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Review

Monosaccharide transporters in plants: structure, function and physiology

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Abstract

Monosaccharide transport across the plant plasma membrane plays an important role both in lower and higher plants. Algae can switch between phototrophic and heterotrophic growth and utilize organic compounds, such as monosaccharides as additional or sole carbon sources. Higher plants represent complex mosaics of phototrophic and heterotrophic cells and tissues and depend on the activity of numerous transporters for the correct partitioning of assimilated carbon between their different organs. The cloning of monosaccharide transporter genes and cDNAs identified closely related integral membrane proteins with 12 transmembrane helices exhibiting significant homology to monosaccharide transporters from yeast, bacteria and mammals. Structural analyses performed with several members of this transporter superfamily identified protein domains or even specific amino acid residues putatively involved in substrate binding and specificity. Expression of plant monosaccharide transporter cDNAs in yeast cells and frog oocytes allowed the characterization of substrate specificities and kinetic parameters. Immunohistochemical studies, *in situ* hybridization analyses and studies performed with transgenic plants expressing reporter genes under the control of promoters from specific monosaccharide transporter genes allowed the localization of the transport proteins or revealed the sites of gene expression. Higher plants possess large families of monosaccharide transporter genes and each of the encoded proteins seems to have a specific function often confined to a limited number of cells and regulated both developmentally and by environmental stimuli. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Monosaccharides represent the principle source of carbon and energy in most heterotrophic organisms and the situation is quite similar in plants. In the reactions of the Calvin cycle and gluconeogenesis, photosynthetically fixed CO₂ is converted into mono-

saccharides, such as glucose or fructose, which represent the central units for carbon metabolism, storage and transport. Frequently it is necessary to convert these units temporarily into a 'masked' form that is osmotically less active and/or not easily accessible for the enzymes of the regular cellular metabolism. Such intermediary storage and transport forms of glucose are starch, sucrose and its derivatives (raffinose, verbascose, etc.) and sugar alcohols. All of these compounds are synthesized from glucose or other monosaccharides. They can be stored for long periods and/or transported over long distances

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without being enzymatically attacked, and eventually they are broken down or retransformed into glucose and other monosaccharides.

Many plant cells, tissues or organs represent non-green, heterotrophic tissues (sinks) that depend on the import of organic carbon from photosynthetically active (source) tissues. It has been shown only recently [1,2] that several sinks possess symplastic connections to the phloem allowing the direct access to transported sucrose and other photoassimilates. Other sinks, however, are symplastically isolated and sucrose is delivered from the phloem into the apoplast of these cells or tissues by a postulated sucrose exporter. The corresponding gene or protein has not been identified to date. Unloaded sucrose can be taken up by the sink cells either directly via plasma membrane-localized, sink-specific sucrose transporters [3–6] or via monosaccharide transporters after extracellular sucrose hydrolysis by cell wall-bound invertases [7–9]. Malfunctioning of this final step results in reduced sink growth causing a phenotype similar to that described for plants impaired in phloem loading [10,11]. Carrot plants defective in their cell wall-bound invertase exhibit not only a reduced development of their tap root, but also a feedback accumulation of carbohydrates in their leaves resulting in a drastically increased leaf-to-root ratio [12] and a mutation in an endosperm-specific cell wall invertase from maize causes aberrant endosperm development [13]. These results underline the important role of extracellular sucrose hydrolysis and subsequent monosaccharide transport for plant development.

Over the last 10 years numerous genes encoding monosaccharide transporters have been cloned. The kinetic properties of the encoded proteins have been studied by heterologous expression or by reconstitution into proteoliposomes and the sites of monosaccharide transporter gene expression were analyzed by in situ hybridization and immunohistochemical techniques [14,15]. All of the transporters characterized so far are energy-dependent H^+ -symporters and accept monosaccharides (hexoses and pentoses) forming a pyranose ring. For this reason we refer to these proteins as monosaccharide transporters rather than transporters for glucose or galactose. This paper tries to summarize the currently available information on the kinetic properties of plant monosaccharide trans-

porters, on their physiological function and on the structure/function relationship.

2. Cloning of plant monosaccharide transporters

Utilization of glucose by lower plants has been studied for many years [16–18]. For the unicellular alga *Chlorella vulgaris* (later renamed to *Chlorella kessleri*) it was shown for the first time that this capability is inducible [19,20] and, after detailed kinetic and biochemical analyses of the *Chlorella* monosaccharide transport system [20–23], this inducibility was the basis for the successful cloning of the first plant sugar transporter gene, *CkHUPI*, by differential screening [24]. The deduced amino acid sequence of this monosaccharide- H^+ symporter revealed homology to facilitative monosaccharide transporters from mammals [25,26] and yeast [27,28] and to H^+ -symporters from bacteria [29,30]. No sequence homology was found to the energy dependent, Na^+ -glucose cotransporters from mammals that belong to a clearly independent family of sugar transport proteins [31]. The *CkHUPI* sequence was then used to screen cDNA and genomic libraries from other organisms and the first sequences from higher plants with significant homology to known transporters were obtained from *Arabidopsis thaliana* and tobacco libraries [32,33]. Since then partial or full length sequences of putative or meanwhile characterized plant monosaccharide transporters have been obtained from numerous plants using heterologous screening [34–36] or PCR-based approaches [35,37–40]. Over the last years several sequencing projects contributed to the continuously growing number of plant monosaccharide transporter sequences.

Prior to the molecular cloning of the first plant monosaccharide transporters it was generally accepted that one plant has one transporter for monosaccharides, one transporter for sucrose and one plasma membrane H^+ -ATPase. People used to talk about the monosaccharide carrier from *Chlorella* [21,24] or about the sucrose carrier from sugar beet [41,42]. Molecular biology revealed that things are much more complicated. Detailed analyses showed that even the unicellular green alga *C. kessleri* has three closely related genes encoding the monosac-

charide transporters, CkHUP1 to CkHUP3 [43,44]. All of these genes are transcribed and their expression levels were shown to be regulated by D-glucose and its analogs. The situation is even more complex in higher plants that possess monosaccharide transporter families of at least eight members (*Ricinus communis*) [38], seven members (*Chenopodium rubrum*) [37] or 14 members (*A. thaliana*) [35]. Several genes coding for *Arabidopsis* monosaccharide transporters were identified during the sequencing of *Arabidopsis* ESTs (expressed sequence tags) or genomic sequences and based on the currently available sequence information one may predict that the entire *Arabidopsis* genome may encode up to 20 different monosaccharide transporter genes.

3. Functional analyses and kinetic properties

Plant monosaccharide-H⁺ symporters were used to establish heterologous expression systems, such as yeast and *Xenopus* oocytes, for the functional characterization of foreign transport proteins [32,43,45,46]. It was not clear at the beginning, whether H⁺-symporters could be properly analyzed in yeast cells, which use mostly facilitative transport systems or in frog oocytes that are energized with a Na⁺-gradient across their plasma membranes. However, already the first experiments showed that both systems were perfectly suited for the functional and kinetic characterization of plant monosaccharide-H⁺ symporters. This allowed for the first time the analysis of single plant transport proteins without the overlapping activities of other homologous transporters possibly expressed in the same plant cell or in the same tissue. Comparison of the activities of independently expressed, recombinant CkHUP1, CkHUP2 and CkHUP3 in *Schizosaccharomyces pombe* revealed that the relative transport rates for different substrates, e.g. for D-glucose, D-galactose, D-xylose, D-mannose, D-fructose, etc., differed drastically. CkHUP1 and CkHUP3 preferentially transported D-glucose, whereas CkHUP2 transported D-galactose much better than D-glucose [43,44]. Together these transport activities represented the properties described earlier for the *Chlorella* transporter in planta [21].

Obviously, such analyses are even more important

for higher plants which have larger gene families with overlapping expression profiles of the individual transporter genes (see below). So far the successful characterization of 11 plant monosaccharide transporters by heterologous expression in yeast and/or oocytes has been reported (Table 1). In addition, the CkHUP1 monosaccharide transporter from *Chlorella* and the AtSTP1 transporter from *Arabidopsis* have been purified to homogeneity, reconstituted into proteoliposomes and analyzed in vitro [48,49]. All of the investigated transport proteins were shown to be sensitive to uncouplers of transmembrane proton gradients, to accumulate their substrates inside the cells to concentrations exceeding the extracellular concentrations, or to be directly driven by an increase of the plasma membrane potential, $\Delta\psi$, or the proton gradient, $\Delta\mu\text{H}^+$. This confirmed former results obtained in planta describing plant monosaccharide transporters as H⁺-symporters [14,15].

Homologous transporter genes characterized in *Escherichia coli* or in *Saccharomyces cerevisiae* are usually expressed in response to the availability of certain monosaccharides in the growth medium. These genes encode proteins with relatively narrow substrate specificities, such as the *S. cerevisiae* galactose permease (ScGalp) [27], the *E. coli* xylose permease (EcXylE) [50], *E. coli* arabinose permease (EcAraE) [51] or the *E. coli* galactose permease (EcGalP) [52]. In contrast, the substrate specificities of plant monosaccharide transporters are relatively broad and all of the characterized proteins can transport various hexoses and pentoses (Table 1). The physiological relevance of this wide substrate specificity is unclear.

The kinetic mechanism of monosaccharide-H⁺ symport has been studied in detail for a single plant transporter, AtSTP1 [53], using the patch clamp technique. The obtained results suggest a 'sequential' mechanism for sugar uptake by AtSTP1, i.e. protons and sugar molecules are imported in two ordered, sequential steps. This differs from the mechanism described for the Na⁺-dependent mammalian glucose symporter that is assumed to import its substrates in a 'simultaneous' mechanism [54,55], i.e. substrate (sugar) and cosubstrate (Na⁺) are transported together. The 'sequential' mechanism described for AtSTP1 differs also from results previously obtained

Table 1
Currently available information on plant monosaccharide-H⁺ symporters

Gene name	Refs.	Functionally characterized in	K _M (μM)	Transported substrates	Site of gene expression
<i>Lower plants:</i>					
<i>CkHUP1</i>	[43,44,46,48]	<i>S. pombe</i> , <i>Xenopus</i> Glc > Frc > Man > Xyl > Gal	Glc: 15/46 alga Frc: 392 Man: 136 Xyl: 725 Gal: 3000		
<i>CkHUP2</i> alga	[44]	<i>S. pombe</i>	Gal: 25		Gal > Glc = Xyl ≫ Man
<i>CkHUP3</i>	[44]	<i>S. pombe</i> Glc > Frc > Man > Xyl > Gal	Gal: 900 alga		
<i>Higher plants:</i>					
<i>AtSTP1</i>	[32,45,49,53]	<i>S. pombe</i> , <i>S. cerevisiae</i> , <i>Xenopus</i> , liposomes	Glc: 20	Glc ≫ Gal ≫ Frc	guard cells
<i>AtSTP2</i>	[36]	<i>S. pombe</i>	Gal: 50	Gal > Xyl > Glc = Man	pollen
<i>AtSTP3</i>	[35]	<i>S. pombe</i>	Glc: 2000	Glc > Xyl > Man > Gal	green leaves, stress
<i>AtSTP4</i>	[34]	<i>S. pombe</i>	Glc: 15	Gal > Glc > Xyl = Man	roots, pollen, stress
<i>AtSTP5-14</i>	[35]	–	–	–	–
<i>LeMST1</i>	AJ010942	–	–	–	–
<i>MtST1</i>	[39]	<i>S. cerevisiae</i>	–	Glc > Frc	roots, leaves, stems, mycorrhiza
<i>NtMST1</i>	[33]	<i>S. cerevisiae</i>	–	Glc ≫ Gal = Xyl	roots
<i>PaMST1</i>	Z83829	–	–	–	–
<i>PhPMT1</i>	[40]	–	–	–	pollen
<i>RcHEX1</i>	[38]	–	–	–	hypocotyl, roots, source leaves
<i>RcHEX3</i>	[38]	<i>S. cerevisiae</i>	Glc: 80	Glc	roots, sink leaves
<i>RcHEX6</i>	[38]	–	–	–	–
<i>SspSGT2</i>	[47]	–	–	–	–
<i>VfSTP1</i> embryo	[4]	<i>S. pombe</i>	Glc: 30		Glc > Man > Gal > Frc
<i>VvSTP1</i>	AJ001061	–	–	–	–

with the *Chlorella* monosaccharide transporter CkHUP1 [23,56] that are also in favor with a ‘simultaneous’ transport mechanism.

4. Homology to other sugar transporters

All plant hexose transporters known so far belong to a large superfamily of transmembrane facilitators (MFS, major facilitator superfamily) [31]. Members of the MFS, also called the uniporter–symporter–

antiporter family, have been found in all living organisms and consists of 17 distinct families [57]. The largest of these families is the sugar porter (SP) family, comprising 133 proteins derived from bacteria, archaea, eukaryotic protists, yeasts, animals and plants [57]. Within the SP family the plant monosaccharide transporters show the strongest homology to yeast hexose transporters (ScHxts, ScGal2), to bacterial sugar-H⁺ symporters (EcAraE, EcXylE; EcGalP) and mammalian permeases (GLUTs).

Interestingly, the plant hexose transporters share

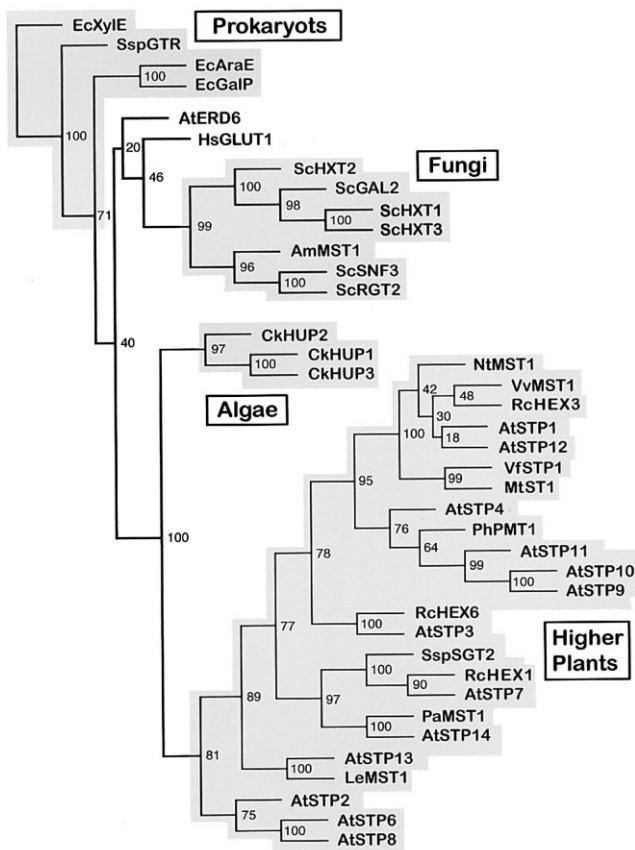


Fig. 1. Phylogenetic tree of plant monosaccharide transporter peptide sequences. Bootstrap values shown at internal nodes indicate the percentage of the occurrence of these nodes in 100 replicates (maximum parsimony) of the data set. The sequence of the *E. coli* xylose permease (EcXylE) was used as outgroup. Accession numbers for non-plant monosaccharide transporter sequences are: EcXylE: P09098; SspGTR: X16472; EcAraE: J03732; EcGalP: AE000377; HsGlut1: 87529; ScHXT1: M82963; ScHXT2: M33270; ScHXT3: L07080; ScSNF3: J03246; ScRGT2: Z74186; AmMST1: Z83828). References or accession numbers for all plant sequences can be obtained from Table 1. Protein sequences were submitted to the BCM Search Launcher (Human Genome Center, Baylor College of Medicine, Houston, TX, <http://kiwi.imgen.bcm.tmc.edu:8088/search-launcher/launcher.html>) for automated multiple alignment using the Clustal W 1.7 program [62] under standard parameters. Maximum parsimony (MP) bootstrap analyses were conducted with the PHYLIP package 3.572c [63]. Addition of taxa was jumbled and repeated three times for each individual bootstrap replication in the MP analysis. Output treefiles were combined with the CONSENSE program and converted into a graphical representation by the Treeview program for Macintosh [64].

no sequence homology with two other families of glucose transport proteins. One is the family of Na^+ -glucose symporters identified in mammals (for review see [58,59]). These Na^+ -driven transporters belong to a separate, small gene family with no homology to plant transporters at either the primary or secondary structural levels. Nevertheless, it is possible that the Na^+ - and H^+ -driven sugar cotransporters share common transport mechanisms ([59], see also above). Glucose uptake systems are also found within the bacterial phosphotransferase family [60,61] that uses a totally different transport mechanism. These transporters show no sequence homology to the plant monosaccharide transporters.

In Fig. 1 a phylogenetic tree is presented using sequences isolated in our lab as well as database-derived sequences to investigate the relationships within the plant monosaccharide transporter family. Obviously, almost all plant monosaccharide transporters form a common cluster (one exception: AtERD6 [65] see below). Within this cluster the three available algal sequences are clearly separated from

all higher plant sequences. Similarly, the included fungal and prokaryotic sequences are grouped separately. The only mammalian sequence within this phylogenetic tree (HsGlut1 from *Homo sapiens*) is not very well separated from these latter groups.

Surprisingly, the *Arabidopsis* AtERD6 protein that is encoded by a dehydration-induced transporter gene [65] with homology to plant monosaccharide transporter genes groups together with the human HsGlut1 facilitator or the bacterial permeases (the bootstrap values at these nodes are below 50 and do therefore not allow a clear assignment). This suggests that, although homologous, AtERD6 is clearly different from the other plant monosaccharide transporters. Unfortunately a clear functional analysis of this protein is still missing. First expression analyses in yeast [65] suggest that AtERD6 may not be a H^+ -symporter for glucose, fructose or xylose. Most strikingly, the 14 *Arabidopsis* AtSTP sequences do not form a clearly separated subgroup but rather are homogeneously distributed throughout the entire higher plant cluster. Within this group the individual, most homologous partner sequences are derived from different plants including monocots (SspSGT2 from sugar cane) [47] and gymnosperms (PaMST1 from *Picea abies*). This suggests that the ancestral organism(s), from which the higher plants branched

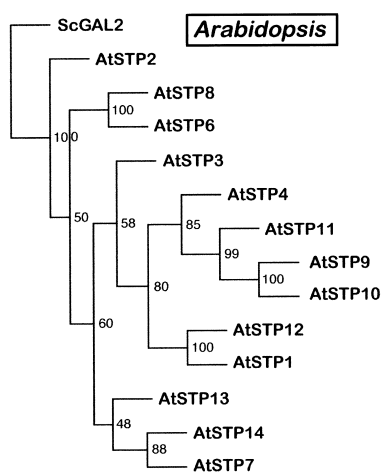


Fig. 2. Phylogenetic tree of *Arabidopsis* monosaccharide transporter peptide sequences. Bootstrap values shown at internal nodes indicate the percentage of the occurrence of these nodes in 100 replicates (maximum parsimony) of the data set. The sequence of the *S. cerevisiae* galactose permease (ScGal2p) was used as outgroup. For additional information see Fig. 1.

off during their evolution, had already several monosaccharide transporter genes for cell type-specific or developmentally regulated monosaccharide transport. This is supported by the finding that already the unicellular *Chlorella* cells possess three different monosaccharide transporter genes (Fig. 1).

Fig. 2 represents a phylogenetic tree of the *Arabidopsis* AtSTP monosaccharide transporter family alone. This tree reveals pairs of highly homologous AtSTP proteins with sequence identities up to 91%. These pairs of transporters, such as AtSTP1 and AtSTP12, AtSTP6 and AtSTP8 or AtSTP9 and AtSTP10, may have evolved by relatively recent gene duplications. Further functional analyses will be necessary to show, if such pairs retained functional similarities above the average.

5. Structure/function

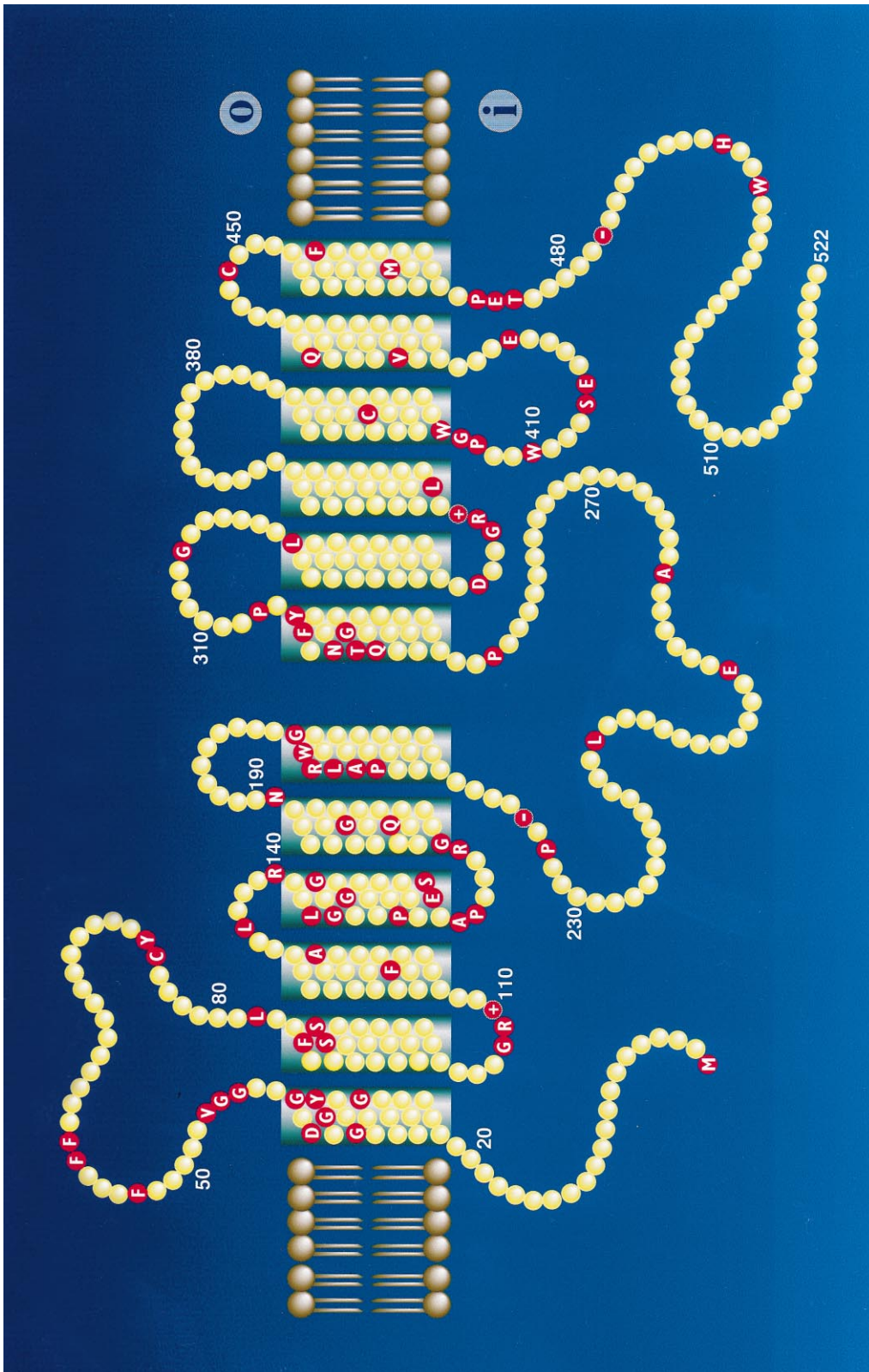
The hydropathy profiles for all plant monosac-

charide transporters are very similar and suggest 12 membrane-spanning domains (Fig. 3), a typical feature of all members of the MFS family [31]. There is considerable homology between the amino-terminal halves and the carboxy-terminal halves of these transport proteins indicating that they may have evolved by gene duplication from an ancestral gene coding for a six-transmembrane helix transporter [31,58,66,67]. This sequence homology is spread throughout the two halves of the protein and it is not possible to identify functionally important domains simply by the occurrence of conserved amino acid residues (Fig. 3).

To date, three-dimensional structural data for sugar transport proteins are not yet available but a number of different approaches using a variety of methods led to the identification of functional important regions/amino acid residues and helped to propose topological models for these proteins. Most of these results were obtained from non-plant transporters [25,29,68], but due to their high degree of sequence similarity with the plant monosaccharide transporters these data will be included in this paper.

The best studied example for sugar transport proteins is the lactose permease from *E. coli*. Using phoA fusion analyses the cytoplasmic localization of the amino and carboxy terminus and the topology of the loops connecting the transmembrane domains was determined [69]. Although the sequence homology of the lactose permease to the plant monosaccharide transporter is not very high the hydropathy profiles of these proteins are very similar. First evidence for a similar topology of the glucose transporters came from studies with the mammalian GLUT1 using peptide specific antibodies [70] and glycosylation scanning mutagenesis [71], which clearly demonstrated that the amino and carboxy termini of this transporter are intracellular and that GLUT1 contains 12 membrane-spanning helices [25]. Immunolocalization with monoclonal antibodies revealed the same topology of both termini also for the plant

Fig. 3. A topological model of the *A. thaliana* monosaccharide transporter AtSTP1 is presented. The positions of the transmembrane helices (the length was set to 21 amino acid residues) were determined by hydrophobicity analyses. Numbers indicate the positions of the respective amino acids in AtSTP1. Conserved amino acid residues in all monosaccharide transporters clustering with the 'algal' or 'higher plant' transporters presented in Fig. 1 (81 residues = 15.5% of all amino acids) as well as conserved negative (2) or positive (2) charges are given in red. The extracellular (o) and the intracellular side (i) of the plasma membrane are indicated.



sucrose- H^+ symporter PmSUC2 [72]. With the increasing number of hexose transporters being isolated from different species the data supporting topological models accumulated over the past few years. Using modified/labeled or inhibitory substrates, site directed and cysteine-scanning mutagenesis hexose transporters from bacteria, yeasts and mammals were extensively studied in order to identify amino acid residues essential for transport function [26,73].

For plant hexose transporters most functional and topological studies were done with the *C. kessleri* CkHUP proteins. Studies with chimeric proteins generated from the glucose specific CkHUP1 and the galactose specific CkHUP2 suggest that the substrate specificity is confined within the amino-terminal halves of these transporters [74] as was shown for the D-arabinitol- H^+ symporter (DalT) and the ribitol- H^+ symporter (RbtT) from *Klebsiella* by the same method [75]. Furthermore, the discrimination between glucose versus galactose in these CkHUP1/CkHUP2 fusion proteins could be narrowed down to a single amino acid residue, asparagine N45 in the first external loop connecting the putative transmembrane helices 1 and 2, by random and site directed mutagenesis [76,77].

However, this was in contrast to the findings for the lactose permease [78] and the yeast hexose transporters Hxt2 and Gal2 [79,80] where the substrate specificity seems to be determined by the carboxy-terminal half. Thus, in algae and yeast and/or in mono and disaccharide transporters the selectivity filters for substrates may either be located at different sites along the translocation path or, and this seems to be more likely, there are two (or more) substrate sites of these proteins involved in substrate recognition and specificity.

The functional expression of the *Chlorella* hexose transporters in *Schizosaccharomyces pombe* allowed further structure/function analyses by screening PCR generated point mutations for the ability to confer resistance to the toxic substrate analogue 2-deoxyglucose [76]. Using this assay, amino acid residues drastically affecting the K_M values could be identified in the first external loop (D44) and in the putative transmembrane helices V (Q179E,N), VII (Q298R, Q299N) and XI (V432L, N436Y) (Fig. 3). Interestingly, D44 which was shown to be essential for transport activity and to alter the pH profile

when replaced by a glutamate residue [77] is conserved in all hexose- H^+ symporters. Moreover, residues Q179 and Q298 are conserved in all hexose transporters further stressing the important role of these amino acid residues in structure and/or function of these transport proteins.

6. Physiological properties

Most higher plants convert the photosynthetically fixed CO_2 into sucrose which then serves as the main form of carbohydrates for long distance transport in the phloem. Certain sinks such as very young, importing leaves [1,2] or root tips [1,81] have direct access to the phloem via numerous large plasmodesmata. Other sinks, however, such as pollen grains and pollen tubes, cells of the anther tapetum, the developing embryo, guard cells are symplastically isolated and can import photoassimilates only with the help of transport proteins in their plasma membranes. Transporters catalyzing the uptake of monosaccharides putatively resulting from extracellular sucrose hydrolysis have been identified in several of these tissues. The *A. thaliana* *AtSTP2* gene is expressed exclusively in pollen grains during a very short period of pollen development [36]. The perfect correlation of *AtSTP2* gene expression with the degradation of callose walls surrounding the pollen tetrads after meiosis suggests that these wall carbohydrates serve as carbon source during early pollen development and that a specific monosaccharide transporter catalyzes the uptake of the resulting glucose units [36]. Pollen-specific expression has also been described for a monosaccharide transporter homolog from *Petunia hybrida*, PhPMT1 [40].

The *Arabidopsis* *AtSTP1* protein has been immunolocalized in the guard cells of various *Arabidopsis* tissues (Stadler and Sauer, unpublished results). Due to the drastic changes in their intracellular osmolarity fully developed guard cells cannot conserve their plasmodesmata, and due to the almost complete lack of Calvin cycle enzymes in guard cell chloroplasts [82,83] these cells depend on the import of carbohydrates from the surrounding apoplast. Expression of the *Arabidopsis* *AtSTP4* gene was found to be specific for root tips and pollen grains [34]. Again these cells or tissues represent heterotrophic sinks. It had

been shown before [81] that carboxyfluorescein, a low molecular weight fluorescent dye that had been loaded into the leaf phloem, can enter several cell layers of the roots of *A. thaliana* by symplastic phloem unloading. However, the very root tips were not accessible to this compound suggesting an apoplastic mechanism for the carbohydrate import into these cells. These cells may depend on the activity of the AtSTP4 transporter. Northern blot analyses suggested that the genes of the tobacco monosaccharide transporter NtMST1 [33] and of the *Medicago truncatula* monosaccharide transporter MtST1 [39] are also most strongly expressed in roots. More detailed analyses of the *MtST1* expression by in situ hybridizations revealed strong expression in the primary phloem fibers and in the region behind the root meristem, most likely the cells of the elongation zone [39].

Expression of plant monosaccharide transporter genes is also regulated by environmental stimuli, such as pathogen infection (*AtSTP4*) [34] or wounding (*AtSTP3* and *AtSTP4*) [34,35]. Analyses of *AtSTP4* expression in elicitor-treated suspension-cultured cells of *Arabidopsis* showed a 50-fold increase compared to untreated control cells [34]. The finding that the expression of a cell wall-bound invertase is also increased in response to stress [7] suggests a close relationship between apoplastic sucrose hydrolysis and monosaccharide uptake during stress response. In fact, treatment of suspension cultured cells of *Chenopodium rubrum* with zeatin resulted in a coordinated increase of both hexose transporter and cell wall invertase mRNA levels [84]. As both, cell wall invertases and monosaccharide transporters stand for sink-specific activities in healthy, unstressed plants, these inductions imply that environmental stresses, such as wounding, infection or elicitor treatment, induce the formation of new sinks.

A two- to four-fold increased expression has also been found for the monosaccharide transporter gene *MtST1* in roots of *Medicago truncatula* after colonization by the mycorrhizal fungi *Glomus versiforme* or *Glomus intraradices* [39]. In colonized roots *MtST1* expression was found not only in phloem fibers and root tips (basal sites of *MtST1* expression; see above), but also in the arbuscule-containing cortical cells. Such an increase in *MtST1* expression was not detected with a *M. truncatula* mutant that terminated

the mycorrhizal association at the appressorial stage. This increase in *MtST1* expression in response to a successful colonization with a mycorrhizal fungus was proposed to be a consequence of the enhanced metabolism of the cortical cells and, thus, as the formation of a new sink [39].

Monosaccharide transport activities have also been reported for mesophyll plasma membranes from source leaves [41,42,85,86]. These cells are photosynthetically active and do certainly not depend on the import of carbohydrates from other tissues. Nevertheless, monosaccharide transporters were also shown to be expressed in green leaves [35,38,39]. It is generally assumed that these transporters play a role in the retrieval of monosaccharides that have been lost from these cells into the apoplast by passive leakage through the plasma membrane.

7. Monosaccharide transporters and sugar sensing

Besides their central role as substrates for carbon metabolism hexoses are important signalling molecules that can alter gene expression levels in lower and higher plants (for reviews see [87–89]). It has been proposed that hexokinases may function as sensors for monosaccharides [90], because *Arabidopsis* plants with increased or decreased hexokinase levels showed a corresponding increase or decrease in their sensitivity towards glucose. This model is supported by the analysis of hexose-mediated gene expression or enzyme activities, where effects were seen only with monosaccharides being substrates for hexokinase, such as mannose or 2-deoxyglucose, but not with 3-*O*-methylglucose or 6-deoxyglucose that are not phosphorylated [91,92]. Other data, however, are not easily explained by a hexokinase-dependent sensing of monosaccharides in the cytoplasm. Whereas the constitutive overexpression of a yeast invertase in the vacuole or apoplast of tobacco plants caused a drastically altered gene expression, bleaching of source leaves and reduced root growth [93,94]. This effect was not detected in tobacco plants expressing the yeast invertase in the cytoplasm [93,94], although hexokinase is a cytosolic enzyme. Moreover, the expression levels of sucrose synthase or apoplastic invertase in *Chenopodium rubrum* were shown to be modulated not only by D-glucose, but also by 6-de-

oxyglucose [8,95], which is not a substrate for the hexokinase. These results could be explained by an extracellular monosaccharide sensor or by monosaccharide sensing during the entry into the cytosol.

To date, direct evidence for a role of plant monosaccharide transporters in sugar sensing is still lacking. In *S. cerevisiae*, however, two plasma membrane-localized hexose transporters, Snf3p and Rgt2p, have been shown to act as glucose sensors [96–98]. Snf3p senses low glucose concentrations, whereas Rgt2p is responsible for the sensing of high glucose concentrations. Both proteins modulate the function of Rgt1p, a protein that functions as activator or repressor of transcription depending on the extracellular glucose concentration [97]. Snf3p and Rgt2p are localized in the yeast plasma membrane and are homologous to the plant monosaccharide transporters (Fig. 1) but possess unusually long C-terminal extensions [96]. Transferring the C-terminal extension of Snf3p to the *S. cerevisiae* monosaccharide transporters Hxt1p or Hxt2p converts these proteins into sensors and restores a mutation in SNF3 [98]. Similar modifications may also allow monosaccharide sensing by transport proteins in plants, but to date such proteins have not been characterized.

8. Conclusions and perspectives

Plants have medium size gene families coding for monosaccharide-H⁺ symporters located in their plasma membranes. Expression of these transporter genes is cell specific and developmentally or environmentally regulated. Most of the so far characterized genes are expressed in cells or tissues that depend on the import of photoassimilates from the green leaves, or their expression is enhanced under conditions of an increased cellular metabolism. This suggests that monosaccharide transporters play an important role for the supply of carbohydrates to non-green, rapidly growing or metabolically hyperactive cells or tissues. Apparently, this is also true for cell wall-bound invertases that hydrolyze sucrose into its component monosaccharides, glucose and fructose, two substrates of the plant monosaccharide transporters. The cooperation of invertases and monosaccharide transporters has been postulated so frequently that

it is generally accepted as a fact. Nevertheless, the potential of a coordinated expression of these two genes in transgenic plants, e.g. for the modulation of sink activity, for an improved stress response or for the formation of new sinks has not been analyzed so far. After the exhausting cloning, characterization and localization work of the last years this will be one of the interesting challenges of the future for applied plant molecular biology.

Hexose transporters are also expected to be found in internal membrane systems, such as in vacuoles or plastids. So far, genes for these transporters have not been identified. A gene product with some homology to the monosaccharide transporters described in this paper has been identified in a vacuolar fraction of sugar beet [99]. However, a functional characterization of this protein has so far not been published.

For basic sciences it will be interesting to understand, why plants possess so many monosaccharide transporter genes. The identification of factors regulating their expression, the precise localization of the encoded proteins and the understanding of their individual roles in plant physiology will not only help to understand the details of carbon allocation in higher plants, but also bring along information about other poorly understood physiological processes, such as seed or pollen development, root growth and growth regulation in general, stomatal opening, plant pathogen interaction, resistance mechanisms, etc.

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