J. Membrane Biol. 162, 177–190 (1998)

The Journal of
Membrane
Biology

Topical Review

Structure and Function of Plasma Membrane Amino Acid, Oligopeptide and Sucrose Transporters from Higher Plants

D. Rentsch[1], K.J. Boorer[2], W.B. Frommer[1]

[1]Botanical Institute, Eberhard-Karls-University, Auf der Morgenstelle 1, D-72076 Tübingen, Germany
[2]Department of Physiology, UCLA School of Medicine, 10833 Le Conte Ave, Los Angeles, CA 90095, USA

Received: 27 June 1997/Revised: 18 September 1997

Introduction

As differentiated multicellular organisms, plants require ways to exchange signals and metabolites between individual cells, tissues and organs. Plants have developed a set of at least three transport routes for uptake or cell-to-cell transport of solutes: (i) direct intercellular connections such as plasmodesmata (ii) endo/exocytosis, and (iii) plasma membrane transport proteins that mediate controlled exchange of solutes into and out of cells.

This review will focus on the molecular biology and structural and functional characteristics of plasma membrane proteins responsible for the transport of sucrose, amino acids and peptides, which represent major transported forms of assimilated carbon and nitrogen in plants. Glucose transporters have been reviewed recently and will thus not be covered here (Sauer et al., 1994*a,b*). A detailed discussion on the physiological role of sucrose or amino acid transport in plants has been published elsewhere (Sauer et al., 1994*a,b;* Ward et al., 1997; Frommer et al., 1994*b;* Rentsch & Frommer, 1996).

Like animals, plants have to transport metabolites for long distances. Assimilates derived from photosynthesis in leaves must be allocated to organs such as roots or flowers that depend on external supplies. The two vascular systems responsible for long distance transport are the xylem and the phloem. Phloem cells remain alive at maturity, forming living conduits, in contrast to xylem, in which long distance transport can be considered as extracellular. Within the phloem sap, the disaccharide sucrose (or derivatives) serves as the major transport metabolite, whereas animals use mainly glucose. The second most abundant class of organic compounds found in the phloem sap are amino acids, which are translocated in high concentrations also in the xylem. Loading of solutes into the phloem in many cases occurs against a concentration gradient and is primarily driven by the electrochemical gradient for protons generated by H^+-ATPases.

Biochemical studies using tissue slices and membrane vesicles revealed the kinetic complexity of metabolite transport. The interpretation of these studies was mainly hampered by the multiplicity of cell types present in one organ and the uncertainty of whether the different transport activities derived from one or more cell types. It was therefore essential to isolate individual carriers and to study their properties, regulation and function before relating them back to the whole plant level.

The recent development of new molecular tools, such as the complementation of uptake-deficient yeast mutants with plant cDNA expression libraries, has circumvented the difficulties of biochemical approaches and enabled the identification of many important transport systems including sucrose, amino acid and oligopeptide transporter genes (reviewed by Frommer and Ninnemann, 1995). To date over 40 genes belonging to more than nine gene families have been functionally ex-

Correspondence to: W.B. Frommer

Key words: Arabidopsis — Solanaceae — Expression cloning — Yeast — Xenopus oocytes — Amino acid permease — Amino acid transport — Plant — Membrane proteins — Sucrose transporter — Peptide transporter

pressed in yeast. They include H^+/ATPases (Gjedde-Palmgren & Christensen, 1994); K^+ channels (Anderson et al., 1992); Cl^- channels (Hechenberger et al., 1996); sulfate transporters (Smith et al., 1995); phosphate transporters (Leggewie, Willmitzer & Riesmeier 1997); ammonium transporters (Ninnemann et al., 1994); hexose transporters (Sauer et al., 1990); sucrose transporters (Riesmeier et al., 1992); amino acid transporters (Frommer et al., 1993, 1995) and oligopeptide transporters (Steiner et al., 1994). To our knowledge, at least 15 sucrose transporter genes from nine different plant species and at least 25 amino acid transporters belonging to two superfamilies from eight different plant species have been identified. A combination of heterologous expression systems such as yeast and *Xenopus* has allowed the characterization of the intrinsic biochemical properties of individual sucrose and amino acid transporters. For instance, substrate specificity, proton-coupling and transport mechanism have been demonstrated for more than nine different plant plasma membrane metabolite carriers to date. The nature of the approach for isolating carrier genes, i.e., complementation of uptake-deficient yeast mutants, could lead to a bias towards identification of transporters for import of solutes into cells. Thermodynamically, all carriers should function in a reversible manner; however *in vivo* the proton cotransporters, in particular, function as import systems. Whether these identified transporters also function in metabolite export (in an uncoupled mode) also remains to be tested. It is possible that new classes of metabolite exporters remain to be discovered.

Primary Sequence and Secondary Structure of Transporters

Sucrose Transporters

A unique feature of plants is that they use the disaccharide sucrose or its derivatives as the major transported form of assimilated carbon. Kinetic studies using leaf discs and plasma membrane vesicles isolated from leaves revealed at least two components of sucrose uptake: saturable high affinity and linear low affinity components (Maynard & Lucas, 1982; Delrot, 1989). Several attempts were made to obtain molecular information about proteins responsible for these sucrose transport activities. Li et al. (1992) identified a 42 kDa plasma membrane protein as a putative candidate for the high affinity sucrose transporter. An antiserum directed against this protein inhibited sucrose transport into plasma membrane vesicles (Gallet et al., 1992). However, attempts to isolate the gene encoding this protein by screening cDNA expression libraries with the antiserum were unsuccessful (W.B. Frommer, R. Lemoine and S. Delrot, *unpublished results*).

As an alternative, complementation of transport mutants was attempted as a means to circumvent the problems associated with biochemical approaches. Since bacterial systems were problematic due to toxicity of overexpressed membrane proteins, alternative expression systems had to be developed. Yeast mutants turned out to be perfect tools to clone sucrose transporters. However, at least at first sight, isolation of sucrose transporter genes by complementation of *Saccharomyces cerevisiae* mutants deficient in sucrose uptake seemed impossible due to the capability of budding yeast to metabolize sucrose extracellularly. Therefore, a yeast strain was modified in such a way that it was deficient in secreted sucrose-hydrolyzing activities, but able to metabolize imported sucrose due to ectopic expression of a sucrose-cleaving activity in the cytosol. This manipulated strain was complemented with plant cDNA expression libraries from spinach and potato to isolate the first sucrose transporter cDNAs (Riesmeier et al., 1992, 1993). Subsequently, these cDNAs were used as probes for isolating corresponding genes from other species, e.g., tobacco, tomato, arabidopsis, and *Plantago* (Gahrtz et al., 1994; Sauer & Stolz, 1994; Weig & Komor, 1996; Kühn et al., 1997).

The sucrose transporter (*SUT*) genes encode highly hydrophobic proteins and belong to the class of metabolite transporters consisting of two sets of six membrane-spanning regions, separated by a large cytoplasmic loop. Currently, the nomenclature seems confusing: genes from different species have been named S21, P62, SUT, SUC or SCR. The first sucrose transporter from potato (designated *s*ucrose *t*ransporter SUT1) is located at the plasma membrane of sieve elements and plays an essential role in long distance transport, as shown by antisense experiments (Riesmeier et al., 1993, 1994; Kühn et al., 1996). In several species, more than one gene has been identified, but in no case was an exhaustive analysis performed. In arabidopsis, two different genes were characterized (Sauer & Stolz, 1994). An additional gene found in the database of expressed sequence tags (accession number # F14112) demonstrates that at least one additional gene is present in the genome of arabidopsis. Similarly, in tomato three different genes have been identified (W.B. Frommer et al., *unpublished results*). Since the SUC2 genes from arabidopsis and *Plantago* seem to be the most highly expressed in the phloem of leaves and since the respective proteins share similar properties, they probably represent functional homologues of SUT1, whereas the other genes probably serve different functions in other cells or organs (Sauer & Stolz, 1994; Gahrtz et al., 1994, 1996). The most distant members of this family display a minimal identity of 40%. Figure 1 shows a phylogenetic analysis based on sequence similarities.

Although no extensive sequence homologies were

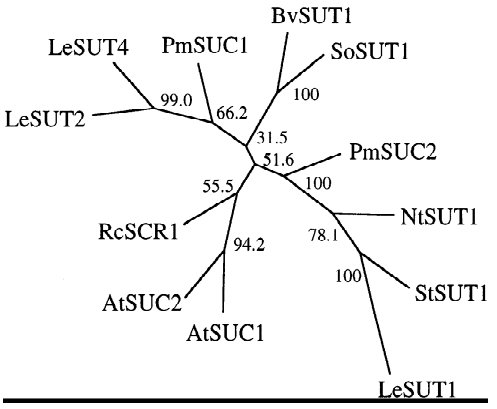

Fig. 1. Computer-aided analysis of the homologies between sucrose transporters. The analysis was performed using the PHYLIP program package (Felsenstein, 1993) with the aligned sequences of AtSUC1-2 (Sauer & Stolz, 1994), BvSUT1 (accession number #1076257), LeSUT1-2,4 (WBF et al., *unpublished*), NtSUT1 (accession number #1076644), PmSUC1-2 (Gahrtz et al., 1994, 1996), RcSCR1 (Weig & Komor, 1996), SoSUT1 (Riesmeier et al., 1992), and StSUT1 (Riesmeier et al., 1993). The comparison was restricted to the region in LeSUT1 from amino acid position 21 to 243. The numbers indicate the occurrence of a given branch in 100 bootstrap replicates of the given data set. LeSUT1 was used as outgroup.

found to the prototype of sugar transporting proteins, the lactose permease from *E. coli*, both proteins have twelve putative membrane-spanning regions separated by a large central loop. However, a conserved [RK]X_{2-3}[RK] motif is located in the second and eighth loop of the sucrose transporters, one of the characteristic features of the MFS (*m*ajor *f*acilitator *s*uperfamily) transporter family (Marger & Saier, 1993; Prive & Kaback, 1996). A structural model has been constructed on the basis of hydropathy analyses and existing models of other transporters, such as lactose and melibiose permeases and mammalian facilitative glucose transporters (Ward et al., 1997). Very little is known about the actual 3-dimensional structures since crystal structures have been obtained for membrane proteins but not for metabolite transporters (Deisenhofer et al., 1995; Ostermeier et al., 1995). As an essential prerequisite for crystallization, several groups have overproduced large quantities of transport proteins. *E. coli* lactose permease or sucrose transporters have been purified using histidine-tags and biotinylation domains (Prive & Kaback, 1996; Sauer & Stolz, 1994; Stolz et al., 1995). Certainly, high resolution structural data from any member of the MFS superfamily may help predict the structure of related transporters. For correlating structure and function, detailed mutagenesis studies have proven extremely valuable. The best studied member of the MFS family regarding structure/function is certainly *E. coli* lactose permease (Frillingos & Kaback, 1996). Based on these extensive data, mutational studies of the sucrose transporters in conjunction with selection in the yeast expression system can be used to identify regions important for substrate recognition and function as was done for plant hexose transporters (Tanner & Caspari, 1996).

Regarding the low affinity component of sucrose uptake, a potential candidate was identified recently. Using photoaffinity labeling techniques, a sucrose binding protein (SBP) has been isolated from soybean cotyledons (Ripp et al., 1988; Warmbrodt et al., 1989; Grimes et al. 1992). SBP is localized at the plasma membrane of the phloem; thus the authors speculate that SBP might be involved in sucrose transport. Furthermore, Harrington et al. (1997) showed that SBP also colocalizes with sucrose/H^+ cotransporters in the plasma membrane of *Vicia faba* transfer cells in developing seeds. A direct function of SBP in sucrose transport was demonstrated using the yeast expression system described above (Overvoorde et al., 1996). Biochemical properties indicate that SBP encodes the linear component of sucrose uptake observed in many transport studies. However, SBP is very hydrophilic and shares similarities with storage proteins. SBP may have a regulatory role in phloem sucrose transport through interaction with SUT1, a mechanism that has also been suggested for the function of the mammalian rBAT protein that seems to activate so far unknown endogenous *Xenopus* oocyte amino acid transporters (Malandro & Kilberg, 1996). Coexpression of SUT1 and SBP in yeast or in oocytes might serve as a suitable approach to test the hypothesis that SBP activates SUTs.

Amino Acid Transporters

The activity of amino acid transporters was first demonstrated using biochemical approaches (Bush, 1993). Again the yeast system has proven to be an efficient system to isolate the respective transporter genes. Three *Saccharomyces cerevisiae* mutants deficient in amino acid uptake were used to identify plant amino acid transporter genes: (i) strain 22574d carrying mutations in the general amino acid, proline and γ-aminobutyric acid permease genes unable to grow on media containing proline, citrulline or γ-aminobutyric acid as the sole nitrogen source (Jauniaux et al., 1987), (ii) strain JT16 lacking functional histidine uptake and biosynthesis for selection on complex media containing histidine (Tanaka & Fink, 1985) and (iii) strain PLAS23-4B carrying a mutation in the *SHR*3 gene and thus defective in correct targeting of yeast endogenous amino acid permeases to the plasma membrane (Ljungdahl et al., 1992; Kuehn et al., 1996).

Suppression of the mutant phenotype enabled the identification of two superfamilies of plant amino acid transporters from cDNA expression libraries under control of yeast promoters. The first superfamily (ATF1; *a*mino acid *t*ransporter *f*amily 1) comprises AAPs (*a*mino acid *p*ermease), ProTs (*p*roline *t*ransporter) and

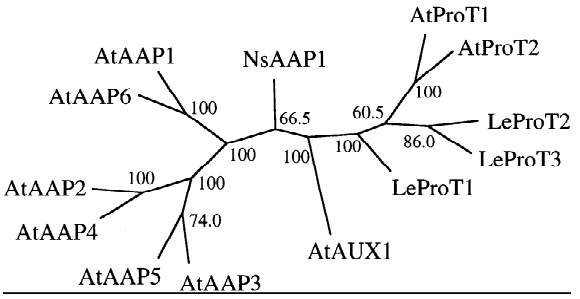

Fig. 2. Computer-aided analysis of the homologies between ATF1 amino acid transporters. The analysis was performed using the PHYLIP program package (Felsenstein, 1993) with the aligned sequences of AtAAP1-6 (Frommer et al., 1993, Kwart et al., 1993, Fischer et al., 1995, Rentsch et al., 1996), NsAAP1 (accession number #U31932), AtProT1-2 (Rentsch et al., 1996), LeProT1-3 (R. Schwacke and D. Rentsch, *unpublished*) and AtAUX1 (Bennett et al., 1996). The comparison was restricted to the region in AtAAP1 from amino acid position 41 to 480. The numbers indicate the occurrence of a given branch in 100 bootstrap replicates of the given data set. AtAUX1 was used as outgroup.

AUX1 (*aux*in resistance). The AAPs comprise both low and high affinity systems and can be subdivided into two subfamilies according to their substrate specificity into general amino acid transporters and systems that recognize preferentially neutral and acidic amino acids (*see below*). AAP1 and NATII isolated by different groups from different arabidopsis ecotypes are almost identical (Frommer et al., 1993; Hsu et al., 1993). The gene family from arabidopsis contains at least 7 members (Kwart et al., 1993; Fischer et al., 1995; Rentsch et al., 1996; D.R. Bush, *unpublished*). Related proteins were also identified from other plant species such as potato, tobacco, rice, *Ricinus communis*, *Brassica napus* and *Lilium longiflorum* (M. Kwart and W.B. Frommer, *unpublished*, accession numbers U31932, D23887, D47073, D21983, 11121, Z68759, 267392, D21814). The second subfamily encodes transporters that prefer proline (ProTs; Rentsch et al., 1996) and the third group is represented by a putative indole acetic acid transporter (Bennett et al., 1996). The three subfamilies share a high degree of similarity within a group but are only distantly related to each other. A phylogenetic tree of the three different subgroups is shown in Fig. 2. The ATF1s share sequence homologies with the neutral amino acid permease MTR from *Neurospora crassa* (Koo & Stuart 1991), but share no significant homologies with any of the yeast or mammalian superfamilies identified so far (*see below*).

Based on hydropathy analyses of the aligned ATF1 members, a structure for an integral membrane protein with 10 membrane-spanning domains was predicted (Fig. 3). According to this model the characterized AAPs contain a number of putative phosphorylation sites on the cytosolic surface of the membrane whereas no N-glycosylation sites were found on the external side. Since the topology of related proteins have not been determined, this model is tentative. Preliminary experimental evidence indicates that AAPs have 11 membrane-spanning domains (D.R. Bush, *unpublished results*).

By complementing the yeast strain deficient in histidine transport, one member of a second superfamily of plant amino acid transporters was identified (AtAAT1; Frommer et al., 1995). Due to conflicts AtAAT1 has been renamed AtCAT1 (*c*ationic *a*mino acid *t*ransporter). AtCAT1 shares significant structural and sequence homologies with the mammalian CAT family of amino acid transporters (*see below*). Low, but significant sequence homologies were also found to yeast YAAP amino acid permeases (André, 1995). In contrast, no significant homologies to the ATF1 family were detectable. These data demonstrate that at least two evolutionarily distinct protein families with at least 11 genes involved in amino acid transport are present in a single plant genome (arabidopsis). *AtCat1* encodes a protein with a calculated molecular mass of 58 kDa and is highly hydrophobic with 14 predicted membrane spanning segments. The structure is remarkably similar to that of the mammalian cationic amino acid transporters (MCAT) for which also 14 membrane-spanning regions were predicted (Fig. 4; Kim et al., 1991). Low and high affinity MCATs have been identified that differ only in the sequence of the eighth hydrophilic loop (Closs et al., 1993). Interchange of domains between the two isoforms showed that this loop is responsible for substrate affinity. Some residues in this region are also conserved in AtCAT1. Thus, interchange of domains by constructing chimeras between different members of this family may allow us to pinpoint sequences involved in the recognition of amino acids by AtCAT1. In contrast, the yeast YAAP amino acid permeases contain only 12 distinct hydrophobic regions. According to a tentative structural model for the mammalian MCATs, both amino- and carboxy-termini are cytosolic (Kim et al., 1991). The MCATs are N-glycosylated in the third extracellular loop, supporting the validity of the model. AtCAT1 contains a conserved N-linked glycosylation sequence in a synonymous region; however glycosylation has not been shown experimentally.

Oligopeptide Transporters

Under certain conditions, plants export oligopeptides derived from proteolysis of storage proteins to increase the transport efficiency of organic nitrogen. Such transport activities were found in a variety of plant tissues and respective *o*ligo*p*eptide *t*ransporter (OPT) genes have been identified (Steiner et al., 1994; Frommer et al., 1994*a;* Rentsch et al., 1995; Song et al., 1996). The OPTs are highly related to the yeast PTR2 and the mammalian PepT H^+-oligopeptide cotransporters (*see below*, Perry et al., 1994; Fei et al., 1994). Surprisingly, the OPTs are also highly homologous to the low affinity

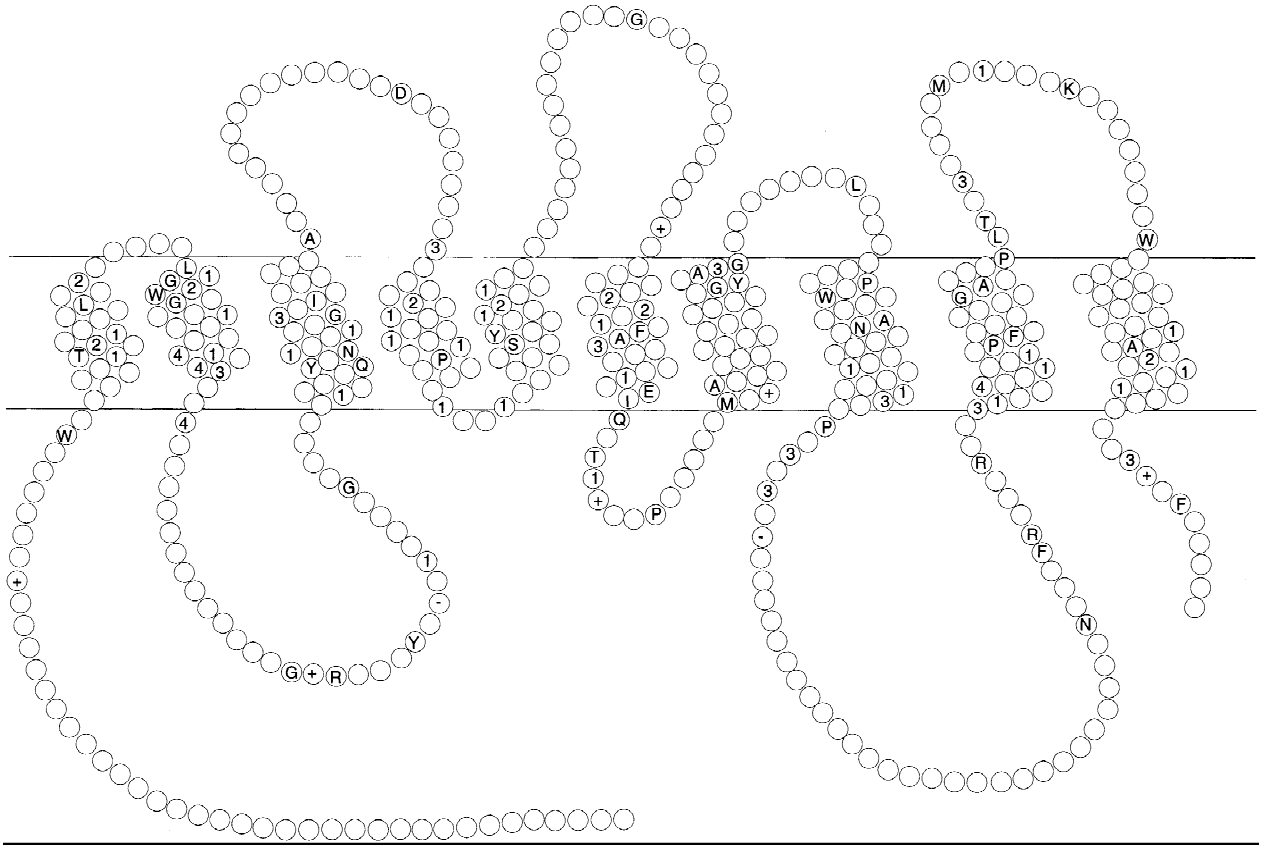

Fig. 3. Structural model of the ATF1 amino acid permeases. The model is based on the aligned sequences of AtAAP1-6 (Frommer et al., 1993, Kwart et al., 1993, Fischer et al., 1995, Rentsch et al., 1996), NsAATP1 (U31932), AtProT1-2 (Rentsch et al., 1996), and LeProT1-3 (R. Schwacke & D. Rentsch, *unpublished;* DNASTAR, London, UK; tmap, Milpetz & Argos, 1995). Conserved residues found in at least 11 or 12 sequences are indicated at corresponding positions in AtAAP1. 1 = I,L,V; 2 = G,A; 3 = F,Y; 4 = S,T; + = K,R,H; − = D,E

nitrate transporters (Tsay et al., 1993; Lauter et al., 1996). A dendrogram and a putative structural model of OPT1 have been described previously (Rentsch et al., 1995).

Comparison with Transporters from Other Eukaryotes

In recent years, genes encoding several amino acid and peptide transporters have been isolated from yeast and animal systems. For comparison, we will briefly summarize the known gene families from these organisms (Table 1).

In yeast, the transporters identified so far fall into two gene families: (i) the YAAP family with 18 related sequences present in the yeast genome, of which ten were shown to transport amino acids and (ii) the yeast CAT (YCAT) family with 6 members which are related to the plant PCAT and animal CAT family and which comprises two methionine permeases, but also GABA and choline transporters (André, 1995 and *personal communication*). Furthermore, the yeast genome contains a single copy gene *PTR2* responsible for high affinity uptake of a large spectrum of oligopeptides (Perry et al. 1994). PTR2 shares significant homologies with the plant oligopeptide transporters.

In animal systems, a large spectrum of amino acid transport systems has been described on the basis of biochemical analyses (e.g., A, L, N, ASC, $b^{0,+}$, y^+L). Recently, some of the respective genes have been identified mainly through oocyte expression cloning, however in no case by yeast complementation (Malandro & Kilberg, 1996). The *c*ationic *a*mino acid *t*ransporter (CAT) family contains integral membrane proteins mediating low- and high-affinity Na^+-independent transport of cationic amino acids at the plasma membrane of many tissues. The arabidopsis AtCAT1 is highly similar to this CAT family. In contrast, none of the plant genes identified so far is related to the following gene families. The glutamate transporter family contains members mediating Na^+-dependent glutamate/aspartate uptake and members with specificity for neutral amino acids. Glutamate transporters are mainly expressed in the central nervous system. The Na^+/Cl^--dependent *p*roline *t*ransporter (PROT) subfamily belongs to a large superfamily

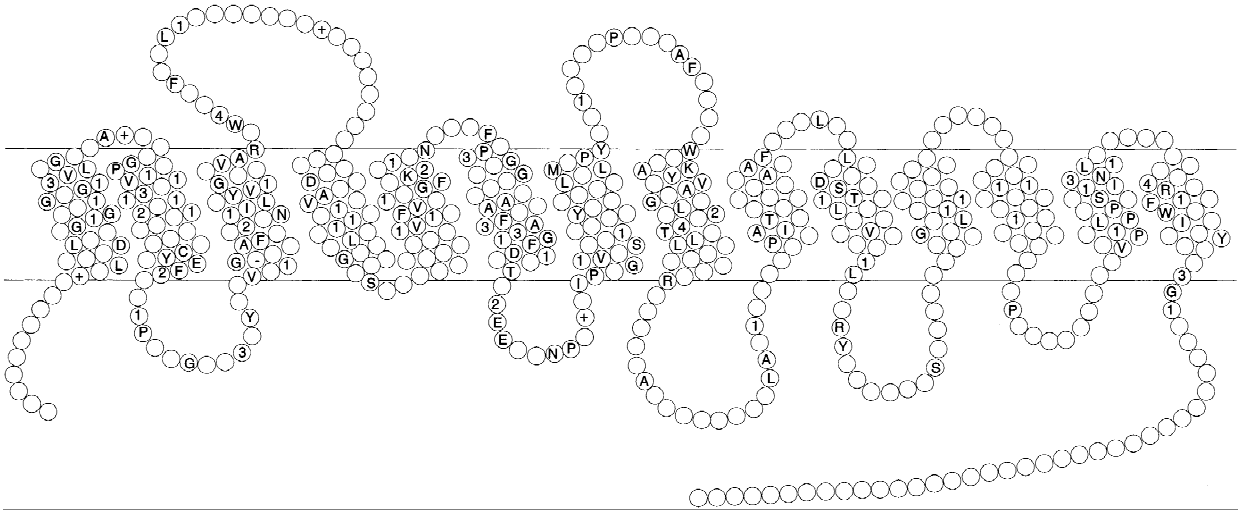

Fig. 4. Structural model of CAT cationic amino acid permeases. The model is based on the aligned sequences of AtAAT1, mCAT1 (Albritton et al., 1989), mCAT2A (Closs et al., 1993) and mCAT3 (accession number #U70859; UWGCG, Devreux et al., 1984; tmap, Milpetz & Argos, 1995). Conserved residues found in all four sequences are indicated at corresponding positions in AtAAT1. 1 = I,L,V; 2 = G,A; 3 = F,Y; 4 = S,T; + = K,R,H; − = D,E

Table 1. Homologous plasma membrane transport proteins from plants, yeast and animals

	Plant	Yeast	Animal
Sucrose	*H^+/sucrose symporters SUT** (*sucrose binding protein SBP*)		
Glucose	*H^+/glucose symporters STP**	HXT hexose transporters	Fac. glucose transporters GLUT* Na^+-glucose symporter SGLT*
Amino acids	*PCAT amino acid transporters*	YCAT YAAP	CAT amino acid transporters*
	H^+/amino acid symporters ATF I (*AAPs**, *ProTs*, Aux1)		Na^+-amino acid symporters GLU/ASPT, GLYT, PROT* rBAT amino acid transporter family*
Oligopeptides	*H^+/oligopeptide symporters* (*OPT*)	PTR2	PEPT H^+/oligopeptide transporters*
Other substrates	ABC type transporters *Sulfate transporters* *Phosphate transporters* *Ammonium transporter AMT* *K^+ channels* (*AKT, KAT**) *Na^+/K^+ symporter* (*HKT1*)* *CLC chloride channel* (*CLCd*) Water channels* *P-type ATPases* (H^+)	ABC type transporters Sulfate transporters Phosphate transporters MEP ammonium transporters CLC Water channels (ORFs) *P-type ATPases* (H^+)	ABC type transporters* Sulfate transporters* Phosphate transporters* K^+-channels (shaker-type)* CLC chloride channels* Water channels* *P-type ATPases* (Na^+/K^+)

(Italicised are genes that were *functionally expressed in yeast mutants;* asterisks indicate functional expression in oocytes*; Aldrich, 1993; Amasheh et al., 1997; Anderson et al., 1992; André, 1995; Bennett et al., 1996; Bissig et al., 1994; Boorer et al., 1994; Boorer et al., 1996*a;* Boorer et al., 1996*b*; Chrispeels & Agre, 1994; DeWitt et al., 1991; Frommer et al., 1993; Frommer et al., 1995; Hastbacka et al., 1994; Hechenberger et al., 1996; Higgins et al., 1990; Kim et al., 1991; Jentsch, 1996; Leggewie et al., 1997; Malandro & Kilberg, 1996; Murer & Biber, 1996; Ninnemann et al., 1994; Overvoorde et al., 1996; Rentsch et al., 1996; Riesmeier et al., 1992; Rubio et al., 1995; Sauer et al., 1990; Sentenac et al., 1992; Smith et al., 1995; Steiner et al., 1994; Thorens, 1996; Wright et al., 1994).

of neurotransmitter transporters and is expressed in the brain. The family also includes the *g*lycine *t*ransporter (GLYT) subfamily expressed in the central nervous system. The rBAT/4F2hc family are type II membrane proteins inducing both neutral (including cystine) and cationic amino acid uptake when expressed in *Xenopus laevis* oocytes. There has been only a single report on the use of yeast as a complementation system for amino acid transporters from human cells; however no transporter gene could be identified (Segel et al., 1992). Mammalian glucose transporters have bene successfully expressed in yeast; however they are generally not correctly targeted to the plasma membrane (Kasahara & Kasahara, 1996). This is reminiscent of the retention of some plant H^+/ATPases in the endoplasmic reticulum of yeast (Gjedde-Palmgren & Christensen, 1994).

Functional Analysis of Plant Transporters in Yeast

As well as serving as an efficient tool for cloning plant transporter genes, the yeast expression system has also enabled investigations into the biochemical properties of individual transporters. For example, the affinity of SUT1 for sucrose, its substrate specificity and inhibitor sensitivity, when expressed in yeast, are similar to characteristics of high affinity sucrose transporters in leaves of a variety of plants (Riesmeier et al., 1992, 1993; Lemoine et al., 1996). Furthermore, the sensitivity of SUT1 mediated sucrose transport in yeast to protonophores indicated a proton-coupled transport mechanism. The oocyte work described below support this finding. Since the MFS superfamily comprises both uniporters and proton-coupled transporters it was presumed that proton binding sites could be derived from sequence comparisons between sym- and uniporters. However, no conserved motifs or charge distribution patterns conserved among cation cotransporters have been identified. Although the MFS family encompasses many different transporters covering a large spectrum of different substrates, it was not possible to derive the substrate recognition domains from the comparisons (Marger & Saier, 1993; Tanner & Caspari, 1996).

The affinity, substrate specificity and inhibitor sensitivity for the ATF1 and PCAT amino acid transporters were determined in yeast. Yeast cells expressing the AAPs actively accumulated amino acids against concentration gradients. The active accumulation was sensitive to protonophores. No amino acid efflux was observed when transport was inhibited by protonophores (Fischer et al., 1995). Regarding substrate specificity of members of the ATF1 family, the ProTs are selective for proline (Rentsch et al., 1996), whereas the AAPs can be categorized into two groups, (i) one for neutral and acidic amino acids and (ii) one also transporting basic amino acids (Fischer et al., 1995). The AAP family contains both low (AAP1-5) and high affinity systems (AAP6). AtCAT1 represents a high affinity system for basic amino acids, but also recognizes neutral and acidic amino acids with lower affinity (Frommer et al., 1995). Substrate specificity and affinity of AtCAT1 are similar to the mammalian homologue MCAT-2B, a high affinity form of the mammalian cationic amino acid transporters (Closs et al., 1993). Transport against a concentration gradient and sensitivity to ATPase inhibitors and protonophores indicate that AtCAT1 may function as a proton symporter. In contrast, MCATs mediate both influx and efflux and seem to function as uniporters (Closs et al., 1993). Detailed electrophysiological and radiotracer experiments using *Xenopus* oocytes should help in solving this putative discrepancy between plant and mammalian CAT transporters.

The numerous amino acid transporter genes that have been identified are compatible with the observation of complex kinetics in plasma membrane vesicles of leaves (Li & Bush, 1990, 1991, 1992). These were originally interpreted as the presence of four defined systems, one for acidic, two for neutral and one for basic amino acids. As the molecular studies show, the situation is much more complex in that plants contain a spectrum of transporters with overlapping specificities. An analysis of the expression pattern of the transporter genes in the plant indicates that the genes identified so far are expressed differentially in the plant (Fischer et al., 1995; Hirner et al., 1997).

When expressed in yeast, the plant oligopeptide transporters (OPT) function as high affinity transporters displaying low selectivity towards amino acid side chains by mediating the transport of di- and tripeptides (Steiner et al., 1994; Rentsch et al., 1995). As was shown for plant amino acid transport systems, the transport of oligopeptides is sensitive to protonophores. The properties found are thus similar to observations from plants and for their mammalian counterparts (Higgins & Payne, 1978; Sopanen 1979; Amasheh et al., 1997). One might assume that OPTs are involved in transferring proteolysis products from areas with high levels of protein degradation, e.g., in germinating seedlings mobilizing storage proteins, during mobilization of vegetative storage proteins in leaves for subsequent pod filling, in mobilization of proteins for export during leaf senescence, or in nutrition in the gut.

Electrophysiological Analysis of Plant Transporters in *Xenopus* Oocytes

As shown above, yeast transport mutants have proven invaluable for the isolation and characterization of plant transport proteins. However, functional analysis of transporters expressed in yeast cells is limited. Growth assays and competition experiments provide information

on substrate specificity but do not allow a distinction between substrates which are transported and those which are inhibitors of transport. Kinetic studies in yeast require the use of radiolabeled substrates and, depending on the apparent affinity of the transporter, are limited by the specific activity of the labeled substrate. H^+-coupled transporters are electrogenic i.e., the transport of substrates is accompanied by a net movement of charge. Thus, substrate-induced changes in membrane potential and substrate-induced currents can be measured using electrophysiological methods. Although difficult to perform, electrophysiological methods have been applied successfully to determine the properties of cloned plant K^+-channels expressed in yeast (Bertl et al., 1995).

Electrophysiological studies on cloned channels and carriers in *Xenopus* oocytes or insect cells have proven to be the most effective tool to study their function (Ward, 1997). Due to their large size (~1.2 mm in diameter) oocytes are amenable to a variety of electrophysiological techniques including the 2-electrode voltage clamp, cut-open oocyte vaseline gap (Taglialatella et al., 1992) and patch clamp methods (Lu et al., 1995; Hilgemann, 1996). Measurement of transport kinetics as a function of membrane voltage and ligand concentrations can be achieved in a single cell with extremely fast temporal resolution (50 microseconds). Provided the 3′-untranslated region of the cDNA encoding the transporter is polyadenylated, high levels of transporter activity can be achieved with little or no interference from endogenous transporter activities. Oocytes are the major expression system for studying transport mechanisms of H^+-coupled sucrose and amino acid cotransporters, mainly due to the absence of endogenous proton-coupled solute transport systems. Oocytes can also tolerate $[H^+]_o$ of at least 10 micromolar (pH 5.0), thus providing a downhill H^+ gradient across the plasma membrane, which is necessary for optimal transport activity of H^+-coupled transporters. The potato H^+/sucrose transporter StSUT1 and two arabidopsis AAP H^+/amino acid cotransporters were characterized by expression in oocytes (Boorer et al., 1996*a*,*b;* Boorer & Fischer, 1997).

Expression of the potato StSUT1 H^+/sucrose transporter in *Xenopus* oocytes and measurement of transporter using the two-electrode voltage-clamp method has enabled the determination of the transport mechanism of StSUT1 (Boorer et al., 1996*b*). The apparent affinity constant for sucrose (0.5–1 mM at 10 mM H^+_o and −150 mV) was similar to that obtained for StSUT1 expressed in yeast, indicating that transporter function is independent of its membrane environment (Riesmeier et al., 1993). The $K^{suc}_{0.5}$ increased (0.5 to 20 mM at −150 mV) when $[H^+]_o$ was decreased from 10 to 0.032 mM. Likewise, the apparent affinity constant for protons $K^H_{0.5}$ increased (0.1 to 2 μM) when $[sucrose]_o$ decreased from 20 to 5 mM. This positive cooperativity between sucrose and protons has important implications for the transport mechanism of StSUT1 and, based on the arguments of Jauch and Luger (1986), suggests that StSUT1 operates via a simultaneous mechanism: both ligands bind to the carrier to form a H^+-substrate-carrier complex which crosses the membrane and releases the substrates at the cytoplasmic surface. A simultaneous binding mechanism was also proposed for the H^+/amino acid cotransporter AAP1 (Boorer et al., 1996*a; see below*). In contrast, the H^+/hexose STP1 cotransporter was shown to operate via a sequential mechanism (Boorer et al., 1994): one ligand (H^+ or sugar) binds to the carrier on the outside, the loaded carrier crosses the membrane, the ligand dissociates and the carrier reorientates in the membrane to repeat the transport cycle with a second substrate. The transport mechanism was also determined by the effect of ligand concentrations on maximal currents. Boorer et al. (1996*b*) showed that a large increase in $[H^+]_o$ (300-fold) gave rise to a small increase (1.4-fold) in the maximal current for sucrose, whereas a 4-fold increase in $[sucrose]_o$ produced an equivalent increase in the maximal current for H^+. Thus protons seem to act as essential activators and bind to StSUT1 before sucrose. The apparent kinetic parameters for StSUT1 were also voltage-dependent. $K^H_{0.5}$ and $K^{suc}_{0.5}$ were relatively voltage-independent when the concentration of the cotransported ligand was saturating, whereas at subsaturating cosubstrate concentrations $K^H_{0.5}$ and $K^{suc}_{0.5}$ increased at depolarized potentials. However, as the membrane potential was hyperpolarized these values decreased and approached those obtained under saturating conditions. These results suggest that StSUT1 is negatively charged and voltage drives the protons into the binding site. Protons bind to StSUT1 before sucrose, and thus, the voltage-dependence of $K^H_{0.5}$ influences the voltage-dependence of $K^{suc}_{0.5}$.

The transport model for StSUT1 was verified by determining rate constants for the partial reaction steps which predicted the effect of ligand concentration and voltage on the experimental data. A further advantage of the oocyte expression system is the opportunity to determine transport stoichiometry directly rather than relying on Hill coefficients obtained from substrate activation curves. The stoichiometry of H^+/sucrose cotransport by simultaneously measuring the amount of charge and radiolabelled sucrose transported into individual, voltage-clamped oocytes was determined to be ~1:1, confirming earlier measurements from plasma membrane vesicles of leaves (Boorer et al., 1996*b;* Bush, 1990; Slone & Buckhout, 1991).

The transport mechanism of the H^+/amino acid cotransporter AAP1 was also studied by expression in oocytes (Boorer et al., 1996*a*). Like StSUT1, transport of amino acids by AAP1 was strictly dependent upon H^+. Neither Na^+ nor Li^+ can be utilized by AAP1 and

StSUT1 to drive substrate transport unlike SGLT1 which can use H^+ and Li^+ to drive sugar transport (Hirayama et al., 1994, 1997). Similar to sucrose transport through StSUT1, amino acid transport by AAP1 was voltage-dependent and the effect of ligand concentration on the kinetic parameters suggested that AAP1 operates via a simultaneous mechanism. However, based on the effect of ligand concentration on maximal currents, it was concluded that protons and amino acids bind randomly to AAP1.

A combination of electrophysiology and freeze fracture electron microscopy was used to determine the unitary functional capacity of AAP1 (Boorer et al., 1996*a*). Expression of AAP1 in oocytes increased the density of particles in the protoplasmic face of the oocyte plasma membrane, which corresponded to an increase in the substrate-induced current. The turnover number of AAP1 i.e., the number of substrate molecules transported per second was >350 sec^{-1}, which is one order of magnitude faster than that of the H^+/hexose transporter STP1 (59 sec^{-1}; Boorer et al., 1994) and SGLT1 (25 sec^{-1}, Panayotova-Heiermann et al., 1994).

The AAPs are peculiar because of their ability to transport anionic, cationic and neutral amino acids (Fischer et al., 1995). Boorer and Fischer (1997) investigated the effect of substrate charge and geometry on the substrate recognition and H^+:amino acid stoichiometry of AAP5. The apparent affinity of AAP5 for amino acids was high for linear, unbranched amino acids such as lysine (0.45 mM), arginine (0.14 mM) and methionine (0.27 mM) and was significantly reduced for substrates with branching at the β-carbon position such as proline (21 mM), valine (33 mM) and isoleucine (42 mM). Detailed kinetic analysis and stoichiometry measurements using lysine, alanine, glutamate and histidine revealed that irrespective of their net charge, one amino acid was cotransported with one proton. Interestingly, lysine was transported in its cationic form whereas histidine and glutamate were transported as neutral species. In addition, the position of the charge in the amino acid side chain was also important for substrate-recognition. So far six amino acid transporters belonging to the AAP gene family have been functionally characterized, all of which differ with respect to their transport kinetics and substrate-specificity (Boorer et al., 1996*a;* Boorer & Fischer, 1997; W.N. Fischer et al., *unpublished data*). Sequence alignments will be used to target amino acid residues for mutagenesis. Kinetic analysis of mutant transporters should then enable identification of amino acid residues involved in substrate recognition, voltage sensing and, in the long term, the correlation between transporter structure and function. The oocyte expression system may also prove useful to study the regulation of plant transporters by protein kinases. The regulation of the SGLT1 (Hirsch et al., 1996) and the Na^+, Cl^- taurine TAUT cotransporter (Loo et al., 1996) was studied by expression in oocytes using the two-electrode voltage clamp method.

Regulation of Transport Activity at Different Levels

Transporters can be regulated at five different levels: (i) gene activity, (ii) mRNA stability, (iii) translatability of the mRNA, (iv) targeting of the protein, and (v) post-translational modification. It is obvious from physiological studies that both sucrose and amino acid transport are regulated by endogenous and environmental signals (Frommer et al., 1994*b*).

Both sucrose and amino acid transporter genes are regulated in a tissue or cell specific manner. For example, the sucrose transporter SUT1 is phloem-associated and the AAPs are expressed differentially in the plant (Riesmeier et al., 1993; Truernit et al., 1995; Fischer et al., 1995). Although *AAP1* and *2* are found in the same organ, the are expressed in different cell types (Hirner et al., 1997). Thus developmental, hormonal and metabolic signals seem to be involved in controlling expression of the genes. Furthermore, the steady state level of *SUT1* mRNA is under developmental and hormonal control (Lemoine et al., 1992; Riesmeier et al., 1993; Harms et al., 1994; Truernit & Sauer 1995). The expression of *SUT1* is regulated diurnally at the mRNA level indicating that both mRNA and protein turnover (*see* below) are regulated and play a role in determining sucrose transport activity. Besides studying regulation at the mRNA and protein level *in planta*, expression in yeast mutants defective in regulation of membrane proteins or expression in oocytes in conjunction with signal transduction intermediates or inhibitors may help elucidating the mechanisms of transporter regulation.

Subcellular Localization and Targeting of Carriers to Plasma Membrane

Plant transporters that complement yeast mutants deficient in nutrient uptake must be located in the plasma membrane. Thus the proteins must either be recognized correctly by the yeast and oocyte targeting machinery or they reach their destination via unspecific mechanisms (Frommer & Ninnemann, 1995). Possibly, only a fraction of the protein reaching the plasma membrane is sufficient for function. Therefore direct proof of a predominant localization at the plasma membrane of yeast, oocytes and most importantly in the plant is necessary. Freeze fracture studies have shown that at least AAP1 is localized at the plasma membrane of *Xenopus* oocytes (Boorer et al., 1996*a*). Immunolocalization allowed us to show that the sucrose transporter SUT1 is localized specifically at the plasma membrane of sieve elements

(Kühn et al., 1997). Plant amino acid or peptide transporters have not been localized. We can thus only speculate that the amino acid and peptide transporters which functionally complement the yeast plasma membrane transport mutants are also targeted to the plasma membrane *in planta.* Immunogold labeling and fusions with markers such as the *green fluorescent protein* in combination with yeast mutants defective in targeting may help in answering these questions. For some proteins, specific receptors are present at the ER that are involved in correct targeting, e.g., SHR3 for amino acid permeases in yeast. The plant ATF1s that at the sequence level are unrelated to yeast YAAPs, are able to suppress the *shr*3 phenotype (Rentsch et al., 1996). Interestingly there are no reports on functional complementation of yeast mutants by mammalian transporters. An explanation could be that some proteins are incompatible with the yeast targeting machinery as observed in the case of the expression of mammalian glucose transporters in yeast (Kasahara & Kasahara, 1996).

For a number of mammalian carriers such as glucose transporters of the intestine or kidney, an asymmetric distribution within a single cell was observed (Thorens, 1996). So far only very few plant transporters have been localized at the subcellular level in plant cells, and asymmetric targeting has not been reported. Despite differences in cell biology, i.e., the lack of tight junctions, it is expected that plant cells also exhibit asymmetric distribution of carriers, especially since many plant cells are polarized e.g., transfer cells in cotyledons and phloem, root hairs or pollen tubes. Transfer cells are especially good candidates for asymmetric carrier distribution since besides being involved in high levels of sucrose and amino acid transport they display an asymmetrically amplified plasma membrane surface similar to microvillar membranes (Offler & Patrick, 1993). Polarized transport processes have also been described in plant cells that must be due to asymmetric distribution of carriers within single cells such as the polar transport of auxins (Lomax et al., 1995).

Intercellular Targeting of Solute Transporters

The phloem consists of several cell types, namely living enucleate sieve elements that function as the actual conduits for sugars and amino acids, neighboring companion cells that are tightly connected with sieve elements via special deltoid plasmodesmata called PPUs (pore plasmodesmal units) and phloem parenchyma cells. The sucrose transporter SUT1 was detected in plasma membranes of enucleate sieve elements of tobacco, potato and tomato (Kühn et al., 1997). *In situ* hybridization at the TEM level showed that *SUT1* mRNA is also localized in sieve elements and is preferentially associated with the orifices of the plasmodesmata. Antisense inhibition of *SUT1* expression in companion cells leads to an inhibition of sucrose export from leaves, indicating that transcription of *SUT1* occurs in companion cells (Kühn et al., 1996). Together with the observed turnover of mRNA and protein (*see below*), this provides strong evidence for the targeting of plant endogenous mRNA and potentially SUT1 protein through phloem plasmodesmata. There are two hypotheses for the intercellular targeting of SUT1: (i) *SUT1* mRNA is guided along the cytoskeleton through PPUs and is translated in sieve elements or (ii) translation occurs in companion cells: the protein is inserted into the ER or plasma membrane and since ER and plasma membrane are continuous through plasmodesmata, SUT1 is transported through the PPUs into sieve elements (Overall & Blackman, 1996). As described above the sucrose binding protein SBP was also found mainly in the plasma membrane of sieve elements (Warmbrodt et al., 1989). This may indicate that SBP is targeted through PPUs to the sieve elements as well. Since translocation of amino acids and oligopeptides is assumed to follow the same route as sucrose, an obvious question arises as to where amino acid and peptide transporters are localized. The analysis of promoter-GUS fusions for the amino acid transporters AAP2 and AtCAT1 demonstrated expression in the vascular system of leaves (Frommer et al., 1995; Hirner et al., 1997).

Regulation of Plant Transport Proteins by Turnover

Inhibition studies using cycloheximide showed that within the plant the turnover of SUT1 protein is in the range of a few hours (Kühn et al., 1997). Similar mechanisms may also be involved in regulating other membrane proteins. Previous studies in maize have shown that the plasma membrane H^+/ATPase turns over in the range of 12 minutes after treatment of the tissue with auxin (Hager et al., 1991). Turnover of amino acid transporters has not yet been tested in plants. The high turnover rate of SUT1 and H^+/ATPases may point to specific mechanisms involved in controlling the number of active carriers in the plasma membrane such as ubiquitination and endocytosis, as was found in case of mammalian glucose transporters or yeast sugar and amino acid permeases (Hein et al., 1995; Thorens, 1996; Medintz et al., 1996). This degradation pathway involves tagging and ubiqitination of the protein, subsequent internalization and degradation. Alternate pathways involve recycling of the internalized proteins to the plasma membrane or degradation without apparent involvement of endocytosis (Hare, 1990; Ooi et al., 1996).

Yeast serves as a model system for studying the turnover of membrane proteins. Posttranslational modification as a trigger for degradation was found in case of the yeast general amino acid permease GAP1 that is inactivated in *npr1* mutants lacking a protein kinase

(Vandenbol et al., 1990). Evidence for a role of ubiquitin was obtained in *npi1* mutants lacking functional ubiquitin protein ligase in which GAP1 is protected from degradation (Hein et al., 1995). Since SUT1 turns over at a high rate, one may speculate that similar regulatory mechanisms are operating. Some of the necessary players have been detected in phloem sap: (i) phosphorylated proteins suggesting the activity of protein kinases in sieve tubes and (ii) ubiquitin which represents one of the major proteinaceous components (Nakamura et al., 1993; Schobert et al., 1995).

Summary and Outlook

Several gene families involved in transport of sugars and amino acids have been identified from fungi, plants and animals. The currently known spectrum of systems is summarized in Table 1. The advanced knowledge in the plant field, i.e., the large number of carrier genes isolated in recent years and the availability of efficient expression systems might be relevant for identifying novel mammalian transporters that have been postulated from biochemical analyses, e.g., systems A and L. Homology searches using plant transporter families that have not yet been found in animals might be the simplest way to proceed (*see* gaps in Table 1). So far no homologues of the Na^+-coupled mammalian transport systems have been found in plants. However the yeast genome contains a few ORFs that share significant homologies with such systems (André, personal communication). The comparatively low abundance of Na^+-coupled transport systems in yeast and plants is mainly due to the fact that while animals preferentially use Na^+-gradients for energization (Na^+/K^+-ATPase), fungi and plants couple secondary active transport mainly to proton gradients (H^+-ATPase). An exception is the mammalian oligopeptide transporter that uses proton gradients for energization (Fei et al., 1994). However, homology searches are not necessarily the best tool to identify the mode of energization since in other systems several carriers have been described that can function both as Na^+- or H^+-cotransporters, such as the *E. coli* melibiose transporters and the mammalian glucose transporter SGLT1 (Henderson, 1990; Hirayama et al., 1994).

The access to transport proteins for sucrose, amino acids and oligopeptides at the molecular level provides a new view of physiology. Multiple sucrose transporters were found, one of them being essential for long distance transport; the function of the others still remains unknown. At least two superfamilies of amino acid transporters with overlapping specificities were identified. The complexity might reflect the need for differential regulation, as was nicely demonstrated in the case of salt stress, where AAPs were downregulated in favor of specific proline transporters (Rentsch et al., 1996). More detailed reviews of the physiological aspects of metabolite transporters has been given elsewhere (Ward et al., 1997; Frommer et al., 1994*b;* Rentsch & Frommer, 1996). This wealth of new information should lead to a better understanding of basic questions regarding structure/function relationships and the control of metabolite trafficking within plants.

We would like to thank Bruno André (Bruxelles) for communicating unpublished observations and Heather Silyn-Roberts for editorial suggestions. We are very grateful for support of our research by grants from Deutsche Forschungsgemeinschaft, Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie and the European Community.

References

Albritton, L.M., Tseng, L., Scadden, D., Cunningham, J.M. 1989. A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. *Cell* **57:**659–666

Aldrich, R. 1993. Potassium channels-advent of a new family. *Nature* **362:**107–108

Amasheh, S., Wenzel, U., Boll, M., Dorn, D., Weber, W.M., Clauss, W., Daniel, H. 1997. Transport of charged dipeptides by the intestinal H^+/peptide symporter PEPT1 expressed in *Xenopus laevis* oocytes. *J. Membrane Biol.* **155:**247–256

Anderson, J.A., Huprikar, S.S., Kochian, L.V., Lucas, W.J., Gaber, R.F. 1992. Functional expression of a probable potassium channel in Saccharomyces cerevisiae Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **89:**3736–3740

André B. 1995. An overview of membrane transport proteins in *Saccharomyces cerevisiae. Yeast* **11:**1575–1611

Bennett, M.J., Marchant, A., Green, H.G., May, S.T., Ward, S.P., Millner, P.A., Walker, A.R., Schulz, B., Feldmann, K.A. 1996. The Arabidopsis AUX1 gene: A permease-like regulator of root gravitropism. *Science* **273:**948–950

Bertl, A., Anderson, J.A., Slayman, C.L., Gaber, R.F. 1995. Use of *Saccharomyces cerevisiae* for patch-clamp analysis of heterologous membrane proteins: Characterization of Kat1, an inward-rectifying K^+ channel from *Arabidopsis thaliana,* and comparison with endogenous yeast channels and carriers. *Proc. Natl. Acad. Sci. USA* **92:**2701–2705

Bissig, M., Hagenbuch, B., Stieger, B., Koller, T., Meier, P. 1994. Functional expression cloning of the canalicular sulfate transport system of rat hepatocytes. *J. Biol Chem.* **269:**3017–3021

Boorer, K.J., Loo, D.D.F., Wright, E.M. 1994. Steady-state and presteady-state kinetics of the H^+/hexose cotransporter (STP1) from *Arabidopsis thaliana* expressed in *Xenopus* oocytes. *J. Biol. Chem.* **269:**20417–20424

Boorer, K.J., Loo, D.D.F., Frommer, W.B., Bush, D.R., Kreman, M., Loo, D.D.F., Wright, E.M. 1996*a.* Kinetics and specificity of a H^+/amino acid transporter from *Arabidopsis thaliana. J. Biol. Chem.* **271:**2213–2220

Boorer, K.J., Loo, D.D.F., Frommer, W.B., Wright, E.M. 1996*b*. Transport mechanism of the cloned potato H^+/sucrose cotransporter StSUC1. *J. Biol. Chem.* **271:**25139–25144

Boorer, K.J., Fischer, W.-N. 1997. Specificity and stoichiometry of the *Arabidopsis* H^+/amino acid transporter AAP5. *J. Biol. Chem.* **272:**13040–13046

Bush, D.R. 1990. Electrogenicity, pH-dependence, and stoichiometry of the proton-sucrose symport. *Plant Physiol.* **93:**1590–1596

Bush, D.R. 1993. Proton-coupled sugar and amino acid transporters in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44:**513–542

Chrispeels, M.J., Agre, P. 1994. Aquaporins: water channel proteins of plant and animal cells. *Trends Biochem. Sci.* **19:**421–425

Closs, E.I., Lyons, C.R., Kelly, C., Cunningham, J.M. 1993. Characterization of the third member of the MCAT family of cationic amino acid transporters. *J. Biol. Chem.* **268:**20796–20800

Deisenhofer, J., Epp, O., Sinning, I., Michel, H. 1995. Crystallographic refinement at 2.3 Å resolution and refined model of the photosynthetic reaction centre from *Rhodopseudomonas viridis. J. Mol. Biol.* **246:**429–457

Delrot, S. 1981. Proton fluxes associated with sugar uptake in *Vicia faba* leaf tissue. *Plant Physiol.* **68:**706–711

Delrot, S. 1989. Phloem loading. *In:* Transport of Photoassimilates. D.A. Baker and J.A. Milburn, editors. pp. 167–205. Longman Scientific, London

Deuvreux, J., Haeberli, P., Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12:**387–395

DeWitt, N.D., Harper, J.F., Sussman, M.R. 1991. Evidence for a plasma membrane proton pump in phloem cells of higher plants. *Plant J.* **1:**121–128

Fei, Y.J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F.H., Romero, M.F., Singh, S.K., Boron, W.F., Hediger, M.A. 1994. Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* **368:**563–566

Felsenstein, J. 1993. PHYLIP (Phylogeny Interference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle

Fischer, W.N., Kwart, M., Hummel, S., Frommer, W.B. 1995. Substrate specificity and expression profile of amino acid transporters (AAPs) in *Arabidopsis. J. Biol Chem.* **270:**16315–16320

Frillingos, S., Kaback, H.R. 1996. Probing the conformation of the lactose permease of *Escherichia coli* by *in situ* site-directed sulfhydryl modification. *Biochem.* **35:**3950–3956

Frommer, W.B., Hummel, S., Riesmeier, J.W. 1993. Yeast expression cloning of a cDNA encoding a broad specificity amino-acid permease from *Arabidopsis thaliana. Proc. Natl. Acad. Sci USA* **90:**5944–5948

Frommer, W.B., Hummel, S., Rentsch, D. 1994*a*. Cloning of an *Arabidopsis* histidine transporting protein related to nitrate and peptide transporters. *FEBS Lett.* **347:**185–189

Frommer, W.B., Kwart, M., Hirner, B., Fischer, W.N., Hummel, S., Ninnemann, O. 1994*b*. Transport of nitrogenous compounds in plants. *Plant Mol. Biol.* **26:**1651–1670

Frommer, W.B., Ninnemann, O. 1995. Heterologous hosts as expression systems for plant genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46:**419–444

Frommer, W.B., Hummel, S., Unseld, M., Ninnemann, O. 1995. Seed and vascular expression of a high affinity transporter for cationic amino acids in *Arabidopsis. Proc. Natl. Acad. Sci. USA* **92:**12036–12040

Gahrtz, M., Stolz, J., Sauer, N. 1994. A phloem-specific sucrose-H^+ symporter from *Plantago major* L. supports the model of apoplastic phloem loading. *Plant J.* **6:**697–706

Gahrtz, M., Schmelzer, E., Stolz, J., Sauer, N. 1996. Expression of the PmSUC1 sucrose carrier gene from *Plantago major* L. is induced during seed development. *Plant J.* **9:**93–100

Gallet, O., Lemoine, R., Gaillard, C., Larsson, C., Delrot, S. 1992. Selective inhibition of active uptake of sucrose into plasma membrane vesicles by polyclonal sera directed against a 42 kilodalton plasma membrane polypeptide. *Plant Physiol.* **98:**17–23

Gjedde-Palmgren, M., Christensen, G. 1994. Functional comparisons between plant plasma membrane H^+-ATPase isoforms expressed in yeast. *J. Biol. Chem.* **269:**3027–3033

Grimes, H.D., Overvoorde, P.J., Ripp, K., Franceschi, V.R., Hitz, W.D. 1992. A 62-kD sucrose binding protein is expressed and localized in tissues actively engaged in sucrose transport. *Plant Cell* **4:**1561–1574

Hager, A., Debus, G., Edel, H.G., Stransky, H., Serrano, R. 1991. Auxin induces exocytosis and the rapid synthesis of a high turnover pool of plasma membrane H^+-ATPase. *Planta* **185:**527–537

Hare, J.F. 1990. Mechanism of protein turnover. *Biophys. Acta* **1031:**71–90

Harms, K., Wöhner, R.V., Schulz, B., Frommer, W.B. 1994. Regulation of two p-type H^+-ATPase genes from potato. *Plant Mol. Biol.* **26:**979–988

Harrington, G.N., Franceschi, V.R., Offler, C.E., Patrick, J.W., Harper, J.F., Frommer, W.B., Tegeder, M., Hitz, W.D. 1997. Cell specific expression of three genes involved in plasma membrane sucrose transport in developing *Vicia faba* seed. *Protoplasma* **197:**160–173

Hästbacka, J., de la Chapelle, A., Mahtani, M.M., Clines, G., Reeve-Daly, M.P., Daly, M., Hamilton, B.A., Kusumi, K., Trivedi, B., Weaver, A., Coloma, A., Lovett, M., Buckler, A., Kaitila, I., Lander, E.S. 1994. The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. *Cell* **78:**1073–1087

Hechenberger, M., Schwappach, B., Fischer, W.N., Frommer, W.B., Jentsch, T.J., Steinmeyer, K. 1996. A family of putative chloride channels from *Arabidopsis* and functional complementation of a yeast strain with a CLC gene disruption. *J. Biol. Chem.* **271:**33632–33638

Hediger, M.A., Coady, M.J., Ikeda, T.S., Wright, E.M. 1987. Expression cloning and cDNA sequencing of the Na^+/glucose cotransporter. *Nature* **330:**379–381

Hein, C., Springael, J.Y., Volland, C., Haguenauer-Tsapis, R., André, B. 1995. NPI1, an essential yeast gene involved in induced degradation of Gap 1 and Fur4 permeates, encodes the Rsp5 ubiquitin-protein ligase. *Mol. Microbiol.* **18:**77–87

Henderson, P.J. 1990. Proton-linked sugar transport systems in bacteria. *J. Bioenerg. Biomembr.* **22:**525–69

Higgins, C.F., Payne, J.W. 1978. Peptide transport by germinating barley embryos. *Planta* **138:**217–221

Higgins, C.F., Hyde, S.C., Mimmack, M.M., Gileadi, U., Gill, D.R., Gallagher, M.P. 1990. Binding protein-dependent transport systems. *J. Bionenerg. Biomembr.* **22:**571–592

Hilgemann, D.W. 1996. Unitary Cardiac Na^+, Ca^{2+} exchange current magnitudes determined from channel-like noise and charge movements of ion transport. *Biophys. J.* **71:**759–768

Hirayama, B.A., Loo, D.D.F., Wright, E.M. 1994. Protons drive sugar transport through the Na^+/glucose cotransporter (SGLT1). *J. Biol. Chem.* **269:**21407–21410

Hirayama, B.A., Loo, D.D.F., Wright, E.M. 1997. Cation effects on protein conformation and transport in the Na^+/glucose cotransporter. *J. Biol. Chem.* **272:**2110–2115

Hirner, B., Fischer, W.N., Rentsch, D., Kwart, M., Frommer, W.B. 1997. Developmental control of H^+/amino acid permease gene expression during seed development of *Arabidopsis. Plant J.* (*in press*)

Hirsch, J.R., Loo, D.D.F., Wright, E.M. 1996. Regulation of Na^+/glucose cotransporter expression by protein kinases in *Xenopus laevis* oocytes. *J. Biol. Chem.* **271:**14740–14746

Hsu, L., Chiou, T., Chen, L., Bush, D.R. 1993. Cloning a plant amino acid transporter by functional complementation of a yeast amino acid transport mutant. *Proc. Natl. Acad. Sci. USA* **90:**7441–7445

Jauch, P., Luger, P. 1986. Electrogenic properties of the sodium-

alanine cotransporter in pancreatic acinar cells. II. Comparison with transport models. *J. Membrane Biol.* **94:**117–127

Jauniaux, J.C., Vandenbol, M., Vissers, S., Broman, K., Grenson, M. 1987. Nitrogen catabolite regulation of proline permease in *Saccharomyces cerevisiae*. Cloning of the *PUT*4 gene and study of *PUT*4 RNA levels in wild-type and mutant strains. *Eur. J. Biochem.* **164:**601–606

Jentsch, T.J. 1996. Chloride channels: a molecular perspective. *Curr. Opin. Neurobiol.* **6:**303–10

Kasahara, T., Kasahara, M. 1996. Expression of the rat GLUT1 glucose transporter in the yeast *Saccharomyces cerevisiae*. *Biochem. J.* **315:**177–182

Kim, J.W., Closs, E.I., Albritton, L.M., Cunningham, J.M. 1991. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. *Nature* **352:**725–728

Koo, K., Stuart, W.D. 1991. Sequence and structure of *mtr*, an amino acid transport gene of *Neurospora crassa*. *Genome* **34:**644–651

Kühn, C., Quick, W.P., Schulz, A., Sonnewald, U., Frommer, W.B. 1996. Companion cell-specific inhibition of the potato sucrose transporter SUT1. *Plant Cell Envir.* **19:**1115–1123

Kühn, C., Franceschi, V.R., Schulz, A., Lemoine, R., Frommer, W.B. 1997. Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. *Science* **275:**1298–1300

Kuehn, M.J., Schekman, R., Ljungdahl, P.O. 1996. Amino acid permeases require COPII components and the ER resident membrane protein Shr3p for packaging into transport vesicles *in vitro*. *J. Cell Biol.* **135:**585–595

Kwart, M., Hirner, B., Hummel, S., Frommer, W.B. 1993. Differential expression of two related amino-acid transporters with differing substrate specificity in *Arabidospsis thaliana*. *Plant J.* **4:**993–1002

Lauter, F.R., Ninnemann, O., Bucher, M., Riesmeier, J.W., Frommer, W.B. 1996. Preferential expression of an ammonium transporter and of two putative nitrate transporters in root hairs of tomato. *Proc. Natl. Acad. Sci. USA* **93:**8139–8144

Leggewie, G., Willmitzer, L., Riesmeier, J.W. 1997. Two cDNAs from potato are able to complement a phosphate uptake deficient yeast mutant: identification of phosphate transporters from higher plants. *Plant Cell* **9:**381–392

Lemoine, R., Gallet, O., Gaillard, C., Frommer, W., Delrot, S. 1992. Plasma membrane vesicles from source and sink leaves: Changes in solute transport and polypeptide composition. *Plant Physiol.* **100:**1150–1156

Lemoine, R., Kühn, C., Thiele, N., Delrot, S., Frommer, W.B. 1996. Antisense inhibition of the sucrose transporter in potato: Effects on amount and activity. *Plant Cell Environ.* **19:**1124–1131

Li, Z.S., Gallet, O., Gaillard, C., Lemoine, R., Delrot, S. 1992. The sucrose carrier of the plant plasmalemma: III. Partial purification and reconstitution of active sucrose transport in liposomes. *Biochim. Biophys. Acta* **1103:**259–267

Li, Z., Bush, D.R. 1990. ΔpH-dependent amino acid transport into plasma membrane vesicles isolated from sugar beet leaves. *Plant Physiol.* **94:**268–277

Li, Z., Bush, D.R. 1991. ΔpH-dependent amino acid transport into plasma membrane vesicles isolated from sugar beet (*Beta vulgaris* L.) leaves. *Plant Physiol.* **96:**1338–1344

Li, Z., Bush, D.R. 1992. Structural determinants in substrate recognition by proton-amino acid symports in plasma membrane vesicles isolated from sugar beet leaves. *Arch. Biochem. Biophys.* **294:**519–526

Ljungdahl, P.O., Gimeno, C.J., Styles, C.A., Fink, G.R. 1992. *SHR*3, a novel component of the secretory pathway specifically required for localization of amino acid permeases in yeast. *Cell* **71:**463–478

Lomax, T.L., Muday, G.K Rubery, P.H. 1995. Auxin transport. *In:* Plant Hormones. P.J. Davies, editor. pp. 509–530 Kluwer Publ. Dordrecht

Loo, D.D.F., Hirsch, J.R., Sarkar, H.K., Wright, E.M. 1996. Regulation of the mouse retinal taurine transporter (TAUT) by protein kinases in *Xenopus* oocytes. *FEBS Lett.* **392:**250–254

Lu, C.C., Kabakov, A., Markin, V.S., Mager, S., Frazier, G.A., Hilgemann, D.W. 1995. Membrane transport mechanisms probed by capacitance measurements with megahertz voltage clamp. *Proc. Natl. Acad. Sci. USA* **92:**11220–11224

Malandro, M.S., Kilberg, M.S. 1996. Molecular biology of mammalian amino acid transporters. *Annu. Rev. Biochem.* **65:**305–336

Marger, M.D., Saier Jr., M.H. 1993. A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. *Trends Biochem. Sci.* **18:**13–20

Maynard, J.W., Lucas, W.J. 1982. Sucrose and glucose uptake in *Beta vulgaris* leaf tissue. A case for a general (apoplastic) retrieval system. *Plant Physiol.* **70:**1436–1443

Medintz, I., Jiang, H., Han, E.K., Cui, W., Michels, C.A. 1996. Characterization of the glucose-induced inactivation of maltose permease in *Saccharomyces cerevisiae*. *J. Bact.* **178:**2245–2254

Milpetz, F., Argos, P. 1995. TMAP: a new email and WWW service for membrane-protein structural predictions. *TIBS* **20:**204–205

Murer, H., Biber, J. 1996. Molecular mechanisms of renal apical Na/phosphate cotransport. *Annu. Rev. Physiol.* **58:**607–18

Nakamura, S., Hayashi, H., Mori, S., Chino, M. 1993. Protein phosphorylation in the sieve tubes of rice plants. *Plant Cell Physiol.* **34:**927–933

Ninnemann, O.W., Jauniaux, J.C., Frommer, W.B. 1994. Identification of a high affinity ammonium transporter from plants. *EMBO J.* **13:**3464–3471

Offler, C.E., Patrick, J.W. 1993. Pathway of photosynthate transfer in the developing seed of *Vicia faba* L: A structural assessment of the role of transfer cells in unloading from the seed coat. *J. Exp. Biol.* **44:**711–724

Ooi, C.E., Rabinovich, E., Dancis, A., Bonifacino, J.S., Klausener, R.D. 1996. Copper-dependent degradation of the *Saccharomyces cerevisiae* plasma membrane copper transporter Ctr1p in the apparent absence of endocytosis. *EMBO J.* **15:**3515–3523

Ostermeier, C., Iwata, S., Ludwig, B., Michel, H. 1995. *Fv* fragment-mediated crystallization of the membrane protein bacterial cytochrome c oxidase. *Nat. Struct. Biol.* **2:**842–846

Overall, R.L., Blackman, L.M. 1996. A model of the macromolecular structure of plasmodesmata. *Trends Plant Sci.* **1:**307–311

Overvoorde, P.J., Frommer, W.B., Grimes, H.D. 1996. A soybean sucrose binding protein independently mediates nonsaturable sucrose uptake in yeast. *Plant Cell* **8:**271–280

Panayotova-Heiermann, M., Loo, D.D.F., Lostao, M.P., Wright, E.M. 1994. Sodium/D-glucose cotransporter charge movements involve polar residues. *J. Biol. Chem.* **269:**21016–21020

Perry, J.R., Basrai, M.A., Steiner, H.Y., Peer, F., Becker, J.M. 1994. Isolation and characterization of a *Saccharomyces cerevisiae* peptide transport gene. *Mol. Cell. Biol.* **14:**104–115

Prive, G.G., Kaback, H.R. 1996. Engineering the *lac* permease for purification and crystallization. *J. Bioenerg. Biomembr.* **28:**29–34

Reizer, J., Finley, K., Kakuda, D., MacLeod, C., Reizer, A., Saier, M.H. 1993. Mammalian integral membrane receptors are homologous to facilitators and antiporters of yeast, fungi and eubacteria. *Protein Sci.* **2:**20–30

Rentsch, D., Laloi, M., Rouhara, I., Schmelzer, E., Delrot, S., Frommer, W.B. 1995. NTR1 encodes a high affinity oligopeptide transporter in *Arabidopsis*. *FEBS Lett.* **370:**264–268

Rentsch, D., Hirner, B., Schmelzer, E., Frommer, W.B. 1996. Salt stress-induced proline transporters and salt stress-repressed broad

specificity amino acid permease genes identified by suppression of an amino acid transport targeting mutant. *Plant Cell* **8:**1437–1446

Rentsch, D., Frommer, W.B. 1996. Molecular approaches towards an understanding of loading and unloading of assimilates in higher plants. *J. Exp. Bot.* **47:**1199–1204

Riesmeier, J.W., Willmitzer, L., Frommer, W.B. 1992. Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. *EMBO J.* **11:**4705–4713

Riesmeier, J.W., Hirner, B., Frommer, W.B. 1993. Expression of the sucrose transporter from potato correlates with the sink-to-source transition in leaves. *Plant Cell* **5:**1591–1598

Riesmeier, J.W., Willmitzer, L., Frommer, W.B. 1994. Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning. *EMBO J.* **13:**1–7

Ripp, K.G., Viitanen, P.V., Hitz, W.D., Franceschi, V.R. 1988. Identification of a membrane protein associated with sucrose transport into cells of developing soybean cotyledons. *Plant Physiol.* **88:**1435–1445

Rubio, R., Gassmann, W., Schroeder, J.I. 1995. Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science* **270:**1660–1663

Sauer, N., Friedländer, K., Gräml-Wicke, U. 1990. Primary structure, genomic organization and heterologous expression of a glucose transporter from *A. thaliana*. *EMBO J.* **9:**3045–3050

Sauer, N., Stolz, J. 1994. SUC1 and SUC2: two sucrose transporters from *Arabidopsis thaliana;* expression and characterization in baker's yeast and identification of the histidine-tagged protein. *Plant J.* **6:**67–77

Sauer, N., Gahrtz, M., Stadler, R., Stolz, J., Truernit, E. 1994*a*. Molecular biology of sugar transporters of the plant plasma membrane. *Symp. Soc. Exp. Biol.* **48:**155–165

Sauer, N., Baier, K., Gahrtz, M., Stadler, R., Stolz, J., Truernit, E. 1994*b*. Sugar transport across the plasma membranes of higher plants. *Plant Mol. Biol.* **26:**1671–1679

Schobert, C., Grossmann, P., Gottschalk, M., Komor, E., Pecsvaradi, A., Nieden, U.Z. 1995. Sieve-tube exudate from Ricinus communis L. seedlings contains ubiquitin and chaperones. *Planta* **196:**205–210

Segel, G.B., Boal, T.R., Cardillo, T.S., Murant, F.C., Lichtman, M.A., Sherman, F. 1992. Isolation of a gene encoding a chaperonin-like protein by complementation of yeast amino acid transport mutants with human cDNA. *Proc. Natl. Acad. Sci. USA* **89:**6060–6064

Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J.M., Gaymard, F., Grignon, C. 1992. Cloning and expression in yeast of a plant potassium ion transport system. *Science* **256:**663–665

Slone, J.H., Buckhout, T.J. 1991. Sucrose-dependent proton transport in plasma-membrane vesicles isolated from sugar-beet leaves (*Beta vulgaris* L.): Evidence in support of the proton-symport model for sucrose transport. *Planta* **183:**584–589

Smith, F.W., Ealing, P.M., Hawkesford, M.J., Clarkson, D.T. 1995. Plant members of a family of sulfate transporters reveal functional subtypes. *Proc. Natl. Acad. Sci. USA* **92:**9373–9377

Song, W., Steiner, H.Y., Zhang, L., Naider, F., Stacey, G., Becker, J.M. 1996. Cloning of a second arabidopsis peptide transport gene. *Plant Physiol.* **110:**171–178

Sopanen, T. 1979. Development of peptide transport activity in barley scutellum during germination. *Plant Physiol.* **64:**570–574

Steiner, H.Y., Song, W., Zhang, L., Naider, F., Becker, J.M., Stacey, G. 1994. An *Arabidopsis* peptide transporter is a member of a novel family of membrane transport proteins. *Plant Cell* **6:**1289–1299

Stolz, J., Darnhofer-Demar, B., Sauer, N. 1995. Rapid purification of a functionally active plant sucrose carrier from transgenic yeast using a bacterial biotin acceptor domain. *FEBS Lett.* **377:**167–171

Taglialatella, M., Toro, L., Stefani, E. 1992. Novel voltage clamp to record small, fast currents from ion channels expressed in *Xenopus* oocytes. *Biophys. J.* **61:**78–82

Tanaka, J., Fink, G.R. 1985. The histidine permease gene (HIP1) of *Saccharomyces cerevisiae*. *Gene* **38:**205–214

Tanner, W., Caspari, T. 1996. Membrane transport carriers. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47:**595–626

Thorens, B. 1996. Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. *Am. J. Physiol.* **270:**541–553

Truernit, E., Sauer, N. 1995. The promoter of the *Arabidopsis thaliana* SUC2 sucrose-H^+ symporter gene directs expression of β-glucuronidase to the phloem: Evidence for phloem loading and unloading by SUC2. *Planta* **196:**564–570

Tsay, Y., Schroeder, J.I., Feldmann, K.A., Crawford, N.M. 1993. The herbicide sensitivity gene *CHL*1 of *Arabidopsis* encodes a nitrate-inducible nitrate transporter. *Cell* **72:**705–713

Vandenbol, M., Jauniaux, J.C., Grenson, M. 1990. The *Saccharomyces cerevisiae NPR1* gene required for the activity of ammonia-sensitive amino acid permeases encodes a protein kinase homologue. *Mol. Gen. Genet.* **222:**393–399

Ward, J.M. 1997. Patch-clamping and other molecular approaches for the study of plasma membrane transporters demystified. *Plant Physiol.* **114:**1151–1159

Ward, J.M., Kühn, C., Tegeder, M., Frommer, W.B. 1997. Sucrose transport in plants. *Int. Rev. Cytol.* (*in press*)

Warmbrodt, R.D., Buckhout, T.J., Hitz, W.D. 1989. Localization of a protein, immunologically similar to a sucrose binding protein from developing soybean cotyledons, on the plasma membrane of sieve tube members of spinach leaves. *Planta* **180:**105–115

Weig, A., Komor, E. 1996. An active sucrose carrier (Scr 1) that is predominantly expressed in the seedling of *Ricinus communis* L. *J. Plant Physiol.* **147:**685–690

Wright, E.M., Loo, D.D.F., Panayotova-Heiermann, M., Lostao, M.P., Hirayama, B.H. 1994. 'Active' sugar transport in eukaryotes. *J. Exp. Biol.* **196:**197–212