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Cutting, ageing and expression of plant membrane transporters

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Abstract

The activity and the expression of sucrose, hexose and amino acid transporters were studied with fresh, cut or aged tissues and plasma membrane vesicles (PMV) of mature sugar beet (*Beta vulgaris* L.) leaves. Cutting and ageing both induced an increase of the transcripts coding for sucrose transporters and hexose transporters. No significant effect could be detected on the amino acid transporter transcripts with the probe used (aap1). A polyclonal serum directed against the *Arabidopsis thaliana* sucrose transporter (AtSUC1) reacted with a 42 kDa band of the sugar beet PMV, confirming previous biochemical identification of this band as a sucrose transporter. ELISA assays run with microsomal fractions and PMV using the AtSUC1 sucrose transporter probe indicated that ageing, and to a lesser extent cutting, increased the amount of sucrose transporter present in the plasma membrane. However, while cutting strongly stimulated proton-motive force driven uptake of sucrose in PMV, ageing only resulted in a slight stimulation. These data give evidence for transcriptional, post-transcriptional and post-translational controls of the activity of the sucrose transporter by mechanical treatments. Proton-motive force driven uptake of 3-*O*-methylglucose and valine in PMV was strongly stimulated in PMV from aged tissues, although previous data had shown that cutting did not affect theses processes. Therefore, the plant cells possess various levels of control mechanisms that allow them to regulate fluxes of the main assimilates across the plasma membrane when their natural environment is directly or indirectly altered. © 1997 Elsevier Science B.V.

Keywords: Plasma membrane; Assimilate transport; Sugar transporter; Amino acid transporter; Wounding; Plant; (Sugar beet)

1. Introduction

Plant productivity depends in part on the ability of the leaf to export the products of assimilative reduction towards the storage organs, via the phloem. This long distance transport depends, in turn, on the activity of membrane transporters which control the export of sugars and amino acids from the assimilating cells towards the free space, their uptake into the conducting complex of the phloem, and their unloading from the phloem towards the receiving organs [1,2]. In the past few years, considerable insight has been gained into the activity of these transporters through the use of PMV [3]. Methods allowing their

Abbreviations: DEPC: diethylpyrocarbonate; 3-OMeG: 3-*O*-methylglucose; PM: plasma membrane; pmf: proton-motive force; PMV: plasma membrane vesicles; SSC: salt sodium citrate buffer

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reconstitution into proteoliposomes have been described [4,5]. After it had been shown that plant hexose transporter could be functionally expressed in yeast [6], a strategy of mutant complementation became possible. This led to the cloning of the sucrose [7,8] and amino acid [9–11] transporters from higher plants.

These recent advances provide a favorable background to study the regulation of sugar and amino acid transport with physiological models such as sink/source transition [12,13]. Another approach to study the regulation of transport consists in artificial perturbations brought to the normal transport physiology of the plant. In this regard, both ageing (incubation of excised tissues for several hours in a simple medium) and wounding are well known to affect dramatically the exchange of organic solutes [14]. In their natural environment, plants undergo a variety of mechanical stresses due to wind, feeding by cattle and insects, agricultural practice (pruning, grafting, mowing, etc.) and attacks by phytopathogens. Cutting (or wounding) and ageing are experimental treatments which may be related to these natural events, which all involve breakage of some cells and modifications of the apoplastic compartment.

Sakr et al. [15] compared the effects of cutting and ageing on sucrose, hexose and amino acid uptake by sugar beet leaf discs. Cutting (i.e., separation of the leaf from the plant, and dipping of the petiole for 12 h in distilled water) selectively increased sucrose uptake into discs made from the detached leaf, compared to discs freshly excised from the plant. Comparison of the uptake in leaf discs with the pmf-driven uptake of sucrose into PMV prepared from fresh or cut leaves indicated that the selective increase in sucrose absorption induced by cutting was actually due to changes in membrane transport activity, and not to increased metabolism inside the cells [15].

A detailed study of the expression and activity of the proton-pumping H^+–ATPase which energizes solute uptake across the PM showed that cutting affected the expression, but not the activity of this enzyme as measured in vivo with leaf tissues and in vitro with purified PMV [16]. Therefore, the increase in sucrose uptake induced by this treatment depended only on a change in the activity of the sucrose transporter, which had previously been demonstrated by uptake measurements with PMV [15].

In contrast, ageing promoted both the expression and the activity of the PM H^+–ATPase, indicating that this enzyme was involved in the strong and general increase of sugar and amino acid uptake observed after ageing in leaf tissues [16]. However, it cannot be excluded that ageing also affects the expression and/or the activity of the transporters.

Cutting and ageing therefore induce distinctive changes in the pattern of solute uptake, and provide useful models to study the regulation of transport. A previous study was focused on the effects of cutting on sucrose transport at the membrane level [15]. The present work further investigates this model by comparing pmf-driven solute uptake in PMV from fresh and aged leaves, and by studying the expression of sucrose, hexose, and amino acid transporters in fresh, cut and aged leaves.

2. Materials and methods

2.1. Plant material

Sugar beet (*Beta vulgaris* L.) plants were grown as described previously [17]. Three kinds of tissues were prepared from mature exporting leaves [16]: Fresh tissues (sampled immediately after separation of the leaf blade from the plant), aged tissues (floated for 12 h in darkness on a medium containing 300 mM mannitol, 0.5 mM $CaCl_2$, 0.25 mM $MgCl_2$), and tissues from cut leaves. In the latter case, the leaves were excised from the plant by cutting their petiole near the crown. The petiole was immersed in distilled water, and the isolated leaves were kept for 12 h in the dark, and saturating humidity, to avoid excessive transpiration.

Highly purified PMV were obtained by phase partitioning of microsomal fractions from fresh, cut and aged tissues. Phase partitioning was run in a Dextran T500/Polyethylene glycol 3350 mixture as described by Lemoine et al. [18]. PMV from fresh, cut and aged tissues, do not differ in purity [16].

2.2. Uptake experiments

Pmf-driven uptake of sucrose, 3-OMeG and valine was studied according to [15], with PMV energized by a combination of transmembrane pH gradient

(ΔpH, pH 7.5 inside, pH 5.5 outside) and transmembrane potential difference ($\Delta\psi$, inside negative, due to the diffusion of internal potassium in the presence of valinomycin). Passive uptake was monitored in parallel experiments with PMV resuspended in an incubation medium that was identical to the equilibration medium, so that no pH or electrical gradient was created across the PM. Uptake experiments were run at 23°C with incubation media containing 1 mM labelled compound at a final specific activity of 13 MBq $mmol^{-1}$. The labelled compounds used, obtained from Amersham France, were as follows: [6,6′(n)-^{3}H]sucrose (or in some cases [U-^{14}C]sucrose), [1-^{3}H]3-*O*-methylglucose and L-[3,4(n)-^{3}H]valine. All uptake data presented report on pmf-driven uptake defined as the difference between uptake measured in the presence of $\Delta pH + \Delta\psi$, and uptake measured in the absence of these gradients.

In vitro measurements of pmf-driven uptake of sugars and amino acids provide a direct means of assessing the activity of the transporters. Indeed, in this system, the pmf driving the activity of these transporters is created by the experimenter, and not linked to the activity of the PM H^+–ATPase which is normally active in vivo.

2.3. RNA preparation and analysis

RNA extraction and Northern blot analysis were run as previously described [16]. The quality and quantity of RNA blotted on the nylon membrane were checked under UV illumination. The RNA were fixed to the membranes at 80°C for 2 h, and prehybridized for 8 h at 42°C in a medium composed of 6 × SSC (90 mM NaCl, 12.5 mM Na citrate, pH 7.0), 50% formamide, 2 × Denhardt's solution and 25 μg ml^{-1} salmon sperm DNA [19]. Hybridation was run for 16 h at 42°C in the same medium after addition of the labelled probe.

The probes used were a 1.5 kb fragment of the BvSut1 clone (*B. vulgaris* sucrose transporter, accession number X 83850), SoSut1 clone (*Spinacia oleacera* sucrose transporter; [7]), stp1 *Arabidopsis thaliana* hexose transporter (1.9 kb; [6]), and aap1 *A. thaliana* amino acid transporters (1.7 kb; [9]). The probes were labeled with [^{32}P]ATP to a specific activity of about 1.2 10^9 cpm μg^{-1} with a random priming labeling kit (Amersham France). For blots with BvSut1, the membranes were washed at 68°C (3 × 20 min) in 2 × SSC and 0.5% SDS. For blots with stp1, the membranes were washed once in 1 × SSC and 0.1% SDS for 30 min at 42°C, and 2 times in 0.1 × SSC and 0.1% SDS for 30 min at 42°C. For blots with aap1, the membranes were washed at 60°C (3 × 20 min) in 2 × SSC and 0.1% SDS. The blots were repeated three times with independent samples.

2.4. Immunological methods

Western blots with PMV from fresh, cut and aged tissues were run using a rabbit polyclonal antiserum directed against a sucrose transporter from *A. thaliana* (AtSUC1, [20]). The blots were made as described in [16], using 50 μg purified PM proteins for each lane, and repeated two times with independent samples. The primary antiserum and the secondary antibodies (horseradish peroxidase-conjugated goat antirabbit antibody) were used at a 1/100 and at a 1/2000 dilution respectively.

The amount of protein reacting with the AtSUC1 antiserum in microsomal fractions and in PMV from fresh, cut and aged tissues was estimated by ELISA as described in [16]. These measurements were run in triplicate on two independent preparations for each set of membranes. The primary antiserum and the secondary antibodies (horseradish peroxidase-conjugated goat antirabbit antibody) were used at a 1/200 and at a 1/2000 dilution respectively.

3. Results

We have previously shown that cutting induces a selective increase in sucrose uptake both in leaf discs and in purified PMV [15]. Fig. 1 shows the time course of pmf-driven uptake of sucrose, 3-OMeG and valine into PMV prepared from fresh or aged sugar beet leaf tissues. Uptake of these substrates in pmf-energized PMV of sugar beet leaves remains linear for at least 1 min ([21–23]. Ageing stimulated pmf-driven uptake of sucrose to some extent (Fig. 1A), although the initial rate of uptake was hardly affected. Pmf-driven uptake of 3-OMeG (Fig. 1B) and valine (Fig. 1C) were strongly enhanced in PMV from aged tissues. The initial rates of uptake were in the same range (5 to 7 nmol after the first min of

incubation) for the three compounds tested in control PMV. The stimulation of the initial rate of uptake induced by ageing was about 3-fold for 3-OMeG and valine. Maximal uptake was reached after 2 min of incubation for sucrose and 3-OMeG, but only after 1 min for valine. After the maximal value was reached, uptake of sugars and valine plateaued at the same value in control PMV, but the concentration of sucrose and 3-OMeG somewhat decreased in aged PMV, indicating partial efflux of the sugars taken up. This efflux did not occur for valine, even in aged PMV.

We have previously shown that the increase of sucrose uptake induced by cutting was due to a significant increase of the V_{max} of the sucrose transporter, and to a small decrease of its K_m [15]. The kinetics of pmf-driven hexose and amino acid uptake

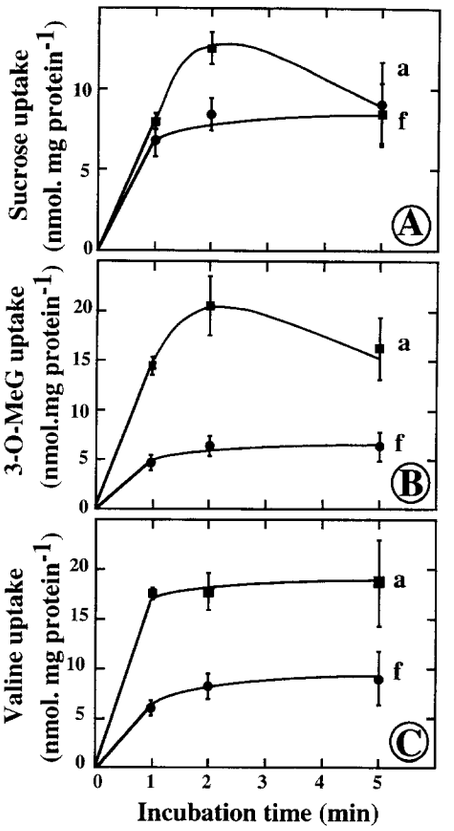

Fig. 1. Time course of pmf-driven sucrose (A), 3-OMeG (B) and valine (C) uptake in PMV from either fresh (f) or aged (a) sugar beet leaves. Means of 12 measurements (from 3 independent experiments) ± SE.

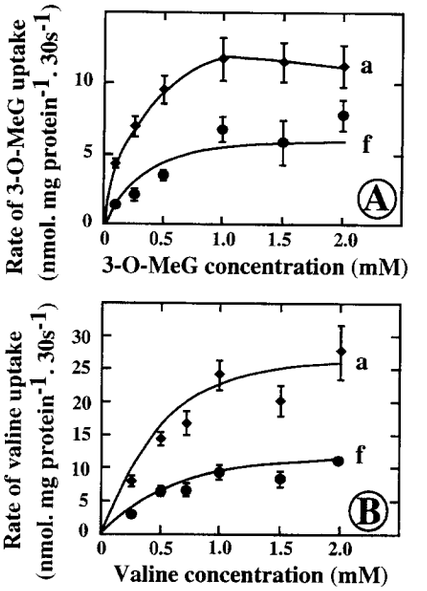

Fig. 2. Kinetics of 3-OMeG (A) and valine (B) uptake in PMV from fresh (f) and aged (a) leaves. Means of 9 measurements (from 3 independent experiments) ± SE. Initial rates of uptake were measured during the first 30 s of incubation.

in PMV prepared from either fresh or aged tissues are shown in Fig. 2. Analysis of the data using Hanes plots (Fig. 3) indicated that ageing decreased the K_m of the transport system mediating pmf-driven uptake of 3-OMeG (0.86 mM vs. 0.37 mM in PMV from fresh and aged tissues, respectively), and increased the apparent V_{max} (20.4 vs. 28.6 nmol 3-OMeG mg protein^{-1} min^{-1} in PMV from fresh and aged tissues, respectively). Ageing resulted in a strong increase of the V_{max} of the valine transport system (74.0 vs. 28.6 nmol valine mg protein^{-1} min^{-1} in PMV from aged and fresh tissues respectively) whereas the K_m was not significantly affected (0.75 mM).

Increase of sucrose uptake induced by cutting was accompanied by a loss of sensitivity to *N*-ethylmaleimide of the uptake system in PMV [15]. In the present paper, we used mersalyl (permeant thiol reagent) and DEPC (imidazole reagent) to test whether changes in the sensitivity to chemical reagents also accompanied the stimulation of hexose and amino acid uptake induced by ageing. The uptake of 3-OMeG exhibited the same sensitivity to mersalyl in fresh and aged PMV (Fig. 4A). However, pmf-driven 3-OMeG uptake was not inhibited by DEPC in fresh

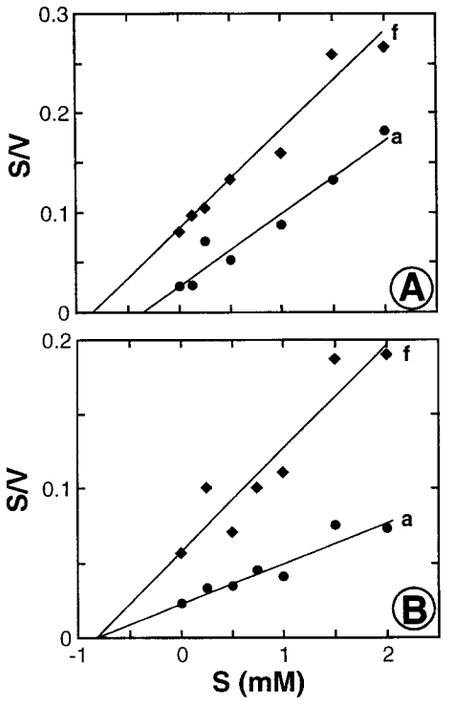

Fig. 3. Hanes plots of 3-OMeG (A) and valine (B) uptake in PMV from fresh (f) and aged (a) leaves. Data are derived from Fig. 2. The intercepts of the lines with the abscissa give K_m, and the intercepts of the lines with the ordinate axis correspond to K_m / V_{max}.

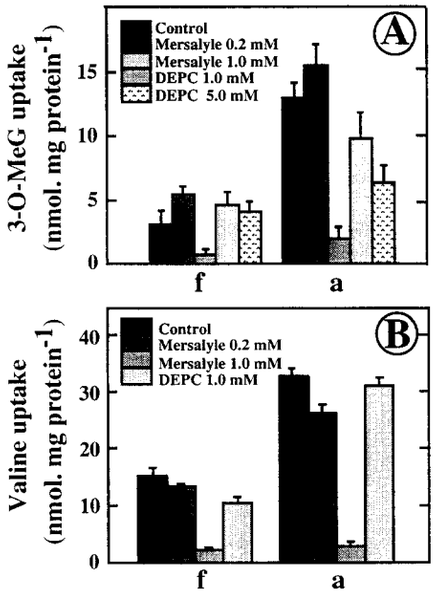

Fig. 4. Sensitivity of pmf-driven uptake of 3-OMeG (A) and valine (B) to mersalyl and DEPC. Means of 12 measurements (from 3 independent experiments) ± SE.

PMV, while it was inhibited 50% by 5 mM DEPC in aged PMV. The sensitivity of pmf-driven valine uptake to mersalyl and DEPC was not significantly affected by ageing (Fig. 4B).

The sensitivity of uptake to various substrates that are possible competitors of the transporters was studied (Fig. 5). Pmf-driven uptake of 3-OMeG was not sensitive to sucrose, either in fresh and aged PMV, confirming previous data obtained with PMV from fresh sugar beet leaves [23]. Glucose was a strong inhibitor of uptake in both sets of vesicles. Fructose did not inhibit 3-OMeG uptake in PMV from fresh leaves (Fig. 5A), as expected from earlier data with fresh leaf discs [24]; however, it was a strong inhibitor in PMV from aged tissues (Fig. 5A). Likewise, the sensitivity of valine uptake to other amino acids was different in fresh and aged PMV. Glu and Leu were much stronger inhibitors of pmf-driven valine uptake in aged PMV than in fresh PMV, while the sensitivity of valine uptake to Arg was not affected by ageing (Fig. 5B).

The effects of cutting and ageing on the amounts of transcripts corresponding to sucrose, glucose and

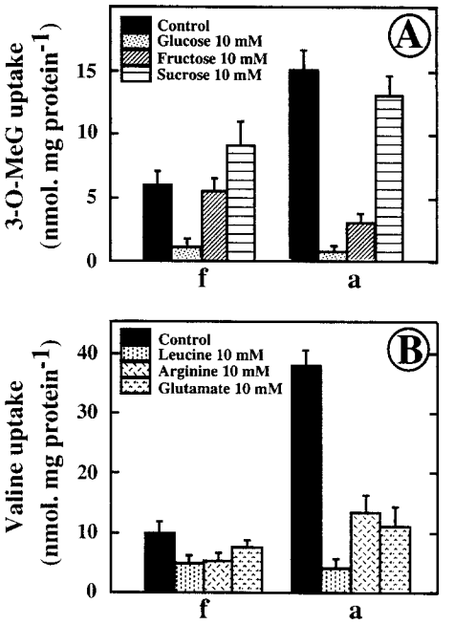

Fig. 5. Effect of various substrates on pmf-driven uptake of 3-OMeG (A) and valine (B) uptake in PMV from fresh and aged tissues. Means of 8 measurements (from 2 independent experiments) ± SE for 3-OMeG and of 12 measurements (from 3 independent experiments) ± SE for valine.

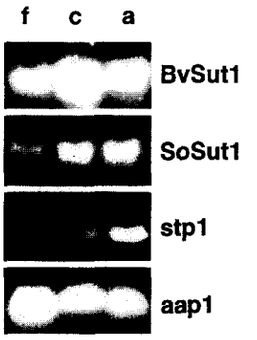

Fig. 6. Northern analysis of RNA extracted from fresh (f), cut (c) or aged (a) leaves, using the BvSut1 (sugar beet sucrose transporter), SoSuT1 (spinach sucrose transporter), stp1 (*A. thaliana* hexose transporter) and aap1 (*A. thaliana* amino acid transporter) probes. Similar patterns were obtained with RNA extracts from 3 independent experiments.

amino acid transporters were studied by Northern blot, using the BvSut1 sucrose transporter probe from sugar beet, the SoSut1 sucrose transporter probe from spinach, the stp hexose transporter probe and the aap1 amino acid transporter probe from *A. thaliana*. Northern blots run with the sucrose transporter probes indicated that both excision and ageing increased the levels of transcripts hybridizing with BvSut1 and SoSut1 (Fig. 6A and B). Both treatments also enhanced the levels of the hexose transporter transcripts (Fig. 6C), but ageing was more efficient than cutting. Cutting and ageing slightly but consistently decreased the levels of the transcripts hybridizing with the aap1 probe (Fig. 6D).

A rabbit polyclonal serum directed against the

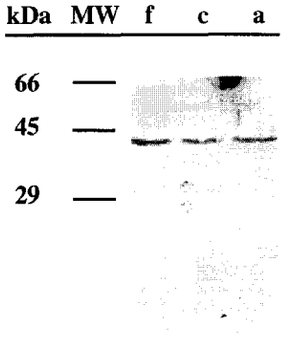

Fig. 7. Western analysis of PM proteins from fresh (f), cut (c) and aged (a) leaves with anti-AtSUC1 serum. 50 μg proteins were deposited in each lane. Similar results were obtained when protein amounts ranging from 25 to 75 μg were deposited. Position of the molecular mass markers is shown on the left.

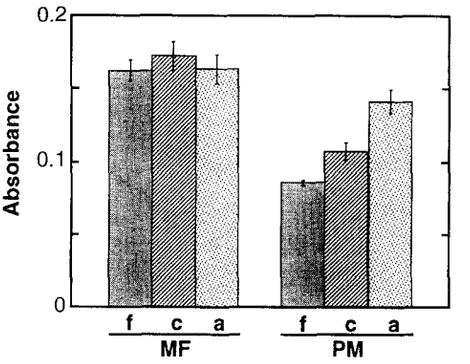

Fig. 8. ELISA estimation of the amount of proteins reacting with the anti-AtSUC1 serum in microsomal and in PMV fractions from fresh (f), cut (c) and aged (a) tissues. Data are means of triplicate ± SE for each sample. The experiment was repeated with another set of samples with similar results.

AtSUC1 sucrose transporter reacted with a single band with an apparent M_r of 42 kDa, in sugar beet PMV prepared from fresh, cut or aged tissues (Fig. 7). In some experiments, a faint additional band at 22 kDa was visible. Visual examination did not allow to detect a difference in the intensity of staining of the 42 kDa band in the three lanes. However, ELISA measurements indicated that ageing and, to a lesser extent, cutting increased the amount of PM material reacting with the AtSUC1 antiserum (Fig. 8). In contrast, none of the treatments affected the immunoreactivity of the microsomal fraction.

4. Discussion

Mechanical stresses induce a wide range of hormonal and biochemical responses allowing the plant to cope with these stresses, to some extent. One of the first targets of environmental perturbation is the plasma membrane whose turn-over appears as a highly dynamic process [25]. Stress-induced changes in membrane transport capacities have been studied mainly with respect to fungal infection. The PM H^+–ATPase is a major target of fungal infection. This ATPase may be activated by elicitor-induced dephosphorylation [26,27]. Fusicoccin, a fungal phytotoxin, may also activate the ATPase, by a mechanism that would result in the displacement of the C-terminal autoinhibitory domain of the enzyme [28,29]. Recent evidence was obtained suggesting that fungal attack may also affect the activity of sugar

transporters. It was shown that gene expression of a sink specific hexose transporter could be induced by wounding, bacterial elicitors and fungal infection [30]. The regulation of membrane activity by phytopathogens may therefore concern the transcriptional and post-translational levels.

Another way to study the effect of stresses on membrane transport is the use of artificial treatments such as cutting and ageing, which both involve the breakage of some cells and alter their natural environment, but may involve different pathways. In our cutting system, a signal (physical or chemical) may be transported over several centimeters from the site of cutting (the petiole) to the leaf blade used to make the measurements. In the ageing system, a stronger mechanical treatment (peeling of the lower epidermis of the blade) is made locally on the samples which are bathed in a large volume of artificial apoplastic medium before the measurements. Both treatments may also induce variations in the nutritional and hormonal status of the tissues, as well as modifications of the intercellular communications.

Table 1
Summary of the effects of cutting and wounding on the activity and expression of the PM H^+–ATPase, of the sucrose, hexose and amino acid transporters of the plasma membrane

	Cut	Aged
PM H^+ – ATPase [a]		
Leaf tissues [c]	114	178
PMV [d]	111	221
Transcripts	+ +	+ + +
Protein [e]	155	200
Sucrose transport		
Leaf tissues [f]	250 [b]	440 [b]
PMV [g]	165 [b]	113
Transcripts	+ + +	+ + +
Protein [h]	120	162
Hexose transport		
Leaf tissues [f]	113 [b]	300 [b]
PMV [g]	50 [b]	353
Transcripts	+ +	+ + +
Valine transport		
Discs [f]	111 [b]	400 [b]
PMV [g]	127 [b]	430
Transcripts	=	=

The table summarizes the data presented in this and in two previous papers ([a] [16]; [b] [15]). All activities are expressed as a percentage of the corresponding activity in samples from fresh leaves (100%), except the transcripts which were estimated visually on Northern blots (=, same amount as in fresh tissues; + +, + + +, increases in the amount of transcripts).

[c] Acidifying activity of leaf tissues measured by titration with NaOH.

[d] Proton pumping in PMV measured by the decrease in amino acridine absorbance.

[e] ATPase amount estimated on PMV by ELISA on PMV with anti-ATPase sera.

[f] Rate of uptake measured in leaf discs.

[g] Rate of pmf-driven uptake measured in PMV.

[h] Sucrose transporter amount estimated on PMV and microsomal fractions (MF) by ELISA with anti-AtSUC1 antiserum.

Previous work has characterized the cutting and ageing system from a physiological point of view, and studied the effect of cutting on sucrose transport at the membrane level [15], as well as the effect of both treatments on PM H^+–ATPase expression and activity [16]. In the present paper, we have further characterized the effects of these treatments on the activity of the sucrose, hexose and amino acid transporters, and we have used molecular and immunological probes to investigate the levels of control involved on the transporters. For convenience, data from the present study, and from Refs. [15,16] are summarized in Table 1.

4.1. Sucrose transport

The initial rate of pmf-driven uptake of sucrose transport into purified PMV from aged tissues was slightly higher than in PMV from fresh tissues (Fig. 1A). This is in contrast with the fact that ageing induce a 3-fold increase of sucrose uptake in leaf discs [15]. These data, together with the demonstration of a strong stimulation of PM–H^+ ATPase activity during ageing [16], strongly suggest that the stimulation of sucrose uptake in aged leaf discs is essentially an indirect consequence of a change in the activity of the ATPase, but not of the sucrose transporter. However, one cannot discard the possibility that a factor involved in post-translational activation of the sucrose transporter is lost during the preparation of the PMV.

Antibodies directed against AtSUC1 react with a single band at 42 kDa in PMV from sugar beet (Fig. 7). This is in good agreement with the former biochemical and immunological identification of a 42 kDa band as a sucrose transporter in the same material [4,17,31–33] and in PMV from broad bean [34].

The discrepancy between the molecular mass predicted by the sequence and the molecular mass observed on the gel is due to the high hydrophobicity of the membrane transporters, which results in abnormal migration patterns [35,36]. In *Arabidopsis*, the AtSUC1 antiserum reacts with a 43 kDa band ([37]; Stadler and Sauer, unpublished).

Both cutting and ageing increased the levels of transcripts hybridizing with sucrose transporters probes from sugar beet and spinach (both belonging to the family of *Chenopodiaceae*). This effect seems specific, since it was not detected after hybridization with the aap1 amino acid transporter probe. Ageing, and to a lesser extent cutting, also increased the amount of protein recognized by the AtSUC1 antiserum (Fig. 8). Yet, pmf-driven uptake of sucrose is hardly stimulated after ageing (Fig. 1), while it is markedly enhanced after cutting (Table 1). Mechanisms allowing post-translational control of the activity of the sucrose transporter therefore exist in the plant cell.

4.2. Hexose transport

Ageing, and to a lesser extent cutting enhanced the levels of hexose transporter transcripts (Fig. 6) in mature blades of sugar beet, which fits well with recent data obtained on wounded tissues of *Arabidopsis* [30]. However, a strong stimulation of pmf-driven uptake of 3-OMeG was observed in purified PMV from aged tissues, but not in PMV from cut tissues (Fig. 1; [15]). These data, as well as those described above on the sucrose transporter, stress the importance of measuring enzyme activities, and not only the transcript levels after various experimental treatments. They are in good agreement with effects of cutting and ageing on hexose uptake in leaf discs [15]. Although no antibodies were available to estimate the amount of hexose transporter present in the PMV, it may be inferred that cutting of the petiole was able to trigger remote transcription of the hexose transporter genes in the leaf blade, but not the synthesis and correct targeting of the corresponding protein. In contrast, ageing, which involves a more drastic and local mechanical treatment of the leaf blade, resulted in a stimulation of hexose transport together with the rise of hexose transporter transcripts. The set of hexose transporters induced by ageing may be different from that normally functioning in fresh tissues, since pmf-driven hexose uptake was sensitive to DEPC and fructose in PMV from aged tissues, but not in PMV from fresh tissues (Figs. 4 and 5). The apparent K_m of the hexose transport system was also affected by ageing (Fig. 3A). Induction of hexose transport activity by mechanical treatment may be related to the induction of a cell-wall invertase [38] and to the elicitor-induced gene expression of an hexose transporter [30] recently described. These processes likely belong to a common set of responses dedicated at limiting pathogen invasion.

4.3. Amino acid transport

Pmf-driven amino acid uptake in PMV was strongly enhanced after ageing (Fig. 1C), but not after cutting [15], which correlates well with the measurements made on leaf discs [15]. No significant change in the transcripts hybridizing with the aap1 probe was observed in Northern blot (Fig. 6). However, one cannot discard the idea that the transcription and synthesis of specific classes of amino acid transporters is induced by ageing, since amino acid transport in plants may be mediated by several members of the AAP [39], and ProT [11] families which differ more or less in substrate specificity. This would explain the different sensitivity to various competing amino acids observed after ageing (Fig. 5). The ageing-induced increase in V_{max} of pmf-driven amino acid transport might be due to a higher density of amino acid transporters in the PM, and/or to a conformational change leading to a better coupling between amino acid transport and the imposed pmf. In the same way as water stress specifically induces proline transporters [11], amino acid transporters mediating the transport of amino compounds appearing after mechanical treatment (for example γ-aminoisobutyric acid) might be induced. This hypothesis has not been tested sofar.

5. Conclusions

The data summarized in Table 1 show that, after mechanical treatment, the plant cell may alter transcriptional, translational and post-translational controls of its membrane enzymes involved in assimilate

transport: viz. the PM H^+–ATPase, the sucrose, hexose and amino acid transporters. Cutting of the petiole induced, at a remote distance, a selective increase of sucrose transport in the leaf blade, observed both in subsequently prepared leaf discs and PMV. It also increased the level of ATPase transcripts, but did not affect the total activity of this enzyme. It may be concluded that the rise in sucrose uptake capacity of the leaf discs, induced by cutting, is only due to a direct change in the activity of the sucrose transport system, and not to an effect on the PM H^+–ATPase activity [16]. The amount of 42 kDa polypeptide reacting with AtSUC1 antiserum is increased only by 20% after cutting, whereas pmf-driven sucrose uptake is increased 65% (Table 1). This suggests that this change in the sucrose transporter activity induced by cutting results from an increased amount of the transporter in the PM, as well from an increased specific activity of the transporter.

Ageing was accompanied by a general and local stimulation of sucrose, hexose and amino acid uptake by leaf discs, the strongest effect being observed with amino acids (valine), and the smallest with sucrose [15]. The data presented here indicate that, for hexose and amino acids, this is at least due partly to an increase in the activity of the corresponding transporters at the membrane level. Yet, only a small change in pmf-driven sucrose uptake could be detected. It may be concluded that the stimulation of sucrose uptake observed in leaf discs after ageing is due mainly to the stimulation of the PM H^+–ATPase, whereas the increased hexose and amino acid transport activities induced by ageing result from a combination of the stimulation of the activity of the ATPase and of the hexose and amino acid transporters. The discrepancy between the increased amount of protein reacting with the AtSUC1 antiserum after ageing, and the activity of sucrose transporter measured in vitro (Table 1) suggests that post-translational processes may regulate the activity of the sucrose transporter.

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