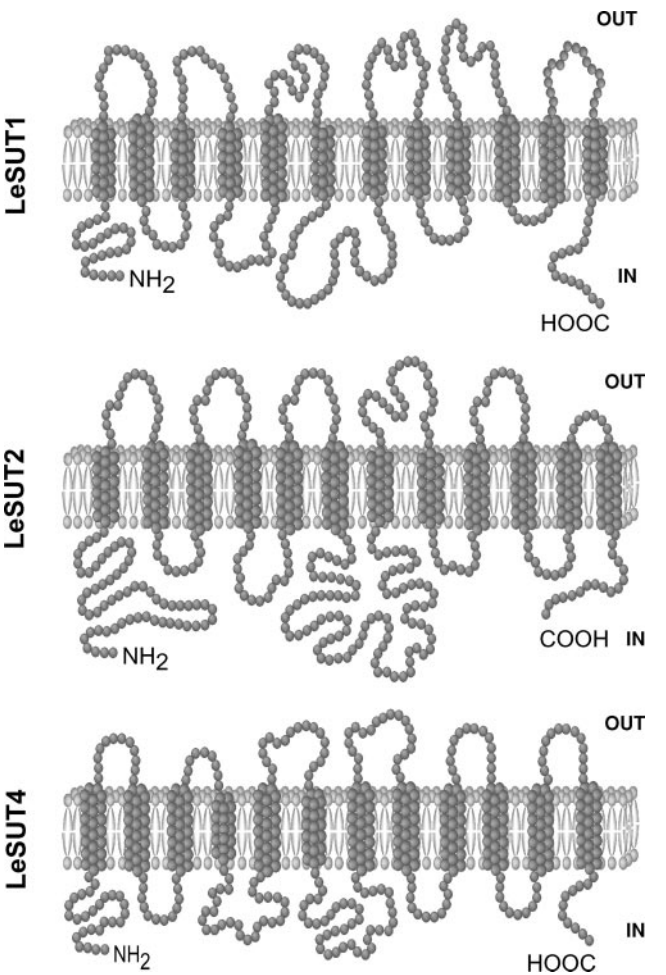


**Figure 2** Structural models of the three types of sucrose transporters: SUT1, SUT2, and SUT4. SUT2 is characterized by an extended sixth loop located in the cytosol and an extended cytosolic N terminus.

Analysis of the *Arabidopsis* and rice genomes shows that SUTs comprise gene families with nine and five members, respectively. To facilitate analysis of SUTs from different species, we performed a phylogenetic analysis after compiling all SUTs for which complete sequences were available (NCBI, August 2003 release; Figure 3). The cellular sucrose importers identified so far have arisen from a single gene family including distant homologs in bacteria, fungi, and animals (56, 121). The proteins fall into three clades with their prototypes SUT1/SUC2 (clade I), SUT2/SUC3 (clade II), and SUT4 (clade III). In dicotyledons, the clades correspond with structural or functional differences: SUT1/SUC2 has a Km (Michaelis-Menten constant) for sucrose of ~1 mM, SUT4 has a tenfold lower affinity, and SUT2, which is also a low-affinity system, has a highly conserved sequence within the extended central loop in most members of the family (Figure 2). Whereas *SUT2* and *SUT4* exist as single copies in dicotyledon genomes, *SUT1* is often present in multiple copies, with *Arabidopsis* standing out with seven members present in this clade. In comparison, the rice genome does not seem to contain proteins corresponding to SUT1/SUC2, whereas the SUT2 clade of rice has been amplified. Some SUT2 proteins from rice contain the extended loop found in dicotyledon SUT2, whereas others lack this domain.

## Sucrose Transporter Clade I (SUT1/SUC2)

Members of the SUT1 subfamily typically show a high affinity for sucrose with a Km between 0.5–2 mM. Solanaceous SUT1 localizes to the SE plasma membrane of leaves, petioles, and stems in source and sink tissues and all along the translocation path (9, 83, 174). Localization of *SUT1* mRNA at the orifices of plasmodesmata also in SE suggests that *SUT1* mRNA synthesizes in CC traffic through plasmodesmata into SE (83). The trafficking of *SUT1* mRNA into SE is supported by the identification of SUT1 mRNA in phloem sap of a wide spectrum of plant species (79, 83, 131). Results from experiments in which SUT1 was fused to green fluorescent proteins are consistent with both RNA- and protein-based trafficking models (87a). The expression of *SUT1/SUC2* along the translocation path suggests a further role in keeping osmotic pressure high in SE by antagonizing loss of sucrose by passive leakage. SUT1/SUC2 must be essential because transgenic lines or mutants with reduced transporter activity display reduced export from leaves coupled to reduced supply of sink organs (25, 63, 124). However, it is still not clear whether the reduced translocation is due to reduced loading or retrieval, nor is it clear whether reduced transporter activity leads to reduced sucrose concentration in the phloem and to reduced transport velocity. More detailed analyses using $^{11}C$ and NMR imaging may help solve these questions (73). Sucrose transport is also required for supplying symplasmically isolated sinks such as pollen and seeds. SUT1/SUC2 paralogs (NtSUT3 and AtSUC1) seem to play a role in nutrition of pollen and derive from gene duplication after separation of the plant families (92, 152). As compared to other plant species, the *Arabidopsis* genome contains additional members of this clade, which suggests that the functions originally mediated by a single SUT1 protein are now distributed across the seven paralogs. Nevertheless, AtSUC2 must retain crucial functions orthologous to SUT1 because

a knockout is lethal (63). In *Arabidopsis*, SUC2 is localized in CC (151), which raises the question of whether other SUC2 paralogs may be targeted to SE, as suggested by the finding that *Plantago* PmSUC2 is present in CC (150), whereas PmSUC1 is in SE (149).

Apoplasmic uptake of nutrients into the developing embryo has best been studied in large-seed legumes (68, 112, 172). In *Vicia*, a SUT1 homolog is expressed in the epidermal cells of the developing cotyledons, where it is probably responsible for high-affinity sucrose uptake into developing embryos (157). Overexpression in the storage parenchyma of cotyledons enhances sugar accumulation, at least during early stages of development (129). Analysis of knock-out mutants in the seven paralogs (clade I) of *Arabidopsis* may help us to understand the role of SUT1/SUC2 members in seed development.

Major unresolved questions remain, e.g., how SUT1s get into SE, why SUTs can be present in either CC or SE, whether homo-oligomerization of SUT1/SUC2 plays a role in regulating sucrose transport (119, 141), how turnover of SUTs is controlled in SE, and whether $H^+$-sucrose cotransporters can also function in a slippage or reverse mode to unload sucrose from the phloem (126).



## Sucrose Transporter Clade II (SUT4)

Tracer studies suggest that sucrose uptake is composed of multiple kinetic components (39, 100) with high-affinity/low-capacity (HALC) and low-affinity/high-capacity (LAHC) systems. Clade I members fit the HALC kinetic parameters, whereas the properties of most SUT4 subfamily members correspond to LAHC (12 mM, AtSUT4) (173). Dicotyledonous species work with only a single-copy member in clade II, whereas at least two paralogs exist in Poaceae. Consistent with a LAHC function, AtSUT4 is expressed in minor veins and sink tissues (174). In barley, *HvSUT2* RNA was detected in endosperm transfer cells (175). In Solanaceae, SUT4 colocalizes with SUT1 and SUT2 in SE (9, 173), and analyses using the split ubiquitin system indicate that SUT4 can interact with SUT1 and SUT2 (119, 141). The functional role of the potential heterodimer remains to be shown. For transporters, affinity often correlates inversely with Vmax, consistent with the hypothesis that SUT4 serves as a high-capacity system for phloem loading and supply of sink organs, whereas SUT1/SUC2 keep apoplasmic sucrose levels low during loading and along the path, and are necessary for loading the phloem at low supply. Knock-out mutants and detailed expression studies in source and sink are required to test these hypotheses. It will also be interesting to learn whether AtSUT4 localizes to SE or CC in *Arabidopsis* (141).

## Sucrose Transporter Clade III (SUT2/SUC3)

The third clade can be divided into two subgroups based on the presence or absence of an extended central loop located in the cytoplasm (Figure 4). Because transport function resides in the hydrophobic transmembrane domains in most membrane proteins, loops serve primarily as linkers. Comparisons between related transporters show that transmembrane domains are often highly conserved whereas

loop sequences vary significantly. The sequence of the extended central loop of SUT2 is highly conserved even between mono- and dicotyledons, suggesting that it serves a specific function, e.g., as an interaction domain for other proteins (Figure 4). Consistent with this, the extended central loop is not required for transport function because its deletion does not affect transport activity (102, 140). Because similar extended interaction domains exist in yeast hexose transporter (HXT)-like proteins, which function as sensors, it has been hypothesized that SUT2 may serve as a sucrose sensor. In bacteria and yeast, it is well established that many transport proteins dispose of sensing functions, transmitting information about the external concentration or metabolite flux to downstream signaling cascades (87) as, e.g., in the glucose phosphate sensor UhpC, which regulates the activity of its close homolog UhpT. UhpC transmits the signal via a histidine-aspartate phospho-relay to regulate transporter activity via a signaling cascade (167). Despite the sensor function, UhpC has retained its transport function (143). Similarly, the bacterial iron citrate transporter FecA mediates transcriptional induction of its own operon via an extended N-terminal domain (22, 23, 48). Transporter-like sensors were also found in yeast, where extracellular hexose levels are measured by HXT-like proteins RGT2 and SNF3, which function as glucose sensors controlling expression of HXT genes (110, 111). The extended C-terminal domains of SNF3 and RGT2 contain conserved motifs that serve as signaling domains (110). Typically, transporter-like sensors show low expression and no detectable or only weak transport activity (10, 140). Also, expression levels of *SUT2* genes characterized by the extended loop are low at the RNA level, and the codon bias suggests low translation efficiency. A crude analysis of a knock-out mutant in the *Arabidopsis SUT2* gene did not reveal obvious phenotypic effects (10). Based on this, the authors argue that SUT2 does not play a role as a sensor because more dramatic phenotypes would be expected for such a sensor. However, consistent with the function of sensors in fine tuning transport and metabolism, a complete knock-out of the sensors SNF3, RGT2, or SSY1 did not lead to dramatic phenotypes in yeast (110). A more detailed analysis of SUT2 knock-out mutants reveals significant changes with respect to sugar transport and potentially also sugar sensing (W.X. Schulze & W.B. Frommer, unpublished). In tomato, all three principal sucrose transporters SUT1, SUT2, and SUT4 colocalize in enucleate SE. In *Arabidopsis*, GUS analysis suggests that transcription of *AtSUC2*, *AtSUT2*, and *AtSUT4* occurs in CC, whereas immunolocalization indicates that SUT2 is present in bundle sheath (102, 141). Further work is required to solve these potential discrepancies.

As postulated in many other organisms, the measurement of apoplasmic sugar concentrations by plasma membrane–localized sensors seems a feasible mechanism for adjusting sucrose fluxes in plants (87). This hypothesis is especially appealing because plants have several SUTs, differing in affinity and capacity [0.5 mM (179) to 11 mM (9)]. External sensors would permit acclimation to specific requirements by regulating the relative abundance and activity of the different carriers. Additional work is required to determine whether SUT2 members serve as sensors or simply as a second set of low-affinity transporters. In case SUT2 does not act as a sucrose sensor, other membrane proteins must be postulated for this

function because physiological analyses strongly suggest that plasma membrane–bound sugar-/sucrose-sensing pathways exist in plants (99).

The second subgroup of the SUT2 clade in monocots SUTs carries no extended central loop and may have a function equivalent to dicotyledon SUT1/SUC2 members. Expression profile, expression level, and transport properties suggest that the SUT2 clade member OsSUT1 from rice, which lacks the extended loop, serves as an ortholog of SUT1/SUC2. Like many other SUT1/SUC2, *HvSUT1* mRNA was detected in phloem sap (42), suggesting that monocots use SUT2 members present at the SE plasma membrane for phloem loading. Thus, Poaceae may have duplicated SUT2, lost the central SUT2 loop in one copy and used this SUT2 derivative for SUT1 function, whereas the SUT1 homolog became redundant and was lost. Analysis of other genomes such as from gymnosperm, ferns, and mosses will shed more light on the evolution of the SUT family.



## REGULATION OF SUCROSE TRANSPORT

It is obvious that sucrose plays a crucial role in determining shoot-to-root ratio (139), and affects developmental processes such as the onset of flowering (34, 69). In addition, communication between sources and sinks is necessary to coordinate photosynthesis and transport of sucrose with the requirements of various sink organs (115). Regulation of sucrose transport is thus a conceivable mechanism to control a variety of whole-plant responses. Due to the inherent variability of environmental parameters, it is anticipated that sucrose transport must be regulated in a wide dynamic range (87). Environmental factors and endogenous signals affect sugar export from leaves by regulating sucrose transport (27, 53, 87). Overexpression of pyruvate decarboxylase in potato increased sucrose export tenfold, underlining the capacity for upregulating transport (155). Expression of SUTs StSUT1 and its *Arabidopsis* ortholog AtSUC2 is under developmental control, and both are induced during the sink-to-source transition in leaves (122, 160). As with many other transport systems, regulation by the substrate would be a logical mechanism to control transport activity according to substrate availability. Consistent with a substrate-regulated mechanism, SUT2 is induced (9), whereas other SUT genes are downregulated by sucrose (32, 165). Besides regulation at the transcriptional level, post-translational mechanisms also affect transport activity, e.g., via modification of SUT activity by phosphorylation (127), or modification of protein abundance at the plasma membrane (2, 85). Protein turnover can be influenced by synthesis, degradation, or cycling of transporters. Another possibility for regulating transporter activity is oligomerization, as described for the bacterial lacS and animal GLUT1 carriers (64, 166, 180). This type of regulation is also feasible for SUTs because they turn over rapidly (83, 165) and because they have the potential to form oligomers. It is also conceivable that regulation occurs within the membrane because SUTs can form homo- and heteromeric complexes (119, 120); this would also provide a direct means for regulation within enucleate SE. A more detailed analysis is required to determine the mechanisms for sensing and signal transduction, and to establish the link to whole-plant physiology.

## CELLULAR EFFLUX AND VACUOLAR TRANSPORT OF SUCROSE

As described above, phloem loading and retrieval of sucrose from the apoplasm are mediated by the $H^+$-coupled cotransporters of the SUT family. The apoplasmic step leads us to a loose end, namely how sucrose is released into the apoplasm before being loaded into the SE-CC. Principally, two routes for efflux are possible: by exocytosis or via efflux transporters at the plasma membrane (Figure 1). There is currently insufficient knowledge in this area for a conclusive answer. Echeverria (44) summarized the evidence for a vesicular efflux pathway, and suggested that vesicle formation from the vacuole, direct trafficking to the plasma membrane, and release of the cargo into the apoplasmic space might represent a feasible mechanism for efflux of solutes including sucrose. No matter which mechanism is involved, transporters are required, e.g. vesicular transport carriers for loading the vesicles or the vacuole. Sucrose accumulates to high levels in vacuoles. Transport systems for both uptake and release of sucrose have been described biochemically (44, 59). Because all three SUTs are present on the plasma membrane, and none of them were found in cells involved in efflux of sucrose, other still unknown proteins must be responsible for vacuolar uptake and release of sucrose. Because respective assays for identifying vacuolar transporter genes are currently not available, genomic and proteomic approaches or the use of novel tools may be means toward the identification.

Alternatively, novel plasma membrane sucrose transporters functioning as uniporters might equilibrate sucrose between cytosol and apoplasm, as occurred in animals with glucose transport from the intestine into the bloodstream. However, this model has recently been challenged because deleting the major glucose facilitator GLUT2 in mice had only marginal effects on glucose efflux from hepatocytes (158). A thorough analysis of potential alternative efflux mechanisms led to the suggestion that glucose-6-P is imported into the ER (endoplasmic reticulum), where it is dephosphorylated and exported by vesicles trafficking to the plasma membrane. Analysis of the effects of ectopic expression of yeast invertase in plant vacuoles provides hints for the presence of sucrose in the ER (70).

An alternative mechanism for sucrose efflux is the slippage mode of $H^+$-coupled SUTs (126). Ritchie and coworkers (126) found $H^+$-coupled sucrose transporters on the efflux side in seed coats of legumes, and suggested that at low extracellular concentrations a SUT member may work as a cotransporter, whereas at high concentrations it switches into a slippage mode. This hypothesis might explain why SUTs are expressed all along the translocation path, even into sink organs, consistent with the finding that inhibition of SUT1 expression specifically in developing tubers negatively affects tuber development during early stages of development (84).

A fourth possibility is that efflux occurs by specific effluxers that either use an antiport mechanism or are directly energized by ATP. A sugar effluxer belonging to the GPH family was recently identified in bacteria (95). Thus, members of the large monosaccharide transporter (MST) family described below may have functions in sugar efflux. However, functional characterization is more complicated than for

import carriers, which were identified by growth assays in yeast mutants. Attempts to clone effluxes using suppression cloning in yeast in the presence of toxic analogs have failed so far (S. Lalonde & W.B. Frommer, unpublished results). Novel tools such as the nanosensors described below may help fuse the loose ends.

## MONOSACCHARIDE TRANSPORTERS (MSTs)

Efflux of sucrose into the apoplasm is also required for an alternative path for sugars imported into cells via cell wall invertases. Invertase secreted into the cell wall space hydrolyzes sucrose, thus requiring subsequent uptake of the hexoses by MSTs. This alternative path occurs during pathogen infection and in certain sink tissues, e.g., in pollen nutrition (146). The hexose uptake route not only provides a means to enhance sink supply by steepening the sugar gradients, but also plays a role in controlling cell division and storage (19). Ruan et al. (130) showed that the difference in sugar content between tomato varieties is independent of sugar export rates from leaves, but correlates well with hexose uptake activity in fruits. This finding suggests that hexose transport limits sugar content. Monosaccharide transport activities have been identified in a variety of plant species (62, 100). In contrast to the HXTs of yeast, which function as uniporters, *Chlorella* and *Arabidopsis* HXTs, belonging to Clade I (STP), function as $H^+$-cotransporters (5, 135). Despite this difference in the transport mechanism, yeast and plant transporter genes are homologous and encode proteins composed of 12 membrane-spanning domains as part of the GPH:cation symporter family. When comparing the transporters of 4 completely sequenced eukaryotic genomes, *Saccharomyces cerevisiae*, *Homo sapiens*, *Arabidopsis thaliana*, and *Oryza sativa*, MSTs can be distinguished according to phylogeny, substrate spectrum, transport mechanism, and cell specificity. Thirteen clusters were recognized in the MST superfamily, with 66 and 22 putative MSTs in the *Arabidopsis* and rice genomes, respectively (Figure 5). The availability of many transporters mediating monosaccharide transport may not be unexpected, considering the complex requirements for intercellular, intracellular, and long-distance transport, and the high number of paralogs found in animals and yeast.

### Monosaccharide Transporter Clade I (STP)

The best-characterized clade of MSTs are the STPs that function as $H^+$-cotransporters (5, 16). The STP clade comprises 14 members in *Arabidopsis* and 13 in rice (159). The expression pattern of various plant STPs suggests that they function primarily in hexose uptake in sink tissues. *AtSTP2* is only expressed in pollen grains during a short developmental period and its function may be related to the callose degradation around the pollen tetrads, suggesting that callose is used as a carbon source (162). *AtSTP4* is specifically expressed in root tips and pollen grains (161). AtSTP1 seems to be responsible for the import of carbohydrates into guard cells from the surrounding apoplasm (28). In addition to the regulation

by developmental factors, AtSTP genes are regulated by external stimuli such as pathogen infection or wounding (161), which allows flexible reallocation of fixed carbon within organs. AtSTP1, which mediates transport of glucose, galactose, xylose, and mannose, but not fructose, accounts for most AtSTP activity in vegetative tissues, and its activity is markedly repressed by treatment with exogenous sugars. *AtSTP1* is also expressed in developing seeds, where it might be responsible for the uptake of glucose. A knock-out mutation in *AtSTP1* results in decreased uptake of exogenously supplied monosaccharides into seedlings (146). AtSTP1 thus seems to function in the acquisition of apoplasmic sugars when other carbohydrate resources are limited and in the salvage of cell-wall-derived sugars. Furthermore, AtSTP4 is expressed at infection sites of fungal biotrophs, an induced type of sink metabolism (52a). A more detailed analysis of knock-out mutants for all 14 STP genes in *Arabidopsis* will provide us with a clearer picture of the role of $H^+$-coupled monosaccharide transport.



## Monosaccharide Transporter Clade II (Sugar Alcohol Transporters)

Plants produce a variety of sugar alcohols (polyols) structurally similar to monosaccharides (107). Polyols accumulate in response to stress, but in some plants polyols replace sucrose as the major transport form for carbohydrates. For example, celery transports significant amounts of mannitol, whereas Rosaceae like apple or cherry transport primarily sorbitol in their phloem (107). Using yeast suppression cloning, mannitol (*AgMaT1*) and sorbitol transporter (*PcSOT1*) genes have been cloned (58, 107). Consistent with a function in loading the phloem and sink supply, AgMaT1 is mainly expressed in mature leaf phloem, whereas PcSOTs seem to fulfill roles during leaf and fruit development (58). Although neither *Arabidopsis* nor rice transport large amounts of polyols, they dispose of five and three polyol transporter-like proteins, respectively (Figure 5). The wide distribution of sugar alcohol transporters in plants, yeast, and humans suggests that polyol transport is common in all eukaryotes.

## Monosaccharide Transporter Clade III (MSSP-Like Transporters)

In addition to the partially characterized plasma membrane monosaccharide and polyol transporter clades, *Arabidopsis* contains a significant number of additional clades, many of which have not been studied yet. Clade III comprises proteins characterized by an extended central loop, a topology reminiscent of SUT2. Two cDNAs were submitted to the EMBL database as putative monosaccharide sensing proteins (AtMSSP) (E. Neuhaus, unpublished data). Clade III includes three *Arabidopsis* as well as two rice genes (Figure 5). The two AtMSSPs and one rice homolog (OsAAG46115) may follow the secretory pathway based on three prediction programs (http://aramemnon.botanik.uni-koeln.de/) (142). The presence of an extended domain may be taken as an indication that these proteins serve as membrane-bound sugar sensors similar to SNF3 and RGT2 in yeast (110, 111).

## Monosaccharide Transporter Clade IV (Human GLUT6/8-Like Transporters)

HsGlut6 and HsGlut8, two members of the human glucose transporter family (GLUT) mediate monosaccharide transport. Both proteins seem to be located in intracellular compartments due to internalization motifs, but at least GLUT8 can be released to the plasma membrane by insulin. Nineteen *Arabidopsis* and a single rice homolog fall into the same clade. None of the plant proteins have been analyzed regarding transport of subcellular localization. The *Arabidopsis AtSFP1* is expressed in seedlings and senescent leaves (116), which suggests a role in sugar transport during senescence. AtSFP2, which is 85% identical to AtSFP1, is constitutively expressed in both sink and source tissues. The activity in source tissue is not unique to SFP2, where MSTs may play a role in the retrieval of monosaccharides lost to the apoplasm by passive leakage or released by apoplasmic invertase (28). It is tempting to speculate that SFPs and GLUT6 and -8 are uniporters facilitating monosaccharide transport either across the plasma membrane or in intracellular compartments. More detailed localization studies, analysis of knock-out mutants, and a detailed biochemical characterization are needed before we can assign functions to the different clade IV proteins.

## Monosaccharide Transporter Clade V [$H^+$-Myo-Inositol Transporters (HMITs)]

Inositol and its phosphorylated derivatives play important roles in brain function as osmolytes and second messengers or regulators of endo- and exocytosis. Accumulation of myo-inositol in plants exposed to salt stress and translocation of myo-inositol in xylem and phloem suggests the presence of specific carriers for acclimation under stress conditions. $H^+$-myo-inositol cotransporters (HMITs) (SLC2a13) have been cloned from mammals and plants and are distantly related to MSTs (163). These transporters are also homologous to $H^+$- and $Na^+$-myo-inositol transporters from yeast (31). The two *Mesembryanthemum* transporters MITR1 and MITR2 locate to the tonoplast, where they are expected to function as $H^+$/symporters. However, to assess their actual function, one must unambiguously determine the transport mechanism. Whereas *Arabidopsis* has four HMIT homologs, rice does not seem to have related genes in this clade (Figure 5). The absence of HMIT homologs in rice and their putative role in salt stress resistance in *Mesembryanthemum* suggest that myo-inositol transport is not a general function necessary for all plants, but is probably a function required mainly in species able to respond to salt stress. Although plant MITRs complement myo-inositol uptake–deficient yeast mutants, the yeast homologs fall into a distinct clade (clade XIII) (106).

## Monosaccharide Transporter Clade VI (GlcT-Like Transporters)

Plant cells are highly compartmentalized and glucose has been detected in cytosol, vacuole, and plastids. Respective chloroplast glucose transporters were identified

by biochemical and proteomic approaches (49, 78, 171). AtpGlcT1 is a typical member of the plasma membrane MSTs, which acquired chloroplast signal sequence. The only rice protein in this clade is most closely related to pGlcT1. The other three *Arabidopsis* sequences in this clade may be the consequence of recent gene duplications events, but they appear to have only a weak mitochondrial targeting peptides. The proposed function of pGlcT is to catalyze the efflux of glucose derived from the amylolytic breakdown of transitory starch (171), whereas the function of the paralogs remains to be shown.



## Monosaccharide Transporter Clade VII (Human GLUT10/12-Like Transporters)

Clade VII includes HsGLUT10 and HsGLUT12, two glucose uniporters and two uncharacterized proteins in *Arabidopsis* and rice genomes, respectively (Figure 5). When expressed in *Xenopus* oocytes, human GLUT10 (SLC2a10) mediates 2-deoxy-glucose transport with an apparent Km of ~0.3 mM (35), and both glucose and galactose compete for 2-deoxy-glucose uptake. GLUT12 facilitates transport of glucose with preference for glucose over other hexoses (128). As with Clade IV, it is tempting to speculate whether clade VII transporters retained their uniport mechanism like the mammalian homologs. Determining the actual cellular and subcellular localization and the analysis of knock-out mutants will help to assign functions to these proteins and provide more insight into the missing parts of the sugar transport machinery.

## Monosaccharide Transporter Clade VIII (Plant-Specific Transporters)

The MST clade VIII includes six *Arabidopsis* genes with no homology to any currently characterized proteins (Figure 5). As suggested for clade IV and VII, these transporters may function either in efflux of monosaccharides or in vacuolar transport.

## Monosaccharide Transporter Clade IX (Phosphate Transporter-Like Proteins)

Plant phosphate transporters of the PHT1 group form the ninth clade in this protein family (Figure 5) (117). These proteins contain a conserved domain corresponding to the sugar transporter signature (Pfam 0083). A second group of phosphate transporters present in bacteria, plants, and animals is more distantly related to the PHT1 family as compared to the MST clades, which suggests multiple independent events leading to the two groups of phosphatae transporting MSTs.

### Monosaccharide Transporter Clade X (Yeast Monosaccharide Transporters and Sensors)

All of the yeast homologs fall into three distinct clades (X, XII, and XIII), with the majority belonging to the HXT family (clade X). The high degree of amplification seems related to yeast's large dependence on efficient monosaccharide uptake in the natural habitats. Clade X comprises 22 proteins (for review see 4, 14, 105), with at least 18 members belonging to the HXT subfamily (HXT1-17, GAL2). The individual HXTs differ considerably with respect to their kinetic properties, with Km values for glucose uptake ranging from 1 mM for HXT7 to 100 mM for HXT1 (118). The expression of many *HXT* genes is regulated by the available external glucose concentration, and the transporter homologs SNF3 and RGT2, which are also part of clade X function as sensors triggering a signal cascade fine-tuning *HXT* gene expression (see hypothetical SUT2 function above) (110, 111). Because the sugar sensors reside in the same clade as the other HXTs of yeast, sensing functions must have developed independently in the different organisms. Clade XIII corresponds to the yeast inositol transporters (see clade V), whereas clade XII contains a unique as yet uncharacterized yeast protein (*ScYFL040W*).

### Monosaccharide Transporters Clade XI (Human GLUT1-Like)

The human SLC2 family of facilitated hexose and polyol transporters contains 13 members (for review see 164), the glucose transporters GLUT 1–12 and HMIT. The GLUTs seem to function mainly as plasma membrane uniporters for hexoses. For GLUT 1–4, 6, 8, and 10 transport of glucose has been demonstrated, whereas GLUT5 is specific for fructose. The genes are differentially expressed and it will be interesting to see why mammals—in contrast to yeast—dispose of a very diversified set of transporters dispersing into the different clades as outlined above. Deleting the important liver and $\beta$-cell glucose transporter GLUT2 in mice was lethal. As for plants, a systematic knock-out analysis will be the best approach to define the physiological function for the individual carriers.

## OLIGOSACCHARIDE TRANSPORT

Some plants classified as symplasmic loaders are thought to mediate phloem loading using diffusion of solutes by way of plasmodesmal connections. According to Turgeon's polymer trap model galactinol synthase catalyzes the formation of raffinose from sucrose and galactinol in the CC [here: intermediary cells (IC)] of these so-called symplasmic loaders (65). Raffinose is supposed to be trapped in the IC-SE complex, because its larger radius prevents diffusion back into IC. Thus the plasmodesmal size exclusion limit differentiates between sucrose and raffinose, permitting passage of sucrose but not of the larger raffinose, thereby concentrating oligosaccharides in the IC-SE complex. Consistent with the transport mechanism found in apoplasmic loaders, none of the SUT sucrose transporters seems able to

transport raffinose. However, still uncharacterized carriers might be responsible for oligosaccharide transport.

## AMINO ACID AND PEPTIDE TRANSPORT

Plants are capable of taking up amino acids as nitrogen source, but more importantly amino acids seem to represent the principal long-distance transport form for organic nitrogen. Proteogenic amino acids accumulate in phloem (100–200 mM) and xylem (at levels approximately tenfold less than in phloem) sap, where amines and acidic amino acids predominate. Overall, long-distance transport of organic nitrogen in the plant must be at least as complex as sugar transport, because the transport substrates are highly diverse, encompassing the 20 proteogenic amino acids plus a variety of amino acid analogs including GABA, oligopeptides, as well as a many yet uncharacterized N-containing compounds found in phloem sap. Moreover, unlike sucrose, amino acids are transported in not only the phloem but also the xylem, requiring complex exchange mechanisms between phloem and xylem (8). Physiological studies using whole tissues, individual cells, or plasma membrane vesicles indicated that amino acid transport in plants is mediated by carriers with overlapping specificity, coupling electrochemical potential gradient (of $H^+$) to secondary active accumulation of amino acids (26, 51).

If we take also into account that nitrogen metabolism is highly compartmentalized within a cell, then we must expect many different transporter genes. Amino acid transporters for cellular import were initially identified by suppression cloning in yeast. Not including mitochondrial transporters, the *Arabidopsis* genome contains 53 putative amino acid transporters, whereas the rice genome has ~59 (thorough annotation is required before an exact number can be provided) (177). Amino acid transporters can be grouped into at least four superfamilies: (*a*) the amino acid–polyamine-choline transporter superfamily (APC), (*b*) the amino acid transporter superfamily 1 (ATF1), (*c*) the amino acid transporters belonging to the major facilitator superfamily (MFS), and (*d*) members of the mitochondrial MC family.

### Amino Acid–Polyamine-Choline Transporter Superfamily

APC homologs are found in fungi, animals, and plants (177). The 24 APC members from yeast contain 12 putative membrane-spanning domains, each covering a different, but in most cases overlapping, spectrum of substrates. Phylogenetic analyses and computer-aided prediction of secondary structures were used to categorize APC transporters into subgroups (http://aramemnon.botanik.uni-koeln.de/) (142). Members of the subfamily of cationic amino acid transporters (CATs) from plants and animals are characterized by 14 putative transmembrane domains. The second subfamily comprises proteins with 12 putative transmembrane domains. Amino acid transport mediated by members of the APC family is diverse, using $Na^+$ or $H^+$ coupling by sym- or antiport and serving functions in uptake and nutrition. To date, only one plant member has been characterized in more detail (54a), and analyses of knock-out mutants are not yet available.

## Amino Acid Transporter Superfamily 1

ATF1 members were originally described in plants but were later also found in yeast and animals. They are the most extensively characterized family. ATF1 contains plant-specific sub-branches as well as branches that overlap with yeast and human counterparts (177). ATF1 members contain 9–11 putative membrane-spanning domains with cytosolic N and extracellular C termini (30).

The members of the best-studied subfamily of amino acid permeases (AAPs) are preferentially expressed in vascular tissue and mediate $Na^+$-independent, proton-coupled uptake of a wide spectrum of amino acids (52). A direct role for AAPs in interorgan transport of amino acids is supported by the finding that potato tubers of StAAP1 antisense lines contain lower free amino acid content (80). Other members of this family are expressed in seeds, suggesting a role in seed supply. AtLHT1, a member of a second ATF1 subfamily (31a), transports lysine, histidine, and other amino acids. LHT1 is present in all tissues, with strongest expression in young leaves, flowers, and siliques. A third subfamily comprises putative transporters for the plant hormone auxin, which is structurally related to tryptophan (98a). ANT1, a member of a fourth subfamily, mediates transport of neutral amino acids (31b). In contrast with the plant carriers described above, which transport diverse amino acids, proline transporters (ProTs) preferentially transport proline, betaine, and GABA (121a). ProTs are ubiquitously expressed but accumulate under salt stress, implying a role in stress adaptation.

Besides a role in plasma membrane amino acid transport, certain ATF1 members fulfill functions in import or export of solutes in intracellular vesicles and vacuoles. They have been described in animals (100a) and yeast (131a), and homologs are found in plants (177).

## Amino Acid Transporters within the Major Facilitator Superfamily

In animals, some members of the MFS family have been identified as amino acid transporters: (*a*) $H^+$/monocarboxylate transporters (MCT), homologs not analyzed yet are present in yeast, (77) and (*b*) vesicular glutamate transporters (VGT), whichare closely related to inorganic phosphate transporters, with homologs of unknown function in *Arabidopsis*. These findings suggest that yeast and plants may use homologs for intracellular amino acid transport, possibly in the context of vesicular and vacuolar transport. This may lead to an even higher complexity of amino acid transport in yeast, similar to that originally anticipated for plant systems.

## Cellular Export of Amino Acids

As outlined for sugar transport, amino acid efflux systems in the apoplasm are required in the release into the apoplasm before the $H^+$-coupled cotransporters can load the phloem. Similar efflux systems are also required for loading of the xylem. Multiple possibilities of how efflux may be achieved exist. These include vesicular transport or carrier-mediated efflux at the plasma membrane. Vesicular export is

well established for amino acids and analogs functioning as neurotransmitters in nerve cells. Carriers for vesicular transport from animals share homologs with those in plants, providing a model for vesicular transport in plants. Mammalian VGAT concentrates GABA in synaptic vesicles of inhibitory synapses, whereas BNP1 and VGLUT1 load glutamate into respective vesicles (for a review see Reference 177). Furthermore, certain mammalian amino acid transporters such as SN1 have been implicated in cellular export at the plasma membrane, suggesting that corresponding plant homologs of the ATF1 family may fulfill similar functions (177). Functional analysis of the plant homologs is required to test these hypotheses.

Potential insights into the physiology of efflux may be derived from the use of nectaries or hydathodes as model organs for secretion. The recent analysis of an *Arabidopsis* mutant hyper-secreting glutamine from hydathodes led to the identification of a new class of putative membrane proteins possibly involved in this process (G. Pilot & W. Frommer, unpublished results). Understanding these proteins, which contain only a single transmembrane domain, may help researchers to identify the mechanism for amino acid export from plant cells.

However, the high complexity of the amino acid transporter families is a major obstacle that has prevented significant progress in our understanding of amino acid fluxes in plants. Only a systematic analysis of all proteins in these three families will help us to comprehend the processes that control loading and unloading of amino acids from phloem and xylem as well as the exchange between the two systems along the path. Furthermore, tools that allow determination of amino acid concentrations at the sites of transport and that permit correlation of dynamic changes of amino acid concentrations in subcellular compartments with transporter characteristics and localization are highly desirable in this context.



## Amino Acid Sensing

In animals, amino acid derivatives are important signaling molecules. Cells must determine the extracellular availability of substrates to regulate uptake and release systems accordingly. Thus, specific receptors or sensors are required at the plasma membrane. An emerging concept is that members of the transporter families have evolved into extracellular solute sensors, i.e., the yeast SSY1 serving as an amino acid sensor (12). It is possible that members of the plant ATF families have also developed sensing properties by acquiring domains able to couple to signaling cascades. However, none of the plant homologs contains extended domains that may serve as obvious interaction domains. Ionotrophic receptors are well-characterized sensor systems. In animal brains, ionotrophic glutamate receptors (iGluRs) function as glutamate-activated ion channels in rapid synaptic transmission. Surprisingly, a large family of putative iGluRs was also found in plants (88). Recent analyses using plants in which expression of the putative *GluR1.1* was inhibited indicate that it regulates distinct C- and N-metabolic enzymes [hexokinase 1 (HXK1) and zeaxanthin epoxidase (ABA1)] at the transcriptional level (76a). However, the exact mechanism by which iGluRs control C:N signaling remains to be established. Higher resolution mapping of amino acid

distribution will be required to advance the fields of amino acid transport and sensing.

## Transporters for Other Nitrogenous Compounds

Metabolomic analysis of phloem sap from Cucurbits detected a large number of unknown nitrogen-containing compounds (50). Given the steep concentration gradients between leaf and phloem for some of these compounds, we postulate that carriers may exist for uphill transport of various other compounds besides amino acids. Furthermore, it has long been suspected that small oligopeptides (proteolysis products) function as alternative nitrogen transport forms in the phloem (75, 147). Corresponding transport activities have been found, but peptide levels are difficult to measure in phloem sap owing to the many potential combinations of amino acids in di- or tripeptides. Peptide transporters fall into three families: the ATP binding cassette family (ABC transporters), the peptide transporter (PTR) family (transporting di- and tripeptides as well as nitrate), and the oligopeptide transporter (OPT) family. The *Arabidopsis* genome possesses 10 times more PTRs than any other sequenced organism, i.e., 52 members of the PTR peptide and nitrate transporter family and 9 OPT transporters (148). Their importance for sink nutrition can be deduced from the finding that antisense repression of *AtPTR2* and T-DNA insertion in *AtOPT3* resulted in an arrest of embryo development (148). In Nepenthes, the expression of a PTR (*NaNTR1*) was localized exclusively to the phloem tissue, contrary to the expression pattern of an amino acid transporter supporting a function in phloem transport (138). A full metabolomic analysis of the phloem sap, combined with the identification of the full complement of transporters and definition of their functions and detection methods to assess their roles, is necessary before we can understand transport of nitrogenous compounds. We have to keep in mind that the findings in one model species may not hold true for all plant species because some species use other nitrogen transport forms such as allantoin in legumes (40).



# ENERGIZATION OF TRANSPORT

## Buildup of an Electrochemical Gradient by $H^+$-ATPases

In most cases, import of metabolites into plant cells makes use of a proton gradient across the plasma membrane. Sucrose, monosaccharide, and amino acid transporters of the AAP family function as $H^+$-coupled cotransport systems. The electrochemical gradient generated by $H^+$-ATPases is the motive force for a large set of secondary transporters, which move their substrates against their concentration gradients by a symport, antiport, or uniport mechanism. Therefore, the $H^+$-ATPase behaves as an energizer, converting the chemical energy released by ATP hydrolysis into chemiosmotic energy (6). The complete inventory of plasma membrane $H^+$-ATPase genes comprises 11 genes from *Arabidopsis* and 10 in rice (11). *Arabidopsis* AHA3 was found in the CC and thus might be responsible for

phloem loading (41), whereas others such as AHA10 are present in seeds (66). A complete analysis of all members is required before we can understand their interplay with the different transport systems.

### A Contribution by Potassium Channels to Assimilate Transport

Potassium is the major cation present in the phloem sap. Potassium stimulates phloem loading of sucrose (33, 61). It is assumed that potassium channels found in the phloem are responsible for charge compensation. Recently, a sink/source-regulated, sugar-inducible $K^+$ channel was identified that dominates the electrical properties of the sieve-tube plasma membrane and possibly controls sugar transport via a voltage-dependent process. Analysis of a knock-out mutant indicates that AKT2/3 stabilizes the membrane potential during sucrose proton cotransport due to its peculiar kinetics and voltage dependence (36).



## NOVEL APPROACHES TO CHARACTERIZE TRANSPORT AND SENSING

As outlined above, we still lack knowledge about major parts of the transport machinery, especially the vacuolar importers/exporters, the cellular efflux mechanisms, and the regulation of transport activities, e.g., by metabolites. This can be attributed to methodological problems in the analysis of the dynamic distribution of metabolites. Measuring compartmental distribution of metabolite levels relies primarily on analysis of average levels in whole tissues or organs of both animals and plants. Thus, we only have a crude estimate of local sugar levels, e.g., in the cytosol versus cell walls, and we know little about variation of sugar levels between cell types (114). Dynamic measurements with high spatial resolution are difficult. However, compartmentation of metabolic reactions, transport, and sugar sensing can only be understood if the subcellular distribution of the metabolites can be established by nondestructive dynamic monitoring techniques. Until recently, methods for in vivo metabolite imaging at cellular or subcellular levels were not available. What little information exists derives from methods requiring tissue fixation or fractionation (20, 45), whereas static analysis of metabolite composition in organs, tissues, and cellular compartments often involves cell disruption. A major advance was the development of methods for single-cell sap sampling that enable determination and correlation of metabolite and gene expression levels in individual cells (7, 21, 97). Using elegant enzymatic approaches, researchers determined glucose and sucrose levels on sections, thereby providing insights into the differential role of glucose and sucrose in controlling development of legume seeds (18, 20, 45). However, none of these techniques provides data on the subcellular distribution of the metabolites. An elegant tool involving nonaqueous fractionation was developed for compartmental analysis within the cell (90); it recently combined with mass spectrometric analysis of metabolites (45). To determine the averaged levels of metabolites in the apoplasm, apoplasmic wash fluids were used (96). Nevertheless, noninvasive techniques would be a significant advantage for better

understanding metabolism and compartmentation. Excellent results for metabolite imaging have been obtained with NMR microscopy, but the resolution is not sufficient for a subcellular analysis (81).

An alternative approach has been the use of ratiometric dyes. Based on the fluorescence resonance energy transfer (FRET) $Ca^{+}$-sensor concept, originally developed by Tsien (103), we tested whether bacterial periplasmic binding proteins (PBPs) might be suitable backbones for metabolite nanosensors. PBPs comprise many diverse proteins, each specific for a different metabolite or ion. The proteins bind their substrates with affinities in the nanomolar range and undergo a Venus fly trap–like movement upon binding. Crystal structures for more than a dozen PBPs, several in both bound and unbound states, provide a detailed understanding of the mechanism of binding and the fly trap movement (98). Owing to the high affinity for their substrates, amino acid residues in PBPs involved in binding can be modified to generate low-affinity nanosensor mutants for detecting analytes in a physiologically relevant range. By fusing the maltose binding protein to two green fluorescent protein variants, one at each end, in combination with site-directed mutagenesis, a prototype nanosensor FLIPmal (fluorescent indicator protein for maltose) with a Kd for maltose of 25 $\mu$M was developed (46). This nanosensor enabled us to visualize maltose uptake into the cytosol of living yeast cells transformed with the chimeric nanosensor gene in real time. The increase of maltose levels in the cytosol following uptake into the cell was detected by increased FRET, leading to increased emission of the yellow fluorescent protein and decreased emission of the cyan fluorescent protein.

Although the bacterial periplasmic glucose and ribose binding proteins are unrelated to maltose binding protein at the primary sequence level, and have different structures regarding the position of the termini, the general applicability of the strategy was demonstrated by developing FRET-based nanosensors for glucose and ribose (47, 86). Using a glucose nanosensor with a Kd of 600 $\mu$M, we showed that free glucose exists in animal cells, and that the intracellular concentration of glucose is approximately half the extracellular level (47). The obvious next steps will be to target the nanosensors to different cellular compartments, and to analyze stable transgenic lines expressing the nanosensors, which can then be crossed with transport and signaling mutants to study the effects on compartmentation. We intend to develop amino acid and sucrose sensors to complete this set of tools for analyzing sugar and amino acid transport studies.

In parallel, other groups have developed promising technologies such as in vivo confocal Raman spectroscopy, a noninvasive optical method that generates detailed information about the molecular composition with high spatial resolution (29). This technology can be adapted to determine glucose and amino acid levels (108, 145). Luminescent semiconductor nanocrystals represent another promising technology. Procedures have been developed for using quantum dots to label living cells and for long-term multicolor imaging (74, 170). Recently, a maltose binding protein was coupled to quantum dots and used for FRET imaging (101). Compared to the proteinaceous FLIP nanosensors, such quantum dots may be more sensitive, whereas targeting and in vivo measurements will be more difficult.

## OUTLOOK

Much of the recent work has concentrated on generating an inventory of the different gene families responsible for metabolite transport. Although we have identified many of the transportome's components, the puzzle is still far from complete. Major pieces are still missing, namely systems for vacuolar import/export, mechanisms for cellular efflux, and receptors responsible for metabolite sensing. Regulation is still poorly understood. Only with the complete set of puzzle pieces will we be able to describe where loading actually occurs, why phloem loading requires transporters on either CC and/or SE, and how transport and metabolism are integrated at the whole-plant level. Especially in monosaccharide, amino acid, and PTR systems, these extensive families will require a combination of biochemical analyses and determination of the expression and localization patterns as well as careful studies of the effects of knock-out mutations for each single protein. From there we will be able to return to what is missing in this review—the analysis of physiology and ecophysiology. The new tools for metabolite imaging may help us to design new approaches to assign genes to the missing transport functions and to better understand the area of metabolite sensing. The new tools, including the nanosensors, will also help toward an understanding of the effects observed in knock-out mutants and thus bring us a step closer to understanding assimilate allocation at the whole-plant level. The phylogenetic analyses may be a helpful tool for characterizing ecophysiological aspects. Finally, results from a related area, i.e., the analysis of structure, regulation, and function of plasmodesmata, will help us understand their role in assimilate transport, sink-source communication, and trafficking of transporters to SE.



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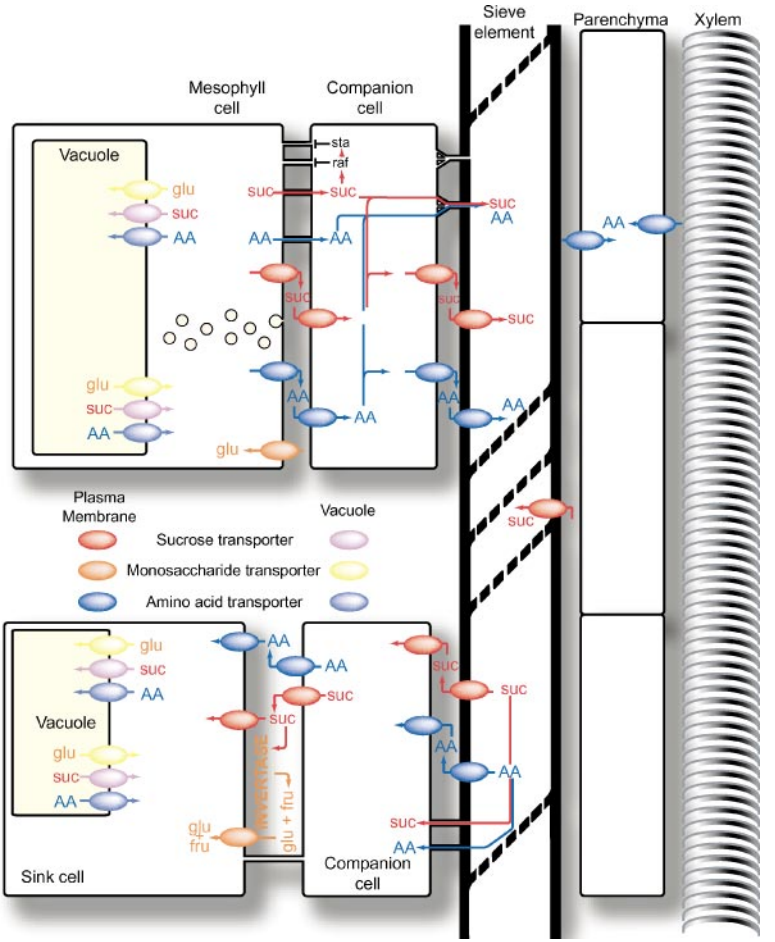


**Figure 1** Long-distance sugar transport via the phloem. From the mesophyll, sucrose and amino acids are loaded into the SE-CC complex either through plasmodesmata or via the apoplasm. The apoplasmic loading mechanism requires export from the mesophyll or the vascular parenchyma and proton-coupled reuptake into the SE-CC complex. Efflux may involve vesicular pathways or plasma membrane transporters. Hydrostatic pressure drives phloem sap movement toward sink tissue. Apoplasmic phloem or post-phloem unloading necessitates exporters at the sink tissue. Import of solutes into sink tissue may occur through plasmodesmata or transporters. In addition to plasmodesmatal- and transporter-mediated uptake, cells in the sink may take up sucrose, subsequent to its hydrolysis by an apoplasmic invertase in the form hexoses. No tonoplast carriers have been identified at the molecular level to date.

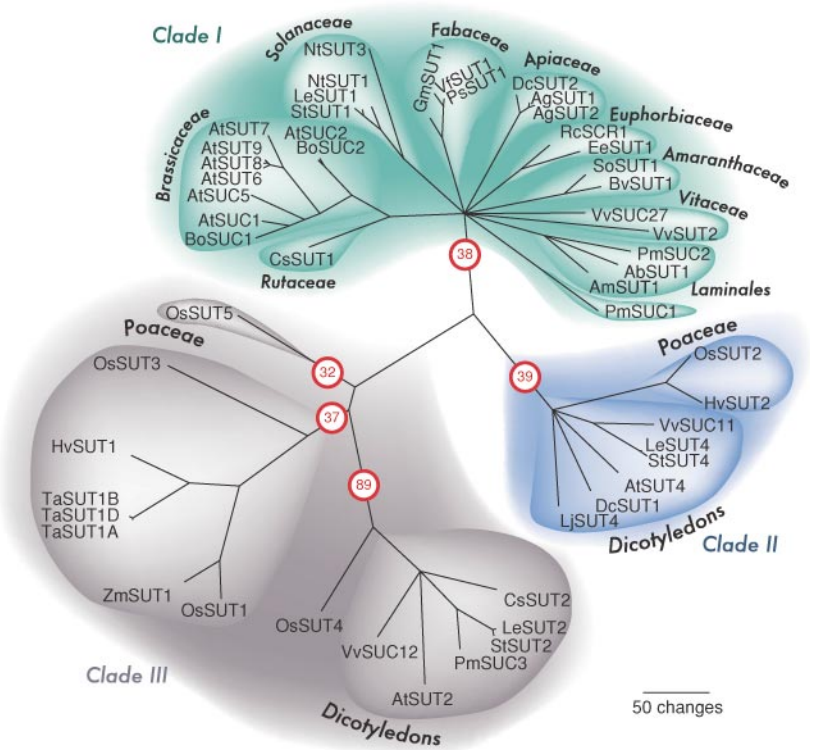


**Figure 3** Phylogenetic tree of the sucrose transporter (SUT) superfamily. Maximum parsimony analyses were performed using PAUP 4.0b10 (154), with all DNA characters unweighted and gaps scored as missing characters. Heuristic tree searches were executed using 1000 random sequence additions and the tree bisection-reconnection branch-swapping algorithm with random sequence analysis. The complete alignment was based on 515 sites; 357 were phylogenetically informative. The SUT family can be divided into 3 clades. The red circles give the average length of the central loop of the corresponding clade. At5g43610, At5g06170, At2g14670, and At1g66570 were newly named as AtSUT6, AtSUT7, AtSUT8, and AtSUT9, respectively. All sequences were obtained from NCBI (http://www.ncbi.nlm.nih.gov) or the Aramemnon database (http://aramemnon.botanik.uni-koeln.de/). Ab, *Asarina barclaina*; Am, *Alonsoa meridionalis*; Ap, *Apium graveolens*; At, *Arabidopsis thaliana*; Bo, *Brassica oleracea*; Bv, *Beta vulgaris*; Cs, *Citrus sinensis*; Dc, *Daucus carota*; Ee, *Euphorbia esula*; Gm, *Glycine max*; Hv, *Hordeum vulgaris*; Le, *Lycopersicon esculentum*; Lj, *Lotus japonicus*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Ps, *Pisum sativum*; Rc, *Ricinus communis*; So, *Spinacea oleracea*; St, *Solanum tuberosum*; Ta, *Triticum aestivum*; Vf, *Vicia faba*; Vv, *Vitis vinifera*; Zm, *Zea mays*.

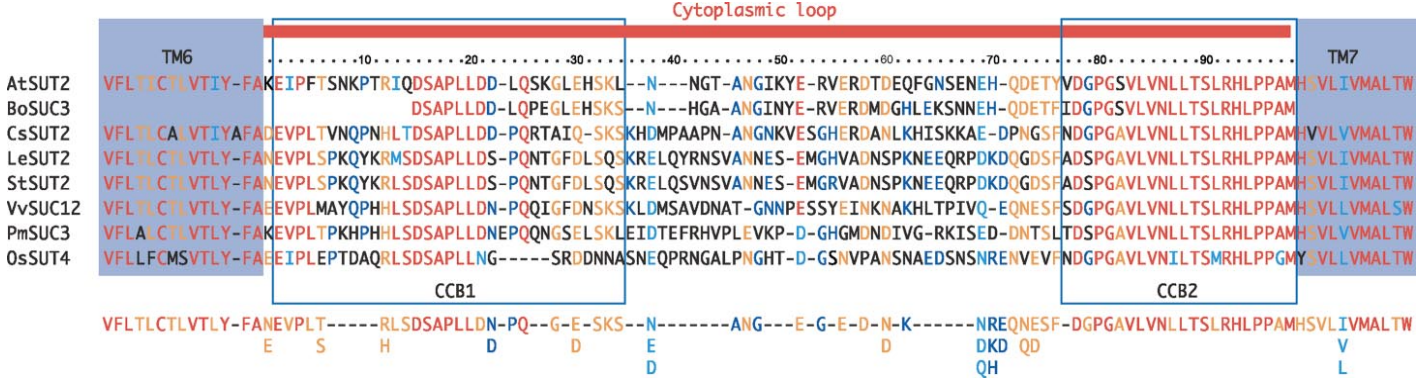


**Figure 4** Alignment of the central loop of SUT2 proteins from *Arabidopsis* (AtSUT2), *Brassica oleraceae* (BoSUC3; partial), *Plantago major* (PmSUC3), *Citrus sinensis* (CsSUT2), *Lycopersicon esculentum* (LeSUT2), *Vitis vinifera* (VvSUC12), and *Oryza sativa* (OsSUT4). Conserved boxes are marked as CCB, perfectly conserved residues are in red, residues conserved in four species are orange, and residues conserved in three species are in dark blue. Numbering is according to AtSUT2 sequence. Boxes and red lines indicate positions of transmembrane domains and the central loop.

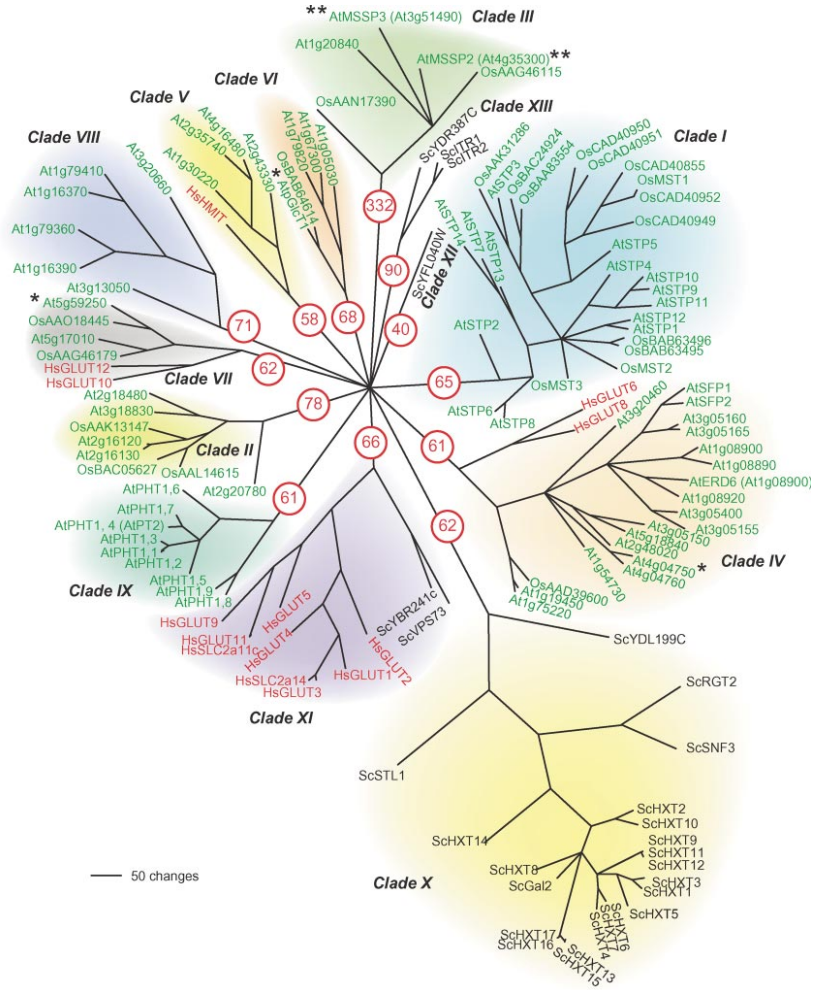


**Figure 5** Phylogenetic tree of the monosaccharide transporter (MST) superfamily. Maximum parsimony analyses were performed using PAUP 4.0b10 (154), with all DNA characters unweighted and gaps scored as missing characters. Heuristic tree searches were executed using 100 random sequence additions and the tree bisection-reconnection branch-swapping algorithm with random sequence analysis. The complete alignment was based on 877 sites; 843 were phylogenetically informative. The MST superfamily can be divided in 13 clades. The red circles give the average length of the central loop of the corresponding clade. The different members of the PHT family (clade IX) have been named according to Rausch & Bucher (117). All sequences were obtained from NCBI (http://www.ncbi.nlm.nih.gov) or the Aramemnon database (http://aramemnon.botanik.uni-koeln.de/). Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*. Asterisk represents predicted chloroplastic signal peptide; double asterisk, the predicted secretory pathway signal peptide.

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