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Minireview

Molecular physiology of higher plant sucrose transporters

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Abstract Sucrose is the primary product of photosynthetic CO_2 fixation that is used for the distribution of assimilated carbon within higher plants. Its partitioning from the site of synthesis to different sites of storage, conversion into other storage compounds or metabolic degradation involves various steps of cell-to-cell movement and transport. Many of these steps occur within symplastic domains, i.e. sucrose moves passively cell-to-cell through plasmodesmata. Some essential steps, however, occur between symplastically isolated cells or tissues. In these cases, sucrose is transiently released into the apoplast and its cell-to-cell transport depends on the activity of plasma membrane-localized, energy dependent, H^+-symporting carrier proteins. This paper reviews the current knowledge of sucrose transporter physiology and molecular biology.
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1. Symplastic movement versus apoplastic transport of sucrose

Due to its stability and due to the limited number of enzymes that catalyze its degradation, the non-reducing disaccharide sucrose is the major form of soluble carbon and energy that is used in higher plants for long-distance transport in the phloem and for long-term storage. In many plants, including the model organisms tobacco (*Nicotiana tabacum* L.; Solanaceae) or Arabidopsis (*Arabidopsis thaliana* L.; Brassicaceae) sucrose is the exclusive or by far dominant form of assimilated carbon that is used for assimilate allocation. In Arabidopsis, for example, sucrose is transported in the phloem together with a small amount of the trisaccharide raffinose [1]. In contrast, plants from other families, such as squash and cucumber (*Cucurbita pepo* L. and *Cucumis sativus* L., Cucurbitaceae [2,3]), mask flower (*Alonsoa meridionalis* O. Kuntze, Scrophulariaceae [4]) or bugleweed (*Ajuga reptans* L., Lamiaceae [5]) have low sucrose concentrations in their phloem and typically high concentrations of raffinose, stachyose and/or verbascose (= raffinose-family oligosaccharides = RFOs). Remarkably, RFOs are synthesized from sucrose within the intermediary cells of the phloem [1]. Finally, species from a third group of families, such as celery (*Apium graveolens* L., Apiaceae [6]), sour cherry (*Prunus cerasus* L., Rosaceae [7]) or common plantain (*Plantago major* L., Plantaginaceae [8]) have sucrose plus polyols in their phloem sap (mannitol or sorbitol), both at similar concentrations. This short overview demonstrates that in all higher plants sucrose plays a slightly different but indispensable role in phloem allocation of assimilated carbon.

Over the last years genes and/or cDNAs for phloem-localized sucrose transporters were cloned from all three groups of plant families, i.e. from species that transport primarily sucrose (e.g. [9,10]), RFOs plus sucrose [11] or sucrose plus polyols (e.g. [6,11]). In RFO plus sucrose transporting plants, the precise role of sucrose transporters in the phloem is still under debate. Typically, these plants have a so-called "open minor vein configuration", i.e. they have numerous functional plasmodesmata connecting the sieve element (SE)–companion cell (CC) complexes and the immediately adjacent cells, and these plants accumulate sucrose in their phloem to concentrations that are only slightly higher than those in the mesophyll (→ symplastic loaders; [4]). In these plants, the main function of phloem sucrose transporters may be the retrieval of sucrose from the apoplast, but inhibitor analyses revealed that at least part of the sucrose loading in these species seems to be carrier-mediated as well [11]. Both other groups of plant families (transport of sucrose plus polyols or transport of sucrose only) possess a so-called "closed minor vein configuration" with only few plasmodesmata between their SE–CC complexes and the adjacent cells, and with far higher sucrose (and polyol) concentrations in their phloem sap than in the mesophyll (= apoplastic loaders). The following paragraph will summarize the multiple roles of sucrose transporters in apoplastic loaders.

2. Phloem-localized sucrose transporters are essential for phloem loading, for maintenance of phloem flux and for sucrose release in apoplastic loaders

Sucrose partitioning between its site of synthesis, the source-leaf mesophyll, and numerous green and non-green sink tissues of an apoplastically loading plant starts in the highly specialized CCs and SEs of the phloem. Membrane-localized, energy-dependent, H^+-symporting sucrose transporters (typically named SUC or SUT proteins for *SUC*ROSE *T*RANSPORTER) are responsible for the loading of sucrose into these cells, for maintaining the high sucrose concentration within the phloem and probably also for the release of sucrose into the apoplast to feed certain sink cells and tissues (= apoplastic sinks, such as guard cells, developing pollen grains, pollen

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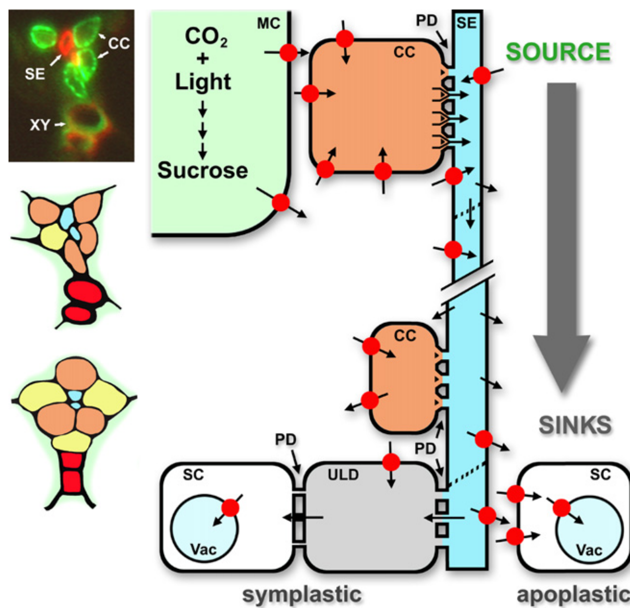

Fig. 1. The path of sucrose from source to sink in an apoplastic loader. The upper left image shows a minor vein from an *Arabidopsis thaliana* source leaf, the main site of sucrose loading into the phloem. Four CCs (green) are fluorescence-labeled with an antiserum against a *Plantago major* sucrose transporter (PmSUC1) that was expressed in the CCs under the control of the *AtSUC2* promoter, two SEs (red) with a fluorescence-labeled anti-SE antiserum (RS6 [36]). The presence of phenolic compounds in the xylem vessels results in yellowish autofluorescence. A schematic drawing of this vein is shown underneath (orange: CC; yellow: parenchyma cell; blue: SE; red: xylem vessels; green: mesophyll). The bottom left drawing shows a minor vein from *Nicotiana tabacum* using the same color-coding. Both, in *Plantago* and tobacco the SEs of minor veins are much smaller than the CCs and are localized in the center of the vein. The image on the right side (same color-coding) shows the path of sucrose from a mesophyll cell into the SE–CC complex and into a symplastic and an apoplastic sink. Sucrose transporters of different cells and membranes are shown as closed circles (red), arrows indicate the direction of sucrose transport. Abbreviations: XY, xylem; SE, sieve element; CC, companion cell; MC, mesophyll cell; PD, plasmodesmata (branched or non-branched); ULD, unloading domain; SC, sink cell; Vac, vacuole.

tubes, the inner integument of the seed coat, or the developing embryo [13]; Fig. 1). Other sinks, however, are connected symplastically to the phloem (= symplastic sinks, such as root tips or the outer integuments of seed coats [13,14]; Fig. 1) by recently discovered, so-called unloading domains (ULDs [13,14]). In these cases, the plasmodesmata between the terminal SEs of the sink phloem and the cells of the ULDs have large size exclusion limits (>60 kDa [13,14]) that allow effective symplastic movement of sucrose into these ULDs. In these cases, phloem-localized sucrose transporters do not seem to be involved and also the subsequent post-phloem transport from the ULDs into the sinks is mediated by plasmodesmata, although with smaller size exclusion limits (∼30 kDa [13–15]).

The different membranes, cells and tissues involved in source-to-sink partitioning of sucrose are shown in Fig. 1. In apoplastic loaders, newly synthesized sucrose is released from the mesophyll into the apoplastic space, where extracellular sucrose concentrations of 2–7 mM were observed [16,17]. This initial step of sucrose partitioning is the least understood and can so far not be attributed to a specific sucrose transporter in any plant species. However, proton-motive force dependent and mesophyll-specific sucrose uptake was shown in leaf plasma membrane vesicles of potato wild type plants [18], and detailed patch clamp analyses demonstrated that the direction of transport by a sucrose carrier may be reversed depending on the direction of the sucrose gradient, the pH or the transmembrane potential [19].

In contrast, the next step, i.e. the loading of sucrose into the SE–CC complex, has been characterized in detail and responsible transporters were identified in several plant species [11,20–25]. First evidence for the important role of these proteins in phloem loading came from promoter/reporter gene analyses and from in situ hybridizations that showed expression of the respective sucrose transporter genes only in the vascular tissue of source leaves and not in sink leaves [26–28]. During sink-to-source transition expression of these genes starts at the tip of the transition leaves and proceeds towards the base of the eventually fully developed leaves [27]. Definite proof for the expected and essential role of these transporters in phloem loading was obtained with transgenic potato plants that expressed the cDNA of the potato phloem loader *StSUT1* in antisense [20]. This result was later confirmed in tobacco plants expressing *NtSUT1* in antisense [24] and in Arabidopsis plants that carried a T-DNA insertion in the *AtSUC2* gene [29]. In all of these plants, export of sucrose from source leaves was partially or completely blocked, and consequently these plants accumulated increased levels of soluble carbohydrates and starch in their source leaves. The reduced carbon export led to chlorotic lesions in the leaves, to inhibition of sink development and consequently to reduced plant growth.

Unexpectedly, promoter/reporter gene analyses as well as immunolocalization studies showed expression of these transporter genes and identified the corresponding proteins also in the phloem of stems, roots and several non-green sink tissues [27,30]. This suggested (i) that phloem-loading sucrose transporters may also be responsible for the retrieval of sucrose from the extracellular space and (ii) that they may even catalyze the export of sucrose from the phloem [27,30]. As mentioned above, patch clamp analyses with *Xenopus laevis* oocytes expressing the cRNA of the maize sucrose transporter ZmSUT1 [19] demonstrated that in principle sucrose transporters can mediate both, sucrose uptake and release.

3. Most sucrose transporter genes are expressed in sink tissues where the encoded proteins may have different functions

In apoplastic sinks (Fig. 1), sucrose that has been released into the extracellular space can (i) be split by cell wall-bound invertases into glucose and fructose for further uptake by monosaccharide transporters or it may be taken up by sink specific sucrose transporters (Fig. 1). In fact, there is evidence that plants are able to choose between these two options depending on the quality of the respective sink tissue. During seed development in fava bean (*Vicia faba* L.) both, a monosaccharide transporter gene (*VfSTP1*) and a sucrose transporter gene (*VfSUT1*) are expressed in the epidermis of the embryo, however, at different developmental stages [31]. While *VfSTP1* is expressed at the pre-storage phase (together with a cell wall invertase gene), when the underlying tissue is still mitotically active, *VfSUT1* is expressed during the following storage phase [31]. This suggests that uptake of monosaccharides produced by extracellular sucrose hydrolysis is the pre-

ferred mechanism for rapidly growing cells, whereas uptake of sucrose may be preferred by non-dividing storage sinks.

Sink-specific expression of sucrose transporter genes has also been observed in numerous other sink organs of different plant species. Examples are *AtSUC1* from Arabidopsis [32], which is expressed in pollen tubes, in funiculi, in the placenta and in the anther connective tissue. The observation that the expression in the connective tissue occurred only during the latest stages of anther development was discussed as a possible role of AtSUC1 in the control of anther dehiscence, which may be mediated by modulating the water potentials in specific cell layers via sucrose accumulation [32]. Another sucrose transporter gene that is expressed in pollen grains and pollen tubes was published for common plantain (*PmSUC1* [33]). This gene is also active in the innermost cell layer of the inner integument, and since this cell layer plays an important role in embryo nutrition, it was discussed that PmSUC1 might catalyze the release of sucrose from the integument towards the growing embryo. Finally, *NtSUT3* from tobacco [34] seems to be expressed exclusively in pollen and pollen tubes. Obviously, all three proteins (AtSUC1, PmSUC1 and NtSUT3) do have a function in pollen tube growth, however, their precise role in pollen tubes is not clear. Of course, sucrose may be an important carbon source for these cells, but it may also have a signaling function for the growing tubes on their way to the egg cell. A complex role of sucrose transport proteins in growing pollen tubes is further supported by the observation that in all plants analyzed [Plantago, Arabidopsis and tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.)] a second sucrose transporter gene is co-expressed in pollen tubes. These additional genes (*PmSUC3* [35], *AtSUC3* [36], *LeSUT2* [37]) encode slightly different sucrose transport proteins that form an independent group in the sucrose transporter phylogenetic tree (see below and Fig. 2).

Sink-specific sucrose transporters were also identified in grape berries (*Vitis vinifera* L., VvSUC11 and VvSUC12 [38]), in the storage parenchyma of sugar cane (*Saccharum hybridum*, ShSUT1 [39]), in the seeds of barley (*Hordeum vulgare* L., [40]), in Arabidopsis flowers (AtSUC9 [41]), in the embryo suspensor (AtSUC3 [13]), or in the endosperm (AtSUC5

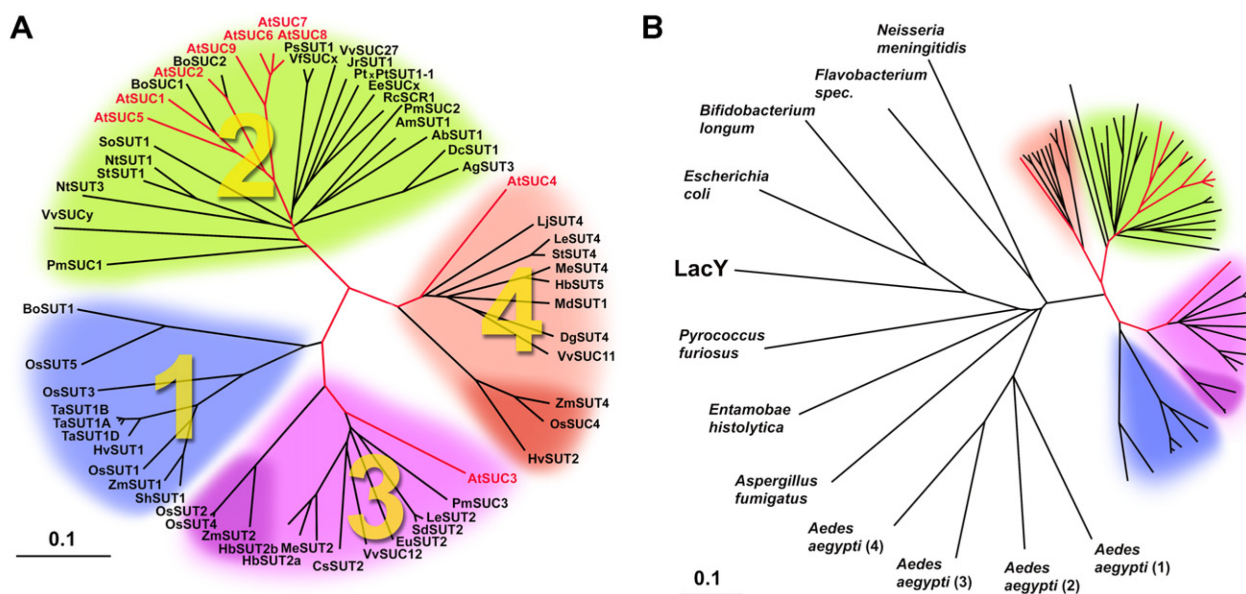

Fig. 2. Phylogenetic trees for confirmed or predicted sucrose transporter sequences from publicly accessible databases. (A) Phylogenetic tree for 62 plant sucrose transporter sequences. The tree reveals four separate groups that are explained and discussed in the text. Accession numbers of presented sucrose transporter sequences are: AbSUT1 (*Asarina barclaiana*; AAF04294), AgSUT3 (*Apium graveolens*; ABB89051), AmSUT1 (*Alonsoa meridionalis*; AAF04295), AtSUC1 (At1g71880), AtSUC2 (At1g22710), AtSUC3 (At2g02860), AtSUC4 (At1g09960), AtSUC5 (At1g71890), AtSUC6 (At5g43610), AtSUC7 (At1g66570), AtSUC8 (At2g14670), AtSUC9 (At5g06170), BoSUC1 (*Brassica oleracea*; AAL58071), BoSUC2 (*B. oleracea*; AAL58072), BoSUT1 (*Bambusa oldhamii*; AAY43226), CsSUT2 (*Citrus sinensis*; AAM29153), DgSUT4 (*Datisca glomerata*; CAG70682), DcSUT1 (*Daucus carota*; BAA89458), EeSUCx (*Euphorbia esula*; AAF65765), EuSUT2 (*Eucommia ulmoides*; AAX49396), HbSUT2a (*Hevea brasiliensis*; ABJ51934), HbSUT2b (*H. brasiliensis*; ABJ51932), HbSUT5 (*H. brasiliensis*; ABK60189), HvSUT1 (*Hordeum vulgare*; CAB75882), HvSUT2 (*H. vulgare*; CAB75881), JrSUT1 (*Juglans regia*; AAU11810), LeSUT2 (*Lycopersicum esculentum*; AAG12987), LeSUT4 (*L. esculentum*; AAG09270), LjSUT4 (*Lotus japonicus*; CAD61275), MdSUT1 (*Malus × domestica*; AAR17700), MeSUT2 (*Manihot esculenta*; ABA08445), MeSUT4 (*M. esculenta*; ABA08443), NtSUT1 (*Nicotiana tabacum*; X82276), NtSUT3 (*N. tabacum*; AAD34610), OsSUT1 (*Oryza sativa*; AAF90181), OsSUT2 (*O. sativa*; AAN15219), OsSUT3 (*O. sativa*; BAB68368), OsSUT4 (*O. sativa*; BAC67164), OsSUC4 (*O. sativa*; Q2QLI1), OsSUT5 (*O. sativa*; BAC67165), PmSUC1 (*Plantago major*; CAI59556), PmSUC2 (*P. major*; X75764), PmSUC3 (*P. major*; CAD58887), Pt × PtSUT1-1 (*Populus tremula × Populus tremuloides*; CAJ33718), PsSUT1 (*Pisum sativum*; AAD41024), RcSCR1 (*Ricinus communis*; CAA83436), SdSUT2 (*Solanum demissum*; AAT40489), ShSUT1 (*Saccharum hybridum*; AAV41028), SoSUT1 (*Spinacea oleracea*; Q03411), StSUT1 (*Solanum tuberosum*; CAA48915), StSUT4 (*S. tuberosum*; AAG25923), TaSUT1A (*Triticum aestivum*; AAM13408), TaSUT1B (*T. aestivum*; AAM13409), TaSUT1D (*T. aestivum*; AAM13410), VfSUCx (*Vicia faba*; CAB07811), VvSUCy (*Vitis vinifera*; AAL32020), VvSUC11 (*V. vinifera*; AAF08329), VvSUC12 (*V. vinifera*; AAF08330), VvSUC27 (*V. vinifera*; AAF08331), ZmSUT1 (*Zea mays*; BAA83501), ZmSUT2 (*Z. mays*; AAS91375), ZmSUT4 (*Z. mays*; AAT51689). **B** small version of the phylogenetic tree shown in **A** containing additional 11 sucrose transporter sequences from bacteria, archaea, fungi and insects plus the *Escherichia coli* lactose permease (LacY). Additional accession numbers are: *Aedes aegypti* (1): EAT35756; *A. aegypti* (2): EAT36400; *A. aegypti* (3): EAT45069; *A. aegypti* (4): EAT36401; *Aspergillus fumigatus*: EAL92728; *Bifidobacterium longum*: NP_695335; *E. coli*: BAB36664; *Entamoeba histolytica*: EAL50988; *Flavobacterium*spec.: EAS20391; *Neisseria meningitides*: NP_273437; *Pyrococcus furiosus*: AAL82126; LacY: AAN78937. Protein sequences were aligned and trees were calculated using the programs CLUSTAL [71] and TREEVIEW [72]. Bars indicate the evolutionary distance.

[42]). Finally, a sucrose transporter gene (*JrSUT1*) has been identified that is expressed in the xylem of walnut (*Juglans regia* L.), where the JrSUT1 protein is thought to be responsible for sucrose transfer from xylem vessels to parenchyma cells during winter time [43].

Mutant analyses of sink-localized sucrose transporters revealed important insights into their possible physiological role in the respective tissues. For example, three allelic mutants of the endosperm-specific Arabidopsis sucrose transporter AtSUC5 were isolated and the effects of these *Atsuc5* mutations on the fatty acid composition in the endosperm and on embryo development were studied [42]. In the *Atsuc5-1* and *Atsuc5-2* mutant lines no *AtSUC5* mRNA could be detected, and compared to *AtSUC5* wild type plants both lines showed a transiently but strongly altered fatty compositions in their endosperm 8 days after fertilization. The concentrations of oleic (18:1) and icosenic (20:1) acid were reduced by a factor of two, whereas palmitic (16:0), linolic (18:2) and linolenic (18:3) acid concentrations were slightly increased (20–30% [42]). Since fatty acid biosynthesis depends on the biotinylation of several proteins and since it has been shown that AtSUC5 mediates the transport of biotin in addition to sucrose (see below and [44]), it is not clear, whether these changes result from a reduced supply of the endosperm tissue with biotin (vitamin and cofactor) or with sucrose (carbon source). Moreover, the defect in the *AtSUC5* gene is associated with a slight delay in embryo development.

In a different approach, tomato plants with reduced expression of *LeSUT2* were analyzed [37]. This sucrose transporter gene is expressed in different floral organs and the protein was also immunolocalized in tomato SEs. Expression of *LeSUT2* mRNA in antisense orientation under the control of the *35S* promoter resulted in fruits with a strongly reduced seed number and in reduced fruit size [37] and was interpreted (i) as evidence for a possible role of LeSUT2 in the release of sucrose from the SEs or (ii) in sucrose import into developing tomato fruits.

Depending on the type of sink tissue, i.e. metabolically active and fast growing sink or fully developed storage sink, sucrose can (i) be degraded and used as energy or carbon source, (ii) may be converted into osmotically inactive storage compounds or (iii) be transported by tonoplast-localized sucrose transporters into the vacuolar lumen (Fig. 1). A first hint towards the identity of tonoplast-localized transporters came from analyses of the tonoplast proteome of barley [45], where the sucrose transporter HvSUT2 [40] was identified. Transient expression analyses of an *HvSUT2* fusion to the open reading frame of the green fluorescent protein (*GFP*) confirmed the targeting of this sucrose transporter to the tonoplast. The identical result was obtained for its Arabidopsis homolog, AtSUC4, in similar analyses. This suggests that tonoplast localization may be a common property of all HvSUT2/AtSUC4-related sucrose transporters that form an independent subgroup within the sucrose transporter phylogenetic tree (see below and Fig. 2).

4. Do different sucrose transporters interact to regulate phloem loading?

As discussed above, immunolocalization and in situ hybridization studies as well as promoter/reporter gene analyses of the different sucrose transporters of single plant species mostly identified the respective proteins or promoter activities in well defined cells or tissues suggesting specific functions in the loading, retrieval or release of sucrose. In solanaceous species, such as tomato, potato or tobacco, however, most or all sucrose transporters were localized exclusively or additionally to the SEs [23,30,37,46,47]. This led to the hypothesis that the different solanaceous transporters, SUT1, SUT2 and SUT4, which were all described as plasma membrane localized transporters, may interact physically to adopt the phloem loading activity of solanaceous SEs to different developmental or environmental conditions. The central idea of this hypothesis was that one of these proteins, SUT2, might act as sucrose sensor [46] that can interact with a high affinity/low capacity transporter, SUT1 [9], and/or with a low affinity/high capacity transporter, SUT4 [47], and regulate the relative activities of these two proteins [47]. In fact, analyses of potential interactions with the split-ubiquitin system in yeast cells supported this idea [48].

Similar co-localization analyses were also performed with the Arabidopsis sucrose transporters AtSUC2, AtSUC3 and AtSUC4 and a capacity to interact was shown for all three transporters and the possible physiological role of such interactions was discussed [49]. However, the plasma membrane sucrose transporters AtSUC2 and AtSUC3 are not co-localized in the same cells (AtSUC2 is in CCs [22] and AtSUC3 in SEs [36]), and the recent finding that AtSUC4 is a tonoplast protein [45] shows that at least the interaction of the *Arabidopsis* transporters is likely to be restricted to the yeast system, where AtSUC4 does not seem to be correctly targeted.

5. Functional characterization: plant sucrose transporters are electrogenic and accept several other substrates besides sucrose

First experimental evidence that higher plant sucrose transport may be electrogenic and depend on protons as co-substrates was obtained 30 years ago in analyses with intact plant tissue [50,51]. However, only after the cloning of the first sucrose transporter cDNAs and the expression of individual cDNAs in bakers' yeast (*Saccharomyces cerevisiae*) it was possible to determine the kinetic properties of individual sucrose transporters. Phloem-loading sucrose transporters, for example, have pH-optima for sucrose uptake at low pH-values, they are sensitive to uncouplers of proton gradients, are inhibited by SH-group inhibitors [e.g. *p*-chloro-mercuriphenylsulphonic acid (PCMPS)] and their K_m-values are in the range of 1–1.5 mM [9–12]. Several of these characteristics supported the idea of proton and membrane potential-driven sucrose uptake, but definite proof was obtained only in analyses of sucrose-induced proton currents in *Xenopus* oocytes. These analyses demonstrated that sucrose and protons are co-transported in a 1:1 stoichiometry, provided detailed information on the affinity of sucrose transporters to protons, and helped to understand the complex interplay of membrane potential, pH-gradient, substrate concentration and the impact of these parameters on the direction of sucrose transport [19,52,53].

Initial analyses of sucrose transporters with respect to their substrate specificities were based mainly on inhibitory effects of candidate substrates on the uptake of ^{14}C-labelled sucrose [9,10,12]. These analyses suggested that maltose might also

be a substrate for plant sucrose transporters and that in general α-glucosides (like sucrose or maltose) and β-glucosides may be substrates accepted as substrates. No evidence was obtained for transport of the trisaccharide raffinose. Additional analyses in *Xenopus* oocytes confirmed these analyses and revealed induction of proton currents also for other naturally occurring glucosides, such as arbutin, an aryl-β-glucoside found for example in coffee plants (*Coffea arabica* L.), and salicin, a saligenine-β-glucoside (a salicylic acid derivative) frequently found in willow (*Salix* spec. [54,55]) or the synthetic sweetener sucralose, a chlorinated sucrose derivative [41].

It came as a surprise, when during a complementation screening for plant biotin transporters in a biotin uptake-defective yeast mutant a cDNA for the Arabidopsis sucrose transporter AtSUC5 was identified [44]. Detailed analyses confirmed that AtSUC5 was in fact able to catalyze uptake of biotin across yeast plasma membranes. Moreover, these analyses revealed that biotin transport is not inhibited by sucrose and that biotin uptake may be a general property of all plant sucrose transporters [44]. The fact that other complementing cDNAs could not be found in this screening suggested that sucrose transporter-mediated transport of biotin may be physiologically relevant.

6. All plants have small families of sucrose transporter genes

In all plants analyzed, small families of sucrose transporter genes were found, e.g. in rice (*Oryza sativa* L.) [57], Arabidopsis [58], grapevine [38] or tomato [37]. The Arabidopsis genome has 9 sucrose transporter-like sequences: *AtSUC1* to *AtSUC9*. Based on analyses of the splice patterns and of nucleotide polymorphisms in different ecotypes, two of these sequences, *AtSUC6* and *AtSUC7*, were characterized as pseudogenes [58], whereas seven sequences encode functional Arabidopsis sucrose transporters. Seven of the nine Arabidopsis SUC sequences share roughly 75% identical amino acids. The two remaining proteins, AtSUC3 and AtSUC4, show only about 50% identity (a) with the other seven and (b) with each other. This was a first indication that Arabidopsis might have three different subtypes of sucrose transporters.

The phylogenetic tree shown in Fig. 2A was calculated for 62 plant sucrose transporters, for which full-length protein sequences were available in public databases. Branches indicating the positions of the nine Arabidopsis sequences are highlighted in red. The 60 sequences fall into four clearly separable groups: Group 1 (blue) contains only sequences from monocot species, whereas group 2 (green) contains only sequences from dicot plants. In contrast, groups 3 (pink) and 4 (red) contain sequences from both, dicot (lighter areas) and monocot plants (darker areas).

Of the monocot sucrose transporters of group 1 so far five proteins were functionally characterized as sucrose transporters (HvSUT1 and HvSUT2 [40], OsSUT1 [59], ShSUT1 [56] and ZmSUT1 [19]). *OsSUT1* was shown to be expressed in CCs [60] and both, OsSUT1 and ZmSUT1, are thought to be responsible for phloem loading [60,61]. For both of these proteins and also for other members of group 1 (e.g. for the wheat transporters TaSUT1A, B and D [62] or the barley transporters HvSUT1 and HvSUT2 [40]) additional functions were suggested or shown in different sink tissues, for example, sucrose import into developing grains. Thus, group 1 seems to represent a group of plasma membrane-localized monocot sucrose transporters that catalyze primarily the uptake of sucrose into phloem and sink cells.

Group 2 represents plasma membrane-localized sucrose transporters from dicots. As in group 1, the functionally characterized and in planta localized sucrose transporters of this group are responsible for phloem loading or for sucrose import into different sink tissues (discussed in detail above). Interestingly, gene duplications seem to have increased the number of group 2 genes in several plant species. Two or more group 2 genes were found in Arabidopsis, common plantain, grapevine, rape (*Brassica napus* L.) and tobacco. Gene duplications took place also in group 1 that contains two clearly different sucrose transporters from rice (OsSUT1 and OsSUT5). In contrast, the extremely similar sucrose transporters from wheat (*Triticum aestivum* L.; TaSUT1A, TaSUT1B and TaSUT1D) are likely to reflect the progenitor genomes of the hexaploid wheat cultivar used to clone these sequences [62]. The gene duplications most likely allowed an optimal adaptation of parameters, such as transcriptional regulation of the genes or pH-dependence and K_m-values of the proteins to the specific requirements of sucrose transporters in phloem CCs or in specific sink tissues.

Transporters of group 3 are easily distinguishable from the transporters of all other groups for several reasons. Firstly, with a total length of roughly 600 amino acids, all group 3-type sucrose transporters (frequently named SUC3/SUT2-type transporters, based on the names of the first characterized transporters of this group: AtSUC3 and LeSUT2) have about 15–20% more amino acids than all other sucrose transporters. Secondly, compared to the transporters of the other groups, group 3-type transporters have clearly higher K_m-values for sucrose uptake. Characterized group 3-type transporters were localized to the SEs in several plant species [35,36,46]. However, these transporters were also found in sink tissues and especially in sink-leaf SEs, where they were discussed as potential efflux carriers that might be involved in phloem unloading into the apoplast [35,37]. Initially, group 3-type transporters were published as sucrose sensors with no transport activity [46], an interpretation that does not seem to be valid any longer.

Transporters of group 4 were originally characterized or at least discussed as plasma membrane localized sucrose transporters (StSUT4 and AtSUC4 [47], HvSUT2 [40]). For StSUT4 and AtSUC4 (also named AtSUT4) this was deduced from functional analyses in the bakers' yeast expression system, where these transporters were targeted to the plasma membrane and catalyzed sucrose uptake from the medium [47]. Like most other transporters from solanaceous plants, StSUT4 was localized to the SEs and was, therefore, published as high capacity transporter of phloem minor veins [47]. However, the recent finding that AtSUC4 and HvSUT2 are tonoplast-localized sucrose transporters [45] puts this interpretation into perspective. Moreover, the localization of group 4-type proteins in SEs is astonishing, as SE do not possess vacuoles. The localization of AtSUC4 and HvSUT2 in the tonoplast [45] and the high levels of *LeSUT4* mRNA in sink tissues suggest, therefore, that group 4-type transporters may represent vacuolar sucrose transporters that are primarily expressed in sink tissues.

In contrast to groups 1 and 2, no gene duplication events seem to have occurred within the groups 3 and 4. Arabidopsis has only a single gene in each of these groups (AtSUC3 in group 3 and AtSUC4 in group 4) and also for most other plant species only one group 3-type or group 4-type transporter has been found so far. An exception is rice with two almost identical sequences in group 4 (OsSUT2 and OsSUT4). These two genes, however, are likely to reflect the polyploidy if rice plants.

Plant sucrose transporters belong to the major facilitator superfamily of transport proteins [63]. At present, hundreds of sequences for transporters representing members of this superfamily can be found in public databases, including sequences from archaea, bacteria, fungi, animals and plants. Database searches with plant sucrose transporter sequences identified accessions that were deposited as sucrose transporter sequences from bacteria, archaea, fungi and insects. Fig. 2B shows a phylogenetic tree that was calculated with the 60 plant sucrose transporters from Fig. 2A, with 11 non-plant sucrose transporter sequences and with the sequence of the lactose permease from *Escherichia coli* (LacY). The plant sucrose transporters form a compact and clearly separated group.

7. Structural analyses of plant sucrose transporters

Independently performed hydropathy analyses with different plant sucrose transporter sequences predicted 12 transmembrane helices for all analyzed proteins (e.g. [9,10]). Immunolocalization studies with site-specific antibodies confirmed the predicted even number of transmembrane helices and demonstrated that N- and C-termini of plasma membrane SUC proteins are on the cytoplasmic side of the membrane [64]. Alignments of available plant sucrose transporter sequences from all four groups shown in Fig. 2A with sequences of other H^+-sugar symporters and alignments of the first and second halves of these sucrose transport proteins supported (i) the membership of plant sucrose transporters in the major facilitator superfamily and (ii) the hypothesis that the members of this superfamily evolved from one or more ancestral transporters with only six transmembrane helices by gene duplication and fusion [63]. Moreover, these sequence analyses allowed to localize variable regions between the transporters of the four groups shown in Fig. 2A and to assign these differences to specific sites in the proteins.

This information was used to draw and compare 2-D models of sucrose transporters of the four different groups (Fig. 3). The most obvious differences were detected in the N-terminal, and C-terminal domains of the transporters and in the loops connecting the predicted transmembrane helices VI and VII (central cytoplasmic loop) or VII and VIII (predicted to face the extracellular space in plasma membrane-localized transporters and the vacuolar lumen in transporters of the tonoplast). The already mentioned 15–20% higher number of amino acids in group 3-type transporters can be attributed almost completely to an elongated N-terminus (about 20 amino acids) and to an enlarged cytoplasmic loop (about 60 amino acids). Moreover, the C-termini of these group 3-type transporters are slightly shorter than those of group 1-type or group 2-type transporters. The predicted vacuolar transporters of group 4 have the shortest C-terminal ends and a very short linker sequence between the transmembrane helices VII and VIII. Therefore, group 4-type transporters usually have the shortest peptide sequence of all sucrose transporter family members of a given plant species.

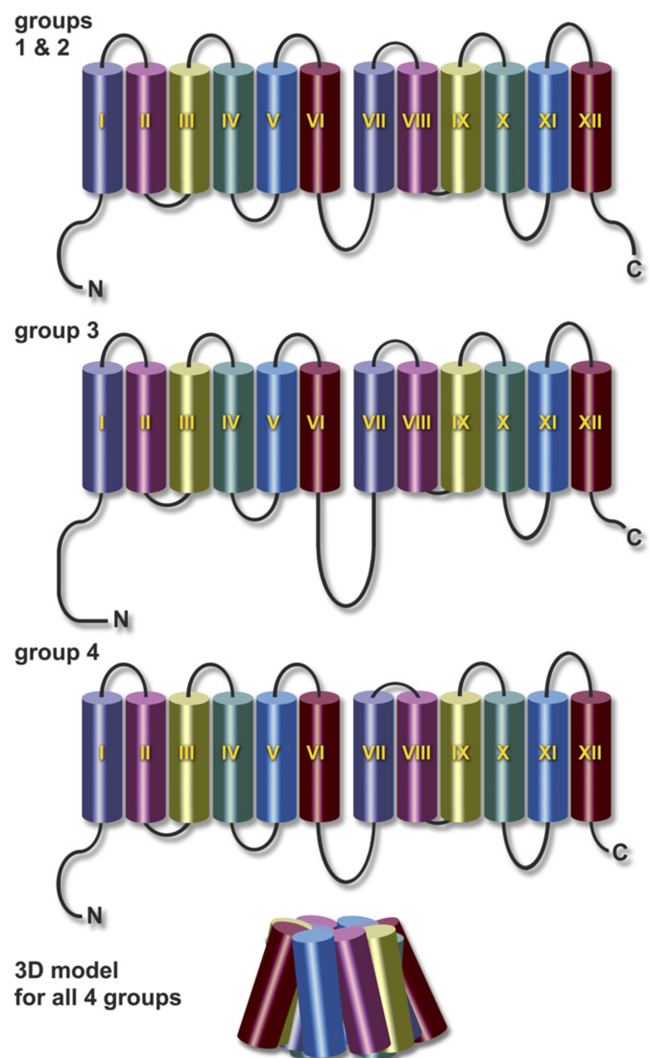

Fig. 3. Two-dimensional models for sucrose transporters belonging to the different subgroups shown in Fig. 2. The top model gives the structure for group 1-type (dicot) and group 2-type (monocot) sucrose transporters of plasma membranes. The second model shows the predicted 2D-structure for group 3-type sucrose transporters of plasma membranes, and the bottom model for tonoplast sucrose transporters of the group 4-type. For plasma membrane-localized transporters, N- and C-termini were shown to be on the cytoplasmic side; for tonoplast transporters this is only predicted. The transmembrane helices are shown in different colors. Identical colors were used for the respective transmembrane helices of the first and the second halves to highlight intra-molecular sequence conservations. The main differences between sucrose transporters of the different groups are outside the predicted membrane spanning regions. The 3D-arrangement of the transmembrane helices was drawn in analogy to the LacY crystal structure.

The similarity of available crystal structures from other members of the major facilitator superfamily (LacY [65] and GlpT [66]) allows to suggest a model showing how the transmembrane helices of plant sucrose transporters could be arranged in the plasma membrane or tonoplast. From the published LacY and GlpT structures it can be deduced that

the transmembrane helices I, IV, VII and X form an hourglass-like structure that shapes the substrate pore that is surrounded by the other eight helices. Assuming that plant sucrose transporters may have a related structure, especially that the same transmembrane helices are involved in pore formation, a crude ''working model'' for further structural analyses can be drawn (Fig. 3 bottom).

8. Regulation of sucrose transport in higher plants

The multiple roles of plant sucrose transporters and especially the central role of sucrose loading into the phloem suggest that sucrose transport may be tightly regulated. In fact, expression of sucrose transporters involved in phloem loading was shown to be developmentally regulated in leaves with young sink leaves showing no expression of *SUC* genes, whereas strong expression is seen in vascular bundles of mature source leaves [27,67]. During this transition from sink to source, expression of *SUC* genes is initiated at the tip and proceeds towards the base of the developing leaf [27,67]. Interestingly, this basipetally progressing *SUC* gene expression does not only depend on the developmental status of the veins, but also on other factors. Partial shading of transition leaves, for example, resulted in an almost complete lack of sucrose transporter gene expression in the minor veins of shaded areas, although the minor veins in these regions were structurally fully developed [67]. Light (or one or several products of photosynthetic CO_2 fixation) was also shown to play an important role for the expression of *LeSUT1* and *StSUT1*, which show reduced expression and reduced protein levels in the dark [23]. Finally, sucrose transporters may be regulated by phytohormones, and stimulatory effects of abscissic acid (ABA) and other hormones on active sucrose transport were described already years ago [68]. However, the direct connection between hormone action and transporter gene expression has so far not been detected.

9. Outlook

Only recently it was published that the maize *tie-died1* mutant (*tdy1*) accumulates sucrose and starch in yellow leaf sectors [69]. These sectors form only, if the plants are exposed to high light during early stages of leaf development. This observed accumulation of carbohydrates is reminiscent of the phenotype observed in dicot plants, where sucrose transporter gene expression is reduced due to T-DNA insertions or antisense inhibition [18,29]. It has been speculated that TDY1 may control induction of sucrose transporter genes that are responsible for high capacity phloem loading [69]. However, direct evidence for a role of TDY1 in sucrose transporter gene regulation is still missing and other explanations may be possible. Nevertheless, this paper touches an important point. At present, very little is known about the regulation of sucrose transport proteins and about the regulation of sucrose transporter gene expression. An important and interesting observation was published only recently for the *Arabidopsis AtSUC9* gene that seems to possess intron sequences that are involved in regulation of gene expression [41]. For a better understanding of such observations or of processes regulating e.g. the sink-to-source transition of a leaf and the concomitant induction of phloem loading it will be essential to understand the underlying regulatory mechanisms.

Moreover, analyses of the *Arabidopsis* phosphoproteome identified potentially phosphorylated residues in the *Arabidopsis* sucrose transporter AtSUC5 [70], however, direct evidence for phosphorylation of AtSUC5 or another sucrose transporter *in planta* or for an interaction with regulatory proteins, e.g. with 14-3-3 proteins, is lacking. Other open questions like the release of sucrose from the mesophyll or controversial results like the lack of CC-specific transporters in Solanaceae have been mentioned in the text. To find answers to these problems will be one of the major challenges in the plant sucrose transporter field for the next years.

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