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Review

Sucrose transporters in plants: update on function and structure

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Abstract

In plants, sucrose is the major transport form for photoassimilated carbon and is both a source of carbon skeletons and energy for plant organs unable to perform photosynthesis (sink organs). As a molecule translocated over distance, sucrose has to pass through a number of membranes. Membrane transport of sucrose has therefore been considered for a long time as a major determinant of plant productivity. After several decades of physiological and biochemical experiments measuring the activity of sucrose carriers, unequivocal evidence came from the first identification of a cDNA coding a sucrose carrier (*SoSUT1*, Riesmeier et al. (1992) EMBO J. 11, 4705–4713). At present 20 different cDNAs encoding sucrose carriers have been identified in different plant species, in both dicots and monocots (one case). The total number is increasing rapidly and most importantly, it can be guessed from the results obtained for *Arabidopsis*, that in each species, sucrose transporters represent a gene family. The sequences are highly conserved and those carriers display the typical 12 transmembrane α-helices of members of the Major Facilitator superfamily. Yeast expression of those carriers indicate that they are all influx carriers, all cotransport sucrose and proton and that their affinity for sucrose is surprisingly similar (0.2–2 mM). All their characteristics are in agreement with those demonstrated at the physiological level in plants. These characteristics are discussed in relation to the function in plants and the few data available on the structure of those transporters in relation to their function are presented.

Keywords: Plant; Membrane transport; Sucrose transporter; Sugar transporter; Structure/function

1. Introduction

Plants are autotrophic organisms that are able to synthesise complex molecules by reducing C, N and S from simple molecules. As a major translocatable product of photosynthesis, sucrose (glucose+fructose) is the main soluble component of the phloem sap [1]. Even in species translocating either derivatives of sucrose (raffinose, stachyose and verbascose) or polyols (mannitol, sorbitol), sucrose is still present in significant amount in the phloem sap. Selection of sucrose as the major transport sugar in plant has been related to its non-reducing nature and relative insensitivity to metabolism [2]. This represents an advantage for a substrate translocated over long distance in the plant [3], allowing transport without the problem of metabolism easily encountered with glucose. The β-fructoside nature of sucrose is unusual. The only other non-reducing disaccharide is trehalose, found in fungi and insect haemolymph [3].

In plants, sucrose is transported from synthesising (source) organs to sink organs where it is stored (as

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sucrose or, e.g., as starch) or metabolised. Sucrose is therefore a source of carbon skeletons but also an energy vector. Recently, sucrose has been considered as a signal molecule involved in the regulation of gene expression by the so-called sugar-sensing pathway [4,5].

In plants, sucrose is transported over long distance in solution in the phloem sap. This flow of sap occurs in a specialised network of cells, called the sieve elements. Sieve elements lose their nucleus and many organelles during differentiation, but stay connected to companion cells, cells with a high metabolic activity. Sieve elements are connected to form sieve tubes that oppose very little resistance to the flow of sap. In most species, at least crop species, the sieve element/companion cell complex (SE-CCC) is symplasmically isolated from the surrounding cells. The high solute content of the phloem sap (sucrose, but also amino acids and ions among other compounds) and the high osmotic pressure (30 bar) of the SE-CCC compared to mesophyll cells (13 bar [6]) has led to the concept of phloem loading. According to this concept, the high osmotic pressure in the SE-CCC is due to an active 'loading' of solutes (mainly sucrose) in those cells. However, this concept may not be universal as, in some species such as willow [7], no solute concentration difference exists between the SE-CCC and the surrounding cells. The movement of the sap in the phloem occurs through mass flow [8,9], the driving force for this movement being the entry of sucrose and subsequently water in the sieve tubes in the source organ while, at the other end of the conduit in the sink organs, the continuous unloading of solutes and water would maintain the flow.

The accumulation of sucrose in the sieve tube requires the presence of a sucrose transporter to drive this active accumulation. This points to the importance of this carrier system for the translocation of solutes from source to sink organs. The existence of a carrier system specific for sucrose and responsible for the entry of sucrose in the phloem has been postulated in the late 1970s [3], the energy for this transport being the proton gradient established by a H^+/ATPase located in the plasma membrane. Although the difference in sucrose concentration between the compartments defined by the different membranes is not precisely known (due to anatomical constraints), the existence of carriers have been postulated (or clearly demonstrated) in the following membranes (Fig. 1). Beginning with the synthesis of sucrose, the first transmembrane event is the transport of sucrose in the vacuole, which determines the pool of sucrose available for export (sucrose is temporarily stored into the vacuole). Then, sucrose has to exit the mesophyll cell (step 2) and, from the apoplasm, enter the phloem cells (step 3). To exit the long distance pathway, several ways are possible as different situations are encountered among species (apoplastic vs. symplastic unloading, for a general review see [10]). When sucrose is unloaded into the apoplasmic space (step 4), it can then be taken up as sucrose into the sink cells (step 5) or cleaved by an invertase to hexoses that are transported by specific carriers [11]. Then sucrose is used for sink growth or development (metabolic sink) or can be stored as sucrose in the vacuoles of the storage cells (sugar beet, sugar cane, step 6 in Fig. 1). There might be some additional steps, such as retrieval along the

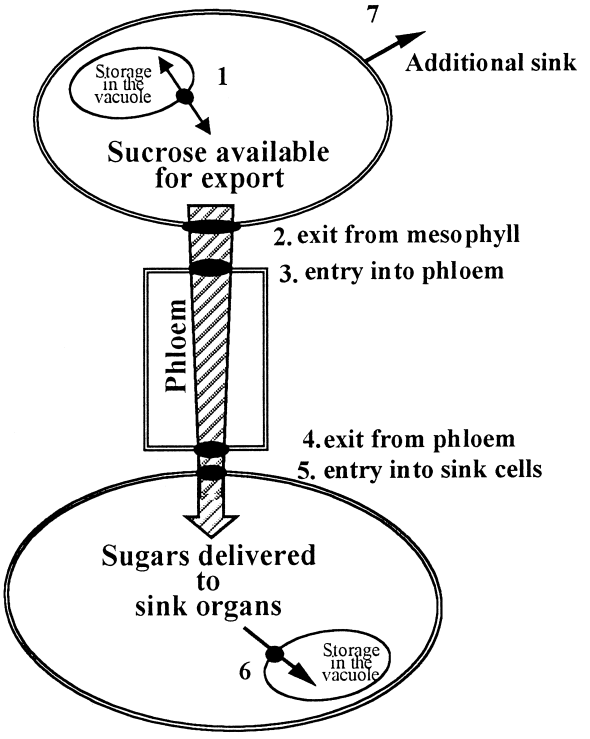

Fig. 1. Transmembrane steps mediated by a sucrose transporter. The flow of sucrose from the source organ (upper part) to the sink organs (lower part) through the phloem is represented as a large arrow, and the numbers refer to the different events of membrane transport discussed in the text.

translocation path; however, the corresponding carriers are responsible for the same type of transport as the one described in step 3. Transmembrane steps have also been involved in the transport of solutes from host plant to fungus (powdery mildew, see [12]) and are represented as step 7 in Fig. 1.

According to the different steps identified, sucrose transporters in plants can be of three types: plasma membrane influx carriers responsible for the entry of sucrose into cells that are of the proton/sucrose symporter type; tonoplast carriers have been proposed to work as sucrose/proton antiporters [13] as the vacuole is acidic compared to the cytoplasm; and finally, plasma membrane efflux carriers responsible, e.g., for the unloading of sucrose in sink organs or for sucrose exit from the mesophyll cells in close vicinity to the phloem (steps 2 and 4). Efflux carriers could, in theory, either be facilitators or antiporters.

In order to get more information on the transport of sucrose in plants, readers are referred to [14] whereas information on the physiological and biochemical characterisation of sucrose transport activities can be found in [15].

2. Identification of sucrose carriers

2.1. *Influx carriers*

The existence of specific carriers responsible for the crossing of sucrose through membranes has been postulated for many years. However, during the 1980s several groups designed strategies to identify precisely these protein(s). One strategy was based on the property of a thiol reagent NEM (*N*-ethylmaleimide) to bind irreversibly to the sucrose carrier, close enough to the binding site so that the presence of sucrose could prevent the binding of NEM. By using radiolabelled NEM for binding experiments in the presence or absence of sucrose, a group of polypeptides (molecular mass of 42 kDa) was identified in the plasma membrane of sugar beet leaves [16]. A similar method had been used before for the identification of the lactose permease from *Escherichia coli* [17]. Antibodies raised against the 42 kDa polypeptides were shown to inhibit specifically the uptake of sucrose [18,19] and the same antibodies were used to immunopurify proteins showing transport activity when reconstituted into liposomes [20]. Due to the presence of several polypeptides in the 42 kDa fraction used, the definitive identification of the sucrose carrier was not possible.

Meanwhile, a second approach based on the photolabelling of the sucrose carrier was developed. A sucrose analogue was designed (6′-deoxy-6′(4-azido-2-hydroxy)-benzamido-sucrose) and shown to competitively inhibit sucrose uptake [21]. This molecule was able to photo-affinity label a 62 kDa protein in soybean [22]. Antibodies raised against this protein showed a correlation between the appearance of a sucrose transport activity and the detection of the 62 kDa protein in different cell types [23]. The corresponding cDNA was cloned from a soybean library but showed no sign of coding a typical membrane protein. Surprisingly, when this cDNA is expressed in yeast, it confers to the yeast the ability to take up sucrose, although in a non-saturable way [24]. The role of the 62 kDa binding protein in sucrose transport (interaction with the H^+/sucrose cotransporter?) is therefore not clear today.

The third and successful trial for the identification of the sucrose carrier was based on a totally different approach. The yeast complementation system had already been used for the identification of several genes (reviewed in [25]). Therefore Riesmeier et al. [26] developed a yeast strain that could only grow on sucrose when complemented with a sucrose carrier. For this purpose they first prepared a yeast strain mutated in the secreted invertase so that sucrose could not be cleaved outside the yeast cell. Then a sucrose metabolising activity (sucrose synthase) was expressed inside the cell. When a sucrose carrier was expressed in this strain, sucrose could enter the cell, be metabolised and support growth. The yeast strain (SUSY7) was complemented with a spinach leaf cDNA library and plated on sucrose as the sole carbon source. Among the growing clones, seven were shown to contain a plasmid with an insert size of 1.95 kb. Apart from conferring the ability to take up sucrose to yeast cells, the protein coded by the cDNA showed some of the typical features of membrane carriers (high overall hydrophobicity and presence of alternating hydrophobic and hydrophilic regions, see below). The first identified sucrose carrier was then called SoSUT1 (for *Spinacia oleracea* SUcrose Transporter). The same method was then used

to identify the sucrose carrier from potato leaves called StSUT1 (*Solanum tuberosum* SUcrose Transporter [27]). All the subsequently identified carriers have been obtained by hybridisation screening or PCR amplification from these initial sequences. All the sucrose carriers cloned to date are listed in Table 1. Four sequences obtained during the sequencing of the *Arabidopsis* genome and showing high identity levels with sucrose carriers have also been included. As can be seen no common nomenclature has been approved yet! There is a majority in favour of using the original name of SUT for SUcrose Transporter, although SUC (for SUcrose Carrier) and Scr (for Sucrose CarrieR) are also found. Once the whole family of sucrose carrier is identified, it will certainly be necessary to have a common nomenclature.The nomenclature used for the four genomic sequences of *Arabidopsis* (AtSUTX1–4) is only indicative. The length of the different carriers is rather similar (around 510 amino acid residues) and this corresponds to a molecular mass of approx. 55 kDa. The longest sequence is one from *Arabidopsis* (AtSUTX4, 594 a.a.) obtained from the genome sequencing programme. The discrepancies between calculated (55 kDa) and measured molecular mass in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (45–48 kDa [28]) are due to the very hydrophobic nature of these proteins.

2.2. Vacuolar carriers

As indicated before, vacuolar carriers are supposed to work as H^+/sucrose antiporters [13]. An immunological approach by Getz et al. [29] gave some indications that the sucrose transport activity from red beet tonoplast was associated with polypeptides in the range 55–60 kDa when reconstituted in proteoliposomes. However, no further characterisation was reported. Only in one case was a protein shown to be associated with the tonoplast [30]. However, the corresponding cDNA is not closely related to all the other sucrose carriers of plant (but there is no indication that a sucrose/proton antiporter and a sucrose/proton symporter should share extensive sequence homologies) and no function could be attributed to this carrier after yeast expression.

Table 1
List of the sucrose transporter sequences available in databases

Name	Species	Length (a.a.)	Accession number	Functional expression/K_m	Ref.
AgSUT1	Celery	512	4091891	Yes/0.14 mM	Noiraud et al., unpublished
AtSUC1	*Arabidopsis*	513	481132	Yes/0.45 mM	[38]
AtSUC2	*Arabidopsis*	512	407092	Yes/0.53 mM	[38]
AtSUTX1	*Arabidopsis*	474	2160188	No	Vystskaia et al., unpublished
AtSUTX2	*Arabidopsis*	513	3287687	No	Rousley et al., unpublished
AtSUTX3	*Arabidopsis*	492	3810593	No	Rousley et al., unpublished
AtSUTX4	*Arabidopsis*	594	3461813	No	Vystskaia et al., unpublished
BvSUT1	Sugar beet	523	633172	No	Westram et al., unpublished
DcSUT1a/b	Carrot	501	2969889, 2969887	Yes/0.5 mM	[64]
DcSUT2	Carrot	515	2969884	Yes/0.7 mM	[64]
LeSUT1	Tomato	511	575299	No	Buerkle and Frommer, unpublished
NtSUT1	Tobacco	507	575351	No	[69]
NtSUT3	Tobacco	520	149981	No	[68]
OsSUT1	Rice	537	2723471	Yes/ND	[71]
PmSUC1	*Plantago*	510	1086253	Yes/0.3 mM	[39]
PmSUC2	*Plantago*	510	415988	Yes/1 mM	[36]
RcSCR1	*Ricinus*	533	542020	Yes/2 mM	[65]
SoSUT1	Spinach	525	549000	Yes/1.5 mM	[26]
StSUT1	Potato	516	542087	Yes/1 mM	[27]
VfSUT1	*Vicia faba*	523	Z93774	Yes/1.4 mM	[67]

Sequences are listed in alphabetical order and, when they are successfully expressed in yeast, the K_m value for sucrose is indicated. ND, not determined.

2.3. Efflux carriers

Although several descriptions of sucrose efflux activities have been reported (e.g., [31]) no such carrier has been identified so far. Some authors have proposed that the influx sucrose carrier could function as an efflux carrier without energisation of the transport, as sucrose would be transported along its concentration gradient [32]. Some data demonstrate the possibility that sucrose transport can occur in the absence of a proton gradient in plasma membrane vesicles from potato [28]. These data could explain the expression of sucrose carriers in the phloem of sink organs [33,34], the high concentration of sucrose in the phloem driving its own efflux outside the conducting tissues. The existence of a carrier involved in sucrose unloading has been postulated from models designed to test the Münch–Horwitz theory [9], indicating that the exit of sucrose has to be rate-limited in order to maintain the movement of the phloem sap. However, this could hold true only if the proton gradient across the plasma membrane is lower than at the loading site.

While studying the uptake of sucrose in plasma membrane vesicles from potato plants where the expression of the sucrose carrier was lowered, it was shown that mesophyll cells are able to take up sucrose [28] confirming data obtained on *Ricinus* cotyledons [35]. However, this could be considered as a retrieval mechanism to pump back sucrose leaked out the mesophyll cells. Therefore the possibility remains that a sucrose carrier is present in the plasma membrane of mesophyll cells, but in the immediate vicinity of the conducting cells, where efflux is occurring. The sucrose concentration gradient across the membrane of such cells (50 mM sucrose inside, less than 1 mM outside [32]) indicates that a facilitator system is capable of allowing sucrose efflux down its gradient, while the very efficient transport system present in the phloem cells keeps the apoplasmic concentration low. In theory, the efflux of sucrose could occur by a proton antiport system, but the direction of sucrose concentration gradient (high sucrose in the cell vs. low sucrose in the apoplasm) is not in favour of an energised step for efflux. However, no such system has been identified in plants so far.

3. Function of the carriers: old and new data

For the rest of this review, only sucrose/H^+ cotransporters will be considered. The most widely used heterologous expression system to characterise those carriers up to now is the yeast *Saccharomyces cerevisiae*. The first sucrose carriers have been isolated by complementation of an invertase deficient yeast mutant [26,27]. Yeast cells have been widely used as they present several advantages for heterologous expression: transformation with foreign cDNAs is routine and very efficient, and growth and processing of cells for sugar uptake measurements are quite simple. For these experiments, minimal material is required and is basically accessible to any laboratory licensed for using radioactive compounds. Moreover, basically any yeast strain is suitable as expression of the endogenous secreted invertase is repressed when glucose is used as the sole carbon source [36]. Sucrose carriers seem to function as monomers in the membrane and are, at least in part, correctly targeted to the plasma membrane in yeast (see [14] for a discussion of this point).

In order to demonstrate that SoSUT1 (the first identified sucrose carrier [26]) was the sucrose transporter extensively studied in plants, several of the properties described in plants were investigated during expression in yeast. Those properties were of three kinds: symport with proton, inhibition by specific reagents and specificity towards sucrose.

3.1. SUTs are proton/sucrose transporters

The first property was demonstrated indirectly by the use of several compounds that collapse the plasma membrane proton gradient established in yeast as in plant cells by a H^+/ATPase of the P-type (for a review see [37]). Therefore protonophores (CCCP, DNP) and inhibitors of ATP generation (antimycin and arsenate) were shown to strongly inhibit the uptake of sucrose into yeast cells expressing SUT [26,38]. Moreover, all SUT carriers successfully expressed in yeast (Table 1) display an optimum activity for external acidic pH (4.5–5.5). However, PmSUC1 and AtSUC1 are less sensitive than other carriers to a rise in pH, having a rather constant activity between pH 4.5–6.5 [38,39]. Sucrose uptake

was also stimulated after addition of glucose to the yeast cells because of glucose stimulation of the H^+/ATPase activity. All these features were in perfect accordance with the properties of sucrose carriers whether studied in leaf discs [40], protoplasts [41] or plasma membrane vesicles (reviewed in [15]). However, more precise data on the kinetics and stoichiometry of the cotransport could only be obtained by oocyte expression [42] as yeast is not suitable for electrophysiological measurements.

As for sucrose transport activities recorded in plants, SUTs were shown to be strongly inhibited by reagents of thiol groups such as PCMBS (para-chloro-mercury-benzene sulfonic acid) and NEM [26,38], and histidine reagents such as DEPC (di-ethyl-pyrocarbonate). The histidyl residue involved in DEPC binding has been determined [43] (see below).

3.2. Specificity of SUTs. Affinity for sucrose

When expressed in yeast, all the sucrose carriers display an affinity for sucrose in the range 0.3–1.5 mM. A slightly higher affinity for sucrose was noted for AgSUT1, the sucrose transporter for celery (N. Noiraud, S. Delrot, R. Lemoine, Plant Physiology, in press). Nevertheless, all K_m determinations were not made with the same yeast strains and the uptake conditions might have been slightly different: therefore one can consider that these K_m values are remarkably similar. Interestingly, the K_m for sucrose of StSUT1 expressed in yeast is very close to the value determined in plasma membrane vesicles from potato leaves [28] and also similar to the value measured after expression in *Xenopus* oocytes [44]. Similar K_m values were also measured for AtSUC1 expressed in yeast [38] and oocytes [45]. It has to be noted that the affinity of the sucrose carriers for sucrose is much lower than the affinity of the monosaccharide transporter for glucose [11] by more than an order of magnitude.

All these carriers would therefore correspond to the high affinity system described in plant fragments (*Vicia faba* leaf discs [40], sugar beet leaf discs [46]). The existence of a lower affinity uptake system for sucrose has been postulated from the saturation curves obtained by these authors. However, the complexity of the living systems used (several types of cells) may well be responsible for these results, as only one high affinity system (superimposed by a diffusional component) could be shown when homogenous plant tissues (such as phloem strand from celery petioles [47] or plasma membrane vesicles [28]) were used. The lower affinity system may well represent the activity of a different transporter located on a different cell type.

All the results obtained by expressing sucrose carriers cDNAs in yeast are in perfect agreement with the already described characteristics of sucrose carriers in plants. They demonstrate that the heterologous expression in yeast cells does not change these major characteristics (dependence on the proton gradient, sensitivity to inhibitors). They also indicate that the plant sucrose carriers are able to function properly in a different lipid environment. However, many data have been accumulated on the specificity of the sucrose carrier towards other substrates (natural or synthetic) and those data have been used to construct a model for the interaction of the sucrose molecule with its own carrier [21,48]. According to this model the substrate recognition by the sucrose carrier occurs through hydrophobic interaction with the fructosyl moiety whereas the hydroxyl groups at position C-3, C-4 and C-5 of the glucopyranosyl moiety confer the specificity for sucrose recognition. However, more recent data also suggest that the glycosyl C-2 hydroxyl is also involved in the substrate specificity [49].

How do these data compare with the results obtained in yeast? Although no large-scale analysis of the specificity of SUTs expressed in yeast has been done yet, a certain number of data are available and are listed in Table 2. All these data have been obtained by studying the effects of the sugars listed on radiolabelled sucrose uptake into yeast cells expressing a sucrose carrier. Sugars were used at concentrations in excess of 10- to 50-fold the sucrose concentration and in optimally energised conditions (external medium acidified by the stimulation of H^+/ATPase activity following glucose addition). Therefore these data are rough estimations as they do not give any indication on the type of inhibition. On the other hand, those data should be taken as confirmation that the sucrose carriers identified are the one already described in plants. Several disaccharides have been tested on the basis of steric con-

Table 2
Substrate specificity of the sucrose transporters expressed in yeast

Substance	Effect	Transporter
Sucrose	Inhibition (isotopic dilution)	All transporters tested
Glucose	Stimulation through ATPase activation	All transporters tested
Maltose	Inhibition (67%), 100-fold excess	AtSUC1 and AtSUC2
	Inhibition (70%), 10-fold excess	PmSUC2
	Inhibition (10%), 3-fold excess	RcScr1
	Inhibition (63%), 50-fold excess	SoSUT1
	Inhibition (10%), 10-fold excess	StSUT1
Isomaltose	Inhibition (12%), 10-fold excess	PmSUC2
Lactose	None, 100-fold excess	AtSUC1 and AtSUC2
	Inhibition (14%), 10-fold excess	PmSUC2
	None, 10-fold excess	SoSUT1
Raffinose	None, 100-fold excess	AtSUC1 and AtSUC2
	Inhibition (12%), 10-fold excess	PmSUC2
	None, 3-fold excess	RcScr1
	None, 10-fold excess	SoSUT1
Trehalose	Inhibition (16%), 10-fold excess	PmSUC2
	None, 50-fold excess	SoSUT1
	None, 10-fold excess	StSUT1
Melibiose	None, 10-fold excess	PmSUC2
Melezitose	None, 10-fold excess	PmSUC2
Palatinose	None, 50-fold excess	SoSUT1
	None, 10-fold excess	StSUT1
Phloridzin	Inhibition (84%), 10-fold excess	SoSUT1
	Inhibition (87%), 10-fold excess	StSUT1
α-Phenylglucoside	Inhibition (80%), 10-fold excess	AtSUC1 and AtSUC2
	Inhibition (93%), 10-fold excess	SoSUT1
	Inhibition (92%), 10-fold excess	StSUT1
β-Phenylglucoside	Inhibition (80%), 10-fold excess	AtSUC1 and AtSUC2

The results are obtained from the papers listed in Table 1.

straints. Out of these only maltose (a dimer of glucose linked in α1–4) is a powerful inhibitor of sucrose uptake (Table 2). Its anomer isomaltose has no effect. None of the trisaccharides tested (raffinose and melezitose) were inhibitory to sucrose uptake. These data are in perfect agreement with the sugar specificity of sucrose carriers in plants. Interestingly, phloridzin, a specific inhibitor of animal Na^+/glucose cotransport shown to inhibit sucrose transport in plants [50], is also a very good inhibitor of SoSUT1 and StSUT1 activities [26,27]. Finally, α-phenylglucoside, one of the substrates used to demonstrate the hydrophobic interaction between the fructosyl moiety of sucrose with its carrier, also appears to be a very powerful inhibitor of sucrose uptake. However, the inhibition by β-phenylglucoside is in contrast to previously published data [51]. This point might deserve further investigation. All these different data confirm that the properties described for sucrose transport activities in plants are the same as the one of SUT carriers expressed in yeast. As the family of sucrose carriers increases (at least in *Arabidopsis*) it will be very interesting to see whether all carriers are equivalent as far as kinetic properties and affinity for sucrose are concerned.

3.3. Other expression systems: oocytes and proteoliposomes

Even if yeast cells represent the most common system for expressing sucrose carriers, other expression systems have been used. As indicated in the review by Miller [42], several sucrose carriers have also been successfully expressed in *Xenopus* oocytes. Oocytes represent a very powerful system to study the kinetics properties of an electrogenic cotransporter. It

has allowed a precise determination of a $1H^+/1$ sucrose stoichiometry in the case of StSUT1 [44] and AtSUC1 [45]. To date, expression of plant sucrose carriers in the baculovirus/insect cells system or COS cells has not been reported.

One interesting experiment has been the purification and reconstitution of the sucrose carrier in proteoliposomes. Stolz et al. [52] have modified the PmSUC2 protein by adding a biotin acceptor domain at the C-terminus. The modified protein was successfully expressed in yeast cells and biotinylated. This allowed the purification of the carrier after solubilisation. Moreover, the activity of the biotinylated PmSUC2 in yeast was comparable to the unmodified protein. The purified protein was then reconstituted in liposomes together with beef heart cytochrome *c* oxidase [53]. In the presence of ascorbate, a proton gradient (outside acidic) is created. After reconstitution in such liposomes, sucrose uptake could be recorded only after addition of the electron donor system for cytochrome *c* oxidase (ascorbate/TMPD/cytochrome *c*), indicating that this uptake is coupled to the proton gradient. However, the time course for the uptake of sucrose is very slow as no saturation occurs after 2 h. This is much slower than when plasma membrane vesicles from yeast cells expressing PmSUC2 were fused to liposomes without prior purification of the carrier [36]. Due to the small size of liposomes, an efficient uptake system (such as the bacterial ones already studied [53]) reaches internal saturation in minutes. This rather long time for equilibration in plant proteoliposomes either indicates that a very small number of carrier molecules were incorporated per liposome, that the carrier was inactivated to a certain extent during the purification and/or that the lipid composition of the liposome membrane was not optimal. However, the fact that plant sucrose carriers can be expressed successfully in yeast cells somehow moderates this latter point. Nevertheless, reconstitution of sucrose carriers into liposomes can be used in the future for a precise investigation of the lipid environment effects on the carrier activities.

3.4. Sucrose transporter in other species

As already indicated in the introduction, sucrose is of major importance for plants as a specific form of long-distance transport that cannot be replaced by another sugar molecule. This is very unique to plants as in other organisms (yeast and bacteria able to grow on sucrose) sucrose is dispensable. In bacteria, for example, a proton/sucrose permease has been identified [54] and the residues involved in substrate recognition and binding have been demonstrated to be rather well conserved with the *E. coli* lactose permease [55]. Therefore, very little homology with plant sucrose carriers is expected (see below).

In yeast where sucrose is externally cleaved by a secreted invertase, the resulting hexoses are taken up into the cells. However, there has been a debate about the possibility for yeast cells to directly take up sucrose without prior cleavage. This was demonstrated in several papers [56,57]. However, no sucrose carrier cloning has been reported yet although a general α-glucoside H^+-cotransporter (AGT1) able to transport trehalose and several other disaccharides have been reported [58,59]. While AGT1 preferentially transport trehalose, it has been recently shown that sucrose is also transported efficiently [60]. However, no clear sequence homologies could be found with plant sucrose carriers.

On chromosome I of *Schizosaccharomyces pombe*, a translated sequence related to the sucrose transporter family (accession no. Z99165) has been identified. This is in fact the most closely related sequence to plant sucrose carriers (*E* value 7e-30). However, to our knowledge, no function in sucrose transport has been attributed to this gene.

3.5. Localisation of sucrose carriers

As already discussed, all sucrose carriers identified so far are proton-coupled sucrose uptake transporters (entry of sucrose into cells by cotransport with protons) and would therefore be involved in step 3 of Fig. 1. Data obtained from either immunolocalisation, in situ hybridisation and promoter–reporter gene expression gave some indications about the cells where those carriers are expressed. The first in situ hybridisation experiments clearly located the expression of StSUT1 in the phloem of potato leaves and stems [27]. Immunolocalisation studies then demonstrated that in solanaceous species (potato, tomato and tobacco) SUT1 is expressed in the plasma membrane of the sieve tube [61]. Interestingly, the authors

also demonstrated that the corresponding mRNA was also detected in the sieve elements, although these cells are devoid of ribosomes. For a discussion of this point the reader should refer to [14,61]. In *Arabidopsis* and *Plantago*, SUC2 (the orthologue of SUT1) has been located to the plasma membrane of companion cells [62]. At the present time, it is not known whether these different results correspond to differences among species. However, a recent report from the group of Sauer [63] indicated that in *Plantago*, the second sucrose transporter identified SUC1 is expressed in the sieve tubes, mainly in the petioles where it would be involved in the retrieval of sucrose (equivalent to step 3 in Fig. 1, but along the translocation pathway). It is therefore possible that this situation is general and occurs in all plant species.

Most of the data also indicate that the sucrose carriers are expressed in the phloem, all along the translocation pathway [27,34] and may be involved in the unloading of sucrose in sinks. At the present time, no carrier involved specifically in the entry of sucrose in the sink (step 5) has been identified, although such a function has been reported for DcSUT2 which is highly expressed in carrot tap roots but not restricted to phloem cells [64]. However, lower but detectable levels of expression where also found in the leaf lamina. In *Ricinus*, a sucrose transporter is predominantly expressed in seedlings but also detected in source leaves [65,66]. In *Vicia faba* seeds, expression of SUT1 was detected but again not exclusively in this organ [67]. There is only one report [68] indicating that a tobacco sucrose transporter-like cDNA is specifically expressed in the pollen grains during maturation and also in the pollen tubes during germination. As for monosaccharide transporters [11], it is expected that some of the new sucrose carriers that are identified will be shown to be specifically expressed in defined organs or cells.

However, the localisation experiments must be interpreted with caution. Due to the very high level of sequence identity, care has to be taken that no cross-hybridisation occurs between the different sucrose carriers of the same plants. This point has been solved by raising antibodies against the less conserved regions of the protein (C-terminus or central loop). Even in that case, the sensitivity level of these methods may still be a problem, because only a highly expressed carrier will give a signal of sufficient intensity to be considered as being above background with confidence. In the case of potato, the study of radiolabelled sucrose uptake in plasma membrane vesicles from leaves of transgenic plants where StSUT1 expression was lowered [28] indicated that StSUT1 (or a very closely related sucrose transporter) is expressed in mesophyll cells, but at a level too low to be detected by other methods. This confirmed data obtained on *Ricinus* cotyledons demonstrating uptake of sucrose in mesophyll cells [35] and RcSUT1 expression in epidermal cells [66]. A description of the effect of antisense repression of NtSUT1 in tobacco has also been reported [69].

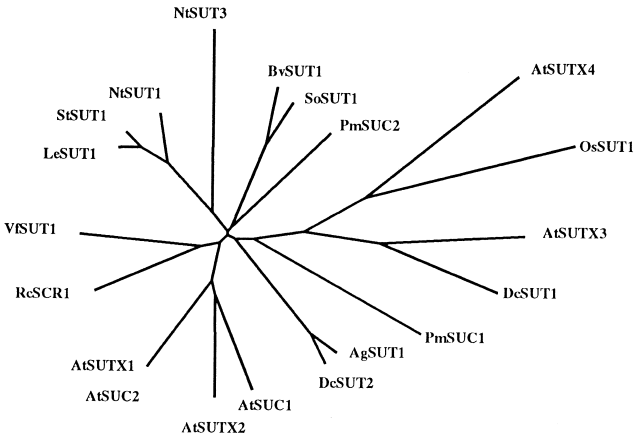

Fig. 2. Phylogenic tree showing the relatedness of the sucrose carriers listed in Table 1, based on the degree of similarity of their sequence. The phylogenic distance is roughly proportional to branch length. The image was generated with the Unrooted software package (pbil.univ-lyon1.fr).

4. Structure/function: the beginning

The number of sucrose carriers identified at the cDNA level is increasing at a fast pace. In the last reviews published on the subject [14,70] 8–12 different sucrose transporters were listed. As seen in Table 1, 20 sucrose carriers are available in the data bases. Two sucrose carriers have been characterised so far in *Arabidopsis* (as in *Plantago*), but it is clear that sucrose carriers make a large gene family and six sequences (Table 1) are present today in the *Arabidopsis* data base. It has to be noted that AtSUC2 and AtSUTX1 differ only by one amino acid. Four of the identified *Arabidopsis* clones cluster together whereas

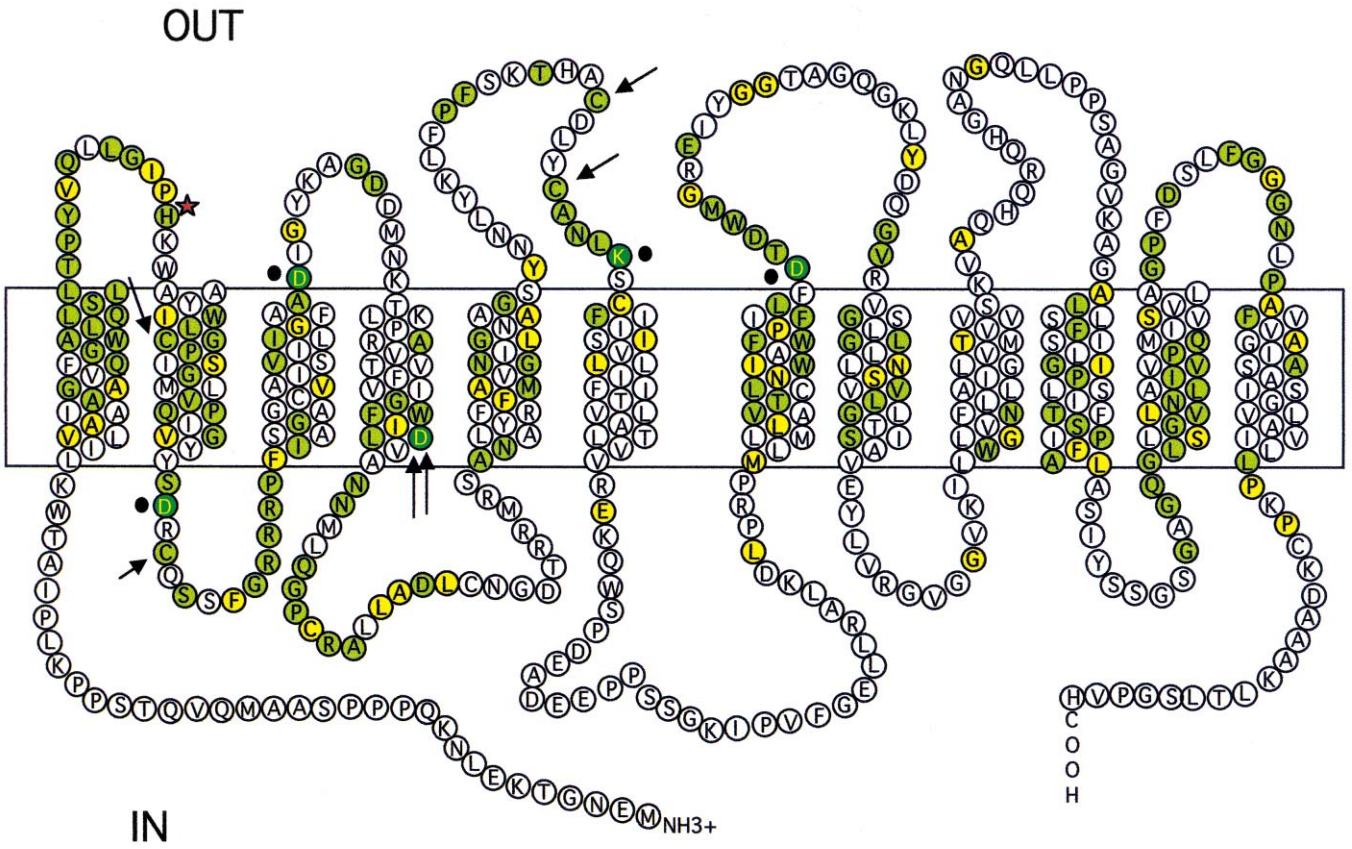

Fig. 3. A tentative prediction for AgSUT1. The transmembrane segments were predicted with HMMTOP [94] after optimisation with all other sucrose transporter sequences available. Sequence alignments were made with the Multialin program at pbil.univ-lyon1.fr [95]. The residues written in green circles are conserved in all 12 sequences shown to function as sucrose transporters (see Table 1) and yellow circles indicate residues conserved in 11 out of 12 of these sequences. Conserved cysteine residues are indicated by arrows whereas points show charged residues located at the border of transmembrane segments (see text). The conserved Asp residue located in a transmembrane segment is indicated by a double arrow. The histidine residue that has been the subject of mutagenesis studies [43] is indicated by a star.

the other two are distantly related (Fig. 2).The next challenge will be to characterise these different clones at the function level. However, for the rest of this review, we will consider the transporters that have been functionally demonstrated to be sucrose carriers (already 12 different clones, see Table 2) by expression in yeast. Since the cloning of the sucrose carrier from rice (OsSUT1 [71]), both monocots and dicots are represented in this list. All these carriers are sequence related (see Fig. 2). The two most distant sequences (OsSUT1 and NtSUT3) still show 37% identity at the protein level. However, DcSUT1 which is located on the closest branch of the unrooted phylogenic tree, already shows 45% identity with NtSUT3.

Although no structural data are available at the present time for any sugar transport protein from any species, one can expect to get valuable information from the sequence comparison of all those 12 well-characterised sucrose carriers. In Fig. 3, a 12-transmembrane α-helices model (two units of six transmembrane segments connected by a central loop) is proposed for AgSUT1. Green circles represent residues that are conserved in all 12 sequences and yellow circles correspond to residues conserved in 11 out of 12 sequences.

As already noted, there is a high conservation of the sequence: 134 conserved amino acids for an average sequence length of 517 which represents 26% of overall conserved amino acids. If the positions where

the residue is conserved in 11 out of 12 sequences compared (yellow in Fig. 3), then 188 residues are conserved (36%). The residues in the first half of the protein are slightly more conserved (30% identity in all 12 sequences, 39% identity in 11 out of 12 sequences) but it is not known if this is relevant to function. The residues predicted to be present in transmembrane regions appear to be more conserved as 79 (out of 250 residues present in putative transmembrane segments) are identical in all 12 sequences compared (32%) and this increases to 43% if one considers residues that are identical in 11 out of 12 sequences. If one would take into account amino acids with similar function, the score would be of course higher as in many positions of putative transmembrane segments, the hydrophobic nature of the residue is conserved among all sequences. Therefore, amino acid conservation occurs in regions which could be related to transmembrane α-helices, whereas a high variability was found in the N- and C-termini and the central loop. The highest conservation is found is transmembrane segments 1, 2 and 11.

4.1. SUTs are members of the sugar transport superfamily

Homologies between sugar carriers from eukaryotes and prokaryotes have been described more than 10 years ago [72,73] and this led to the concept of the Major Facilitator superfamily (MFS) [74]. This is a very old family which appeared in prokaryotes more than 3.5 billion years ago [75]. The common feature for members of this superfamily is that they are all supposed to have 12 transmembrane spanning segments in the form of α-helices (based on hydropathy calculations). It has been proposed that those transporters arose from the duplication and fusion of a primordial gene coding a protein with six transmembrane segments.This explains the presence of conserved motives in both halves of the transporters (see below). Moreover, the fact that the two halves of the *E. coli* lac permease (a member extensively studied of the MSF) can be expressed from different promoters and assemble into the membrane to form a functional transporter is in favour of the duplication theory. Members of this superfamily can be uniporters, symporters or antiporters and are able to transport a variety of metabolites. This superfamily has been divided into five clusters [74] and the plant sucrose transporters (as well as plant monosaccharide transporters) are included into the second cluster composed mainly of sugar uniporter from animals, glucose cotransporter from yeast and plants and sugar/H^+ cotransporter from bacteria. It has to be noted that the *E. coli* lactose permease, which is the cotransporter for which most information is available (see below), is not a member of this cluster. Extensive sequences analysis and comparison between the different proteins of the same cluster has led to a proposed conserved motif that could be used as a signature for the members of this cluster. The initial pattern proposed for the sugar transporter was R-X-G-R-[KR] (the first one is located in the loop between the second and third transmembrane domain whereas the second between transmembrane domains 8 and 9). However, this model has been refined (see ProSite 00216) and now includes extension to this initial motif. The pattern located between transmembrane segment 8 and 9 has been extended to the following: [LIVMSTAG]-[LIVMFSAG]-x(2)-[LIVMSA]-[DE]-x-[LIVMFYWA]-G-R-[RK]-x(4–6)-[GSTA] which gives in plant sucrose transporters: [MSTA]-[S]-x(2)-[LIVM]-[EYQD]x-[LIMF]-[GCAV]-[RK]-x (3)-[GA].

This indicates that the second pattern is not heavily conserved in sucrose carriers. Moreover, a third pattern has been described in the loop between transmembrane segments 4 and 5 but this pattern could not be identified among SUT sequences. It has to be noted that the sequences used to design those consensus patterns included only one plant sucrose carrier (SoSUT1). Due to the increasing number of plant sucrose carriers available, the consensus patterns will certainly be changed to take these sequences into consideration. These conserved sequences have not been related to any particular function, except for the small pattern between the second and third transmembrane segments which is predicted to form a β-turn linking the two α-helices [73].

At the present time, no three-dimensional structure is available for any transporter in the MFS superfamily and therefore all the models proposed are hypothetical. Nevertheless, as pointed out by Tanner and Caspari [76], this did not preclude the collection of an impressive number of data leading to stimulating models. The model in Fig. 3 represents the Ag-

SUT1 transporter as a succession of α-helices spanning the membrane, with the N- and C-termini on the cytoplasmic side. What are the basis for such a representation, commonly used for sugar carriers? Hydropathy analysis indicates the succession of hydrophobic and hydrophilic regions. One example is given in [26]. In the case of sucrose carriers, 12 hydrophobic segments can be identified with reasonable confidence, except in the case of the first two transmembrane segments which appear as a large hydrophobic region. The hydropathy profile for SoSUT1 can be taken as a model for all other sucrose carriers known to date. One constraint is that the segments have to be long enough to cross the membrane, which, in the case of an α-helix, is a minimum of 20 residues. However, it has to be kept in mind that an α-helix is not necessarily perpendicular to the membrane as demonstrated in the case of G-protein-coupled receptors [77]. The length of the transmembrane segments determines the number of residues in the connecting loops. This can also give some indications on the arrangement of the different α-helices in the membrane. As pointed out in [78], a short loop will connect two adjacent transmembrane segments whereas longer loops could indicate that two segments that are adjacent in the sequence could be far apart in the tertiary structure. These authors present a general model for members of the MSF, based mainly on the data obtained on the lac permease of *E. coli*, in which helices 2, 3 and 4, 5 and 6; 8, 9 and 10; 11 and 12 are adjacent. From the model presented here and in other papers for sucrose carriers [14], it is possible to hypothesise that helices 2, 3 and 4 are adjacent and so are helices 11 and 12. However, the loop between helices 1 and 2 is certainly short as indicated in the model proposed for StSUT1 [14] because of the high overall hydrophobicity of this region. It is therefore very speculative at the present time to decide whether the sucrose carrier fit into the model proposed in [78]. A recent model for the animal glucose transporters [79] displays a long connecting loop between helices 1 and 2, almost as long as the central loop, a quite different situation from what is seen in the plant sucrose transporters.

As for the orientation of the protein in the membrane, there is now a general consensus to consider that the N-terminus, the central loop and the C-terminus are all located on the cytoplasmic side. This prediction is based on data obtained from studies on the lac-permease and GLUT1, the glucose transporter from human erythrocytes. At the present time no result contradicts this prediction. Such a prediction will be quite easy to verify by challenging antibodies against the N- or C-terminus, with plant plasma membrane of defined orientation (either right-side out or inside out). This could also be useful to identify which residues are readily located into the membrane (but with some caution in the data interpretation as the epitope recognised by the antibodies (monoclonal) has to be precisely known).

Although there is a general consensus on the α-helix nature of the transmembrane segments, some authors have proposed a different model (consisting of a beta barrel) for the structure of GLUT1 (human glucose transporter) and other known sugar carriers [80]. This controversy will be resolved when the three-dimensional structure of such a protein will be determined.

Apart from being related to members of the sugar transporter cluster, sucrose carriers are not directly sequence related to other carriers. For example, very few common elements exist with the hexose carrier plant gene family [11], with an average of 20% of identical amino acids between sucrose and hexose plant transporters [81]. A conserved stretch of residues common to plant sucrose carriers and melibiose carrier of *E. coli* has been described by Naderi and Saier [82]. Three plant sucrose carrier sequences were used to construct this alignment. The same alignment still hold true when more sucrose carriers sequences are included (data not shown). This region is centred on the first conserved motif of the sugar transport family and comprises residues from the second and third transmembrane segments and the interconnecting loop. Interestingly, this region includes two Asp residues that have been involved in the cation selectivity of the melibiose permease. The melibiose permease is peculiar because this carrier is able to catalyse melibiose (or other α-D-galactosides) accumulation by using either Na^+, H^+ or Li^+ gradients [83]. At the present time there is no indication of sucrose carriers able to use other cation gradient than the proton gradient to drive sucrose uptake. In melibiose permease of *E. coli*, the N-terminal domain has been involved in cation recognition in heli-

ces 2 and 4 [83] whereas in the lac permease, the charged residues involved are located in the second half of the protein (helices 7, 8, 9, 10). In plant sucrose carriers the charged residues that are found in a putative transmembrane segment are in the first half of the protein (see below). This could indicate that melibiose permease could be a better model than the lactose permease for identification of important residues.

Regulation of gene expression by sugars has been extensively studied in the yeast *Saccharomyces cerevisiae*. The number of hexose transporters in yeast is surprisingly high (20) but this feature is more understandable as those carriers display different affinities for glucose, allowing a very rapid adaptation of the yeast to changing external sugar concentration. What is even more interesting in yeast is the identification of two members of the hexose transporter family as glucose sensor (Snf3 and Rgt2, reviewed in [84]). Both carriers display an extended carboxy terminus and this has been related to their function as a sensor. The possibility of finding similar carrier/sensor in plants would open new exciting research area. The conservation of the hexokinase pathway in plant sugar sensing argues in favour of the identification of sucrose sensor/transporter proteins [5,85]. It has to be noted that AtSUTX4 has a longer central loop than other sucrose carriers and a similar feature has been found for a tomato sucrose carrier which colocalises with SUT1 in sieve elements [86]. The authors suggest that this protein could be involved in sugar sensing.

4.2. *Conservation of specific residues and structure/function relations*

As already indicated, many residues are conserved among the SUT sequences identified so far and, for the carriers that have been expressed to date, very similar kinetics (e.g., K_m values) were recorded. The major difference noted is the pH sensitivity of SUC1 and SUC2 in *Arabidopsis* and *Plantago* [38,39] but it is difficult to relate specific residues to the difference in function.

It has been noted for many carriers that the location of cysteine residues is often not conserved, even in member of the same transporter family [87]. This would indicate that, in spite of the sensitivity to thiol reagents such as PCMBS or NEM often reported for transporters, cysteine residues are not essential for the transport function. However, in the plant sucrose transporter family, there are four conserved cysteine residues (indicated by arrows in Fig. 3), three in connecting loops and one in a putative transmembrane segment. This number is quite high. Cysteine mutagenesis and protein chemistry have been extensively and successfully used in the study of different carriers [88]. Therefore, this will certainly be a future direction for the work on sucrose carriers. There are several glycosylation sites that are present in the different sequences but not conserved among them. However, there is no evidence at the present time that sucrose carriers are glycosylated in vivo: when the protein is immunodetected on Western blots [28] it appears as a rather sharp band. Conserved phosphorylation sites can be identified in sucrose carriers sequences, also in non-transmembrane segments. The relevance of phosphorylation to the activity of sucrose carrier is discussed in this issue [42,89].

In the case of a proton coupled cotransport system, negatively charged residues are expected to be present in the transmembrane regions in order to translocate protons. In the case of the lac permease, Glu325 has been involved in the proton translocation and four negatively charged residues have been proposed to be involved in the energy coupling [90]. In the case of StSUT1, only one charged residue (Asp, negative) has been located to a transmembrane region [14]. This Asp residue is conserved among all sequences as shown in Fig. 3 (fourth transmembrane segment, double arrow). Whereas this is the only charged residue present in a transmembrane segment in StSUT1, AgSUT1 has three other (positive) charged residues. Those charged residues are only located in transmembrane segments 4 and 5 whereas they are located in the second half of the protein in *E. coli* lac permease (helices 7–11). The small number of charged residues supposed to be present inside the transmembrane domains of sucrose carriers may indicate a different energy coupling system. It is also possible that as the model proposed will be refined, some of the charged residues that are located at the border of transmembrane segments will be included into them. This could be the case of the Asp residues indicated by dark circles in Fig. 3.

4.3. Mutagenesis on sucrose transporters: the infancy

Much of the information available on the structure of symporters has been obtained from mutagenesis studies. Two different strategies can be used: site-directed mutagenesis on particular residues (charged ones or residues having specialised function such as cysteine) or random mutagenesis. The first one has been extensively used in the case of the lac permease of *E. coli* and most of the information known on its structure derives from such experiments. Random mutagenesis has been used in the case of the *Chlorella* hexose carrier, with great success [91,92]. In that case, the carrier cDNA is amplified by PCR under conditions leading to mutations into the cDNA. A mutagenised library is constructed and inserted into a vector for heterologous expression in yeast. Transformed yeast cells are then plated and screened on the desired medium. The screening can be done for altered affinity for the natural substrate (by changing the substrate concentration into the growth medium) or for changes in the substrate specificity. Once a clone showing growth on the selective medium is identified, the mutation responsible for the phenotype is found by sequencing. Unfortunately, mutagenesis work on the plant sucrose carrier is still in its infancy and only one paper has been published on this topic [43]. The authors have made site-directed mutagenesis on a histidine residue of the sucrose transporter from *Arabidopsis* AtSUC1. This residue (His65 in AtSUC1) is conserved in all sucrose carriers and is located in the first extracellular loop, close to the start of the putative second transmembrane segment (marked with a star in Fig. 3). The rationale for mutating this special residue is that sucrose carrier activity has been shown to be inhibited by DEPC, a chemical modifier of histidine residues. As this residue is the only conserved histidine in all sucrose carriers, it therefore appeared as a good candidate for mutagenesis. Histidine was replaced by hydrophobic (Gly, Leu), polar (Cys, Asn, Gln, Ser, Tyr) or charged residues (negative Asp, or positive Lys, Arg). All the different mutant carriers appeared to be expressed equally in yeast, except for the His65–Cys which was degraded after translation, maybe because of incorrect folding of the protein. When histidine was replaced by a positively charged residue (either Arg or Lys), sucrose transport activity in yeast was significantly increased. Both the V_{max} (up to 14-fold for the Lys substitution) and K_m values were increased in such mutants. However, the specificity of these mutant carriers towards sucrose was not tested. The possibility remains that the observed increase in the uptake rate and decrease in the affinity are the results of a lost of specificity. This point will deserve further investigations. Replacing His with a negatively charged residue (Asp) led to dramatic decrease in the V_{max} (8-fold), which is in accordance with the other results. However, replacing His65 with non-charged residues such as Gly or Ser did not change the kinetic properties compared to the wild type, indicating that the positive charge is not mandatory for the activity. The role of His65 in the translocation process is not clear at the present time. In the *E. coli* permease, there is one His at position 322 which cannot be substituted for any other residue without loss of cotransport function and which is involved in the stabilisation of the helices involved in lactose transport [90]. Nevertheless, it is clear that His65 is important for the overall activity of the sucrose transporter. However, the yeast expression system, although very powerful to screen for mutants, shows its limits when fine kinetics have to be investigated. One has to remember that mutants of the *E. coli* lac permease have been studied extensively on membrane vesicles. The use of the yeast expression system as a first screen combined with oocyte expression will certainly give invaluable information.

The identity of His65 as the target residue for DEPC was confirmed by the fact that the mutant carriers devoid of His65 and still able to transport sucrose were less or not at all sensitive to DEPC. This first attempt to identify a mutant in the transport pathway of sucrose and proton therefore gave very interesting results and calls for more research being done in that area. Screening for mutants with altered specificity is also important as, for example, in *E. coli*, lac permease have been mutated to transport sucrose with a higher affinity than the wild-type carrier [93].

5. Conclusions and future prospects

The existence of several sequences related to al-

ready known sucrose carriers in the *Arabidopsis* database indicate that a whole family of sucrose transporter genes is present in one single plant. The total number is still unknown as it will certainly increase at the same pace as new sequences are released. The next challenge will be to unravel the exact function of the new genes and their role in the plant. It is reasonable to expect that carriers with different specificities and/or kinetic properties will be identified. Carriers with different affinities for sucrose will certainly be found as different cell types encounter different sucrose concentrations. However, as in the case of hexose carriers, the expression pattern of some of these carriers might be very restricted to certain cell types or developmental phase, making their localisation more difficult. However, the knowledge of all the sequences for sucrose transporters will be of considerable interest for the structure/function studies.

Another challenge will be to understand more about the way plants regulate the flow of sucrose both at the whole plant or cellular level. Whether homologous genes to the yeast glucose sensors (Snf3 and Rgt2) are present in plants is a very stimulating problem for the understanding of gene regulation in plants. This will of course relate to former physiological questions such as the priority among different sinks (see Fig. 1) competing for sucrose delivery and use. These problems are related to plant productivity as a high harvest index is obtained when the harvested organs (sinks) received a significant portion of the exported sucrose. It might therefore be conceivable to modify the flow of sucrose to a particular sink by changing the expression of selected carriers and/or sensors. This future direction of work will be reasonable to follow when all carriers are known and precisely characterised.

The other sucrose transporters involved in different transport events (tonoplast carrier, efflux carrier, sink-specific carriers) will also have to be identified. No indication exists, for example, that the tonoplast sucrose carrier is related in sequence to other sucrose carriers, although there are antiporters that are members of the Major Facilitator superfamily, but not in the same cluster. New methods will have to be designed and used for these identifications.

As already noted, the structure/function studies on the sucrose carriers are still in their infancy. Nevertheless, it is obvious that more information is needed on the residues relevant to some of the properties of the sucrose carrier such as selectivity and affinity. This may not only be interesting from a fundamental point of view but also as a possibility to alter the flow of sucrose to sink, or to alter the selectivity of the carrier so that it would accept foreign molecules (xenobiotics or natural) and allow their long distance transport in the plant. This was in fact one of the original aims for the identification of the sucrose carrier back at the beginning of the 1980s. Improving the quality of sinks is also a positive outcome to be expected. However, there is still much information that needs to be obtained. Of course the main problem is the lack of three-dimensional structure elucidated to date. In spite of efforts from several groups to overproduce membrane carriers, no crystallisation of any transport protein could be obtained. However, many data are still to be collected for reaching a knowledge level similar to the lac permease. A thorough review of the different experiments that led to the partial understanding of the structure/function of the lac permease is presented in [90]. Compared to the lac permease, our knowledge on the sucrose carriers is scarce not only on the structure but also on the function. Thanks to the oocyte expression, precise data are becoming available on the cotransport phase with proton. This system will certainly be invaluable for the study of mutagenised carriers altered in the coupling of the two substrates. Much information is surprisingly lacking on the function of the sucrose carrier in the absence of a proton gradient, its kinetics when the sucrose gradient is inverted (efflux?) and its precise selectivity. The lac permease displays the advantage that it can be overexpressed in its own native lipid environment, which allowed a precise study of all its kinetic characteristics. In plants, however, plasma membrane vesicles studies have not given such a level of information (for a review, see [15]) and the heterologous expression in yeast has been mainly used to confirm the nature of such carriers as sucrose transporters. Therefore, the lack of an expression system as convenient and efficient as *E. coli* might impair some of these studies.

It may still be a long time before a paper devoted to sucrose carrier structure will contain more than speculative data, but the prominent role of sucrose

transport and sucrose transporters for the growth of plants will certainly lead to a dramatic increase of our knowledge in the field.

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