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Review

Regulation of sugar, amino acid and peptide plant membrane transporters

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Abstract

During the past few years, various cDNAs encoding the proton cotransporters which mediate the uptake of sucrose, hexoses, amino acids and peptides across the plant plasma membrane have been cloned. This has made possible some preliminary insight into the regulation of the activity of these transporters at various levels. The paper summarises the present status of knowledge and gaps relative to their transcriptional control (organ, tissue and cell specificity, response to the environment) and post-transcriptional control (targeting and turnover, kinetic and thermodynamic control, lipidic environment, phosphorylation). This outline and the description of a few cases (the sink/source transition of the leaf, the pollen grain, the legume seed) serve as a basis for suggesting some directions for future research.

Keywords: Development; Membrane; Plant; Regulation; Secondary transporter; Stress

1. Introduction

The scope of this review is to appraise the present knowledge concerning the regulation of the membrane transporters mediating the influx of sugars, amino acids and peptides across the plasma membrane and tonoplast of plant cells and to suggest some lines of research that might be relevant for future studies. Membrane transport activities are qualitatively important for eukaryotic cells which invest about 12% of their genomic information in transport proteins [1]. Membrane transporters have a dual function in providing part of the nutrients necessary for cell growth and in transducing environmental and endogenous signals. One may, therefore, expect that these activities are controlled in a tight and complex way. Due to the very rapid progress made in the identification of these proton-coupled cotransporters since the early 1990s [2–7], there have been a number of excellent and comprehensive reviews on their identification and functioning ([1,8–16], see also the articles in this special issue), but the topic of transporter regulation has never been specifically addressed to our knowledge. In contrast, there are many data concerning the regulation of the plasma membrane H^+-ATPase (for review [17]) and some of these data will be referred to when they may provide clues for understanding the regulation of transporters. Likewise, ion transporters and aqua-

Abbreviations: NEM, *N*-ethylmaleimide; PMV, plasma membrane vesicles

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porins, reviewed elsewhere [18], are not covered by this paper, except to give some examples of possible ways of regulation.

Throughout its life cycle, the plant is composed of a mosaic of tissues and cells whose heterotrophy/autotrophy for reduced carbon, nitrogen and sulfur may vary spatially and temporally. Morphogenesis, development, and in the end plant productivity therefore depend on a constant and fine regulation of transporters expression and activity, which allows coordinate exchanges between the different cells, tissues and organs. It has long been known from physiological data that long distance transport is controlled by environmental parameters [19] such as light [20], temperature [21,22], osmotic conditions [23–25] and by development [26,27]. Changes in assimilate partitioning are due to modifications of metabolism and/or assimilate transport. According to the mass flow hypothesis, long distance transport of assimilates mainly depends on loading in the source and unloading in the sinks. In many cases, these processes involve efflux of organic solutes to the apoplast and their retrieval either by the conducting complex (in the source) or the accumulating cells (in the sinks). Transmembrane transport events are, therefore, important for the control of long distance transport of assimilates. However, early physiological approaches were not able to identify controls acting on membrane transporter activity, because the necessary tools (molecular and immunological probes) were not available. Studies of sugar and amino acid uptake with organs or tissues also yielded complex kinetics involving one or two saturable phases and a non-saturable linear component (for review, see [28,29]). The interpretation of these kinetics (are they due to different transporters, or to

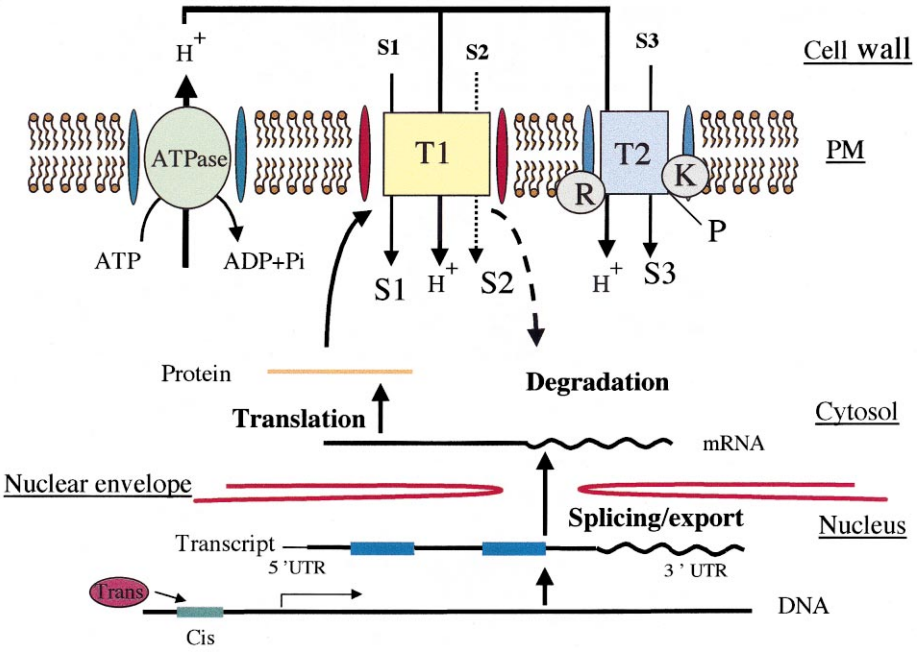

Fig. 1. Possible regulation of plant membrane transporters. The amount of transporter present in the membrane depends on its rate of synthesis, targeting and degradation. At the transcriptional level, *cis* and *trans* elements involved in the control of transporter gene expression during development and in response to the environment have not yet been identified. The role of the untranslated regions has not been studied. The targeting of the transporters to the plasma membrane and their degradation have not been studied, but may benefit of the study of yeast mutants (see Fig. 4). The transporters may be driven by both components ($\Delta\psi$ and ΔpH) of the proton motive force generated by a H^+-pumping ATPase. Several different transporters (T1, T2, T3) present in the same membrane compete for the proton motive force. Some transporters are multifunctional and their activity in vivo depends on the existing substrate gradients (s1/S1; s2/S2), with the substrate usually concentrated in the cell (S1, S2) compared to the external medium (s1, s2). The activity of a transporter may require specific sterols and lipids; it may be regulated by allosteric control by a regulatory protein (R), by ATP, and/or by phosphorylation/dephosphorylation by a kinase (K)/phosphatase. Data regarding these possibilities are summarised in the text.

different conformations of a single protein) were not possible.

Several important breakthroughs made during the last decade have allowed the start of more precise approaches concerning the regulation of transporters, i.e. the preparation and the use of purified plasma membranes and of reconstituted proteoliposomes to study the activity of the transporters in vitro, the cloning of sugar, amino acid and peptide transporters, largely based on yeast complementation, the use of heterologous expression systems to determine the substrate specificity and the thermodynamic aspects of transporter activity (yeasts and *Xenopus* oocytes) and the preparation of transgenic plants expressing various constructs (sense and antisense transporter cDNA under the control of ectopic or tissue specific promoters, promoter of transporter gene fused to reporter genes). However, our understanding of transporter regulation is still in its infancy, due to the complexity revealed by these data.

The regulation of transporter activity under the control of development and in response to biotic and abiotic signals may occur by modifications of transcription (gene activity), mRNA stability, mRNA translation and by post-translational control. Post-translational control includes any factor affecting the amount and activity of the transporter in the membrane, i.e. incorporation by exocytosis, turnover by endocytosis and proteolysis, the components of the proton-motive force (ΔpH and $\Delta\psi$), modification of transporter activity by the lipidic environment, by phosphorylation, redox status or interaction with other proteins.

Fig. 1 shows the main parameters potentially controlling the activity of the plant membrane transporters, and will be used as a frame for the present paper. Before summarising the main data available on these different types of control, it is worth mentioning some aspects of sugar sensing, a concept which was also developed during the last decade and which underlines a new facet of the importance of regulation of sugar transport.

2. Sugar transport and sugar sensing

In addition to the nutritive role of the transporters, there is ample evidence that the sugar and nitrogen status of the cell have profound effects on gene expression. Sugars may affect the expression of many genes involved in essential processes such as photosynthesis, glycolysis, glyoxylate cycle, nitrogen, sucrose and starch metabolism and cell cycle regulation [30,31]. Although most of the initial evidence for sugar sensing was obtained with high sugar concentrations, there are now clear indications that plant cells are able to sense sugars at low levels, either directly from the external medium or through changes in internal metabolic pools. For example, 1 mM glucose was able to repress *RBCS* expression in suspension cells of *Chenopodium rubrum* within 10 h [32]. Carbon and nitrogen metabolism are obviously interdependent (see for example [33]) and the expression of amino acid transporters is induced by glucose and glucose analogues, at least in algae [34].

Sugar-sensing and, therefore, the concentrations of sugars in the apoplast, in the cells and sugar transport may affect morphogenesis by control of cell division and metabolism at the transcriptional, translational and post-translational level. High-resolution mapping of glucose concentrations in tissue slices of broad bean cotyledons was recently achieved by quantitative bioluminescence and single photon imaging [35]. The results indicate the existence of very steep gradients of sugars, which were suggested to control morphogenetic processes as cell division and differentiation [35,36]. In agreement with this hypothesis, several data suggest that sugar transporters may be involved in cell-cycle regulation.

Three pathways have been described for sugar-sensing in plants. The first is an hexokinase-sensing system similar to the one existing in yeasts and mammalian cells and the two others involve transporters, i.e. a hexose transport associated sensor and a sucrose transporter that might be involved in a sucrose-specific pathway [31]. In addition to these multiple pathways, one of the major conceptual difficulties related to sugar-sensing is to understand how a metabolite present at high concentrations in all plant cells may play a role as a signal. In this regard, one may assume that sugar transporters with special substrate recognition or kinetic properties remain to be cloned that might be associated with sugar sensing. For example, although fructose is present at a low level in the intracellular and extracellular compartment of plant cells, no transporter

specific for fructose has yet been identified. In addition, overexpression of hexokinase1 (HXK1), but not glucokinase (GLK), can overcome the defect of glucose repression in the *hxk2* mutant of *Saccharomyces cerevisiae*, although overexpression of either *HXK1* or *GLK* provides similar glucose-phosphorylating activity [37]. Metabolites of fructose such as fructose-2,6-bisphosphate have well known functions as signals [38]. Data from yeasts suggest that a sugar sensor may act as a sugar transporter with a very low transport capacity, and transducing the sugar signal by a conformational change during sugar transport [16,39].

2.1. Sugar sensing and transcriptional control

The sensitivity of the cells to their environment is a necessary adaptive mechanism that has been conserved among prokaryotes and eukaryotes. In *E. coli*, yeasts and *Chlorella*, the sugar transporter genes are induced by their substrate. During the shift of *Chlorella* cells from carbon autotrophy to heterotrophy, hexose transport activity increases more than 200-fold [40]. The *HUP1* mRNA, absent in photosynthetically grown cells, appears within 5 min after addition of sugars [41]. *HUP2* and *HUP3*, two other *HUP* genes very homologous to *HUP1* are induced in response to addition of D-glucose to the medium. While HUP1 and HUP3 preferentially mediate glucose transport, HUP2 is more specific for galactose. The transcripts levels of the three genes reach a maximum 10–30 min after induction, but their pattern of expression slightly differs, with the maximum of *HUP2* expression being sharper and reached later. *HUP3* is induced at a much lower level than *HUP1* and *HUP3*. *HUP1* and *HUP3* genes are connected in tandem and their organisation differs from that of *HUP2* [42]. Altogether, the data show a fine tuning of hexose transporter expression, which concerns the strength and the time course of induction, as well as the substrate specificity of the induced transporters. However, the perception of glucose and the subsequent signal transduction have not been investigated in this model.

In higher plants, analysis of the expression of hexose transporter genes upon addition of glucose to photoautotrophic suspension-culture cells of *C. rubrum* indicated that these genes are constitutively expressed and not regulated by sugar [43]. The authors concluded that differential expression of sugar transporters in higher plants does not depend on substrate induction, but rather reflects tissue specific promoters not regulated by sugars. However, the possibility of sugar carrier genes which are under dual control (tissue-specific expression, and glucose regulation) still exists. Although several genes (sucrose synthase, granule bound starch synthase) were shown to be regulated by glucose in this experimental system, whether the data obtained from suspension cells may be fully extrapolated to the in vivo situation also remains to be ascertained. Analysis of the promoter of *VvHT1*, a hexose transporter gene expressed during grape maturation, reveals the presence of a sucrose box previously identified in several genes (chalcone synthase, sporamin) whose expression is induced by sugars [44]. In tobacco cells expressing the GUS reporter gene under the control of *VvHT1* promoter, GUS activity is enhanced in the presence of sugars (Atanassova et al., unpublished).

Concerning the sucrose transporters, a possible effect of sugar (glucose and sucrose) on the activity of the *AtSUC2* promoter was studied and excluded, both in excised leaves and young seedlings of transgenic *Arabidopsis* plants [45]. However, as stated by the authors, a possible down regulation of *SUC2-GUS* expression in response to sugars might have been overlooked due to the stability of the GUS protein. The *StSUT1* transcript is not inducible by feeding 6% sucrose to infiltrated leaves [46]. In contrast, feeding of excised sugar beet leaves with 100 mM sucrose (but not glucose) for 24 h, selectively decreased the expression of the sucrose transporter gene and the sucrose transport activity of plasma membrane vesicles (PMV) prepared from the leaves [47], suggesting a down regulation of *BvSUT1* expression by its substrate. This regulation seems specific inasmuch as the transport of glucose and alanine was not affected by sucrose treatments. Unfortunately, the sucrose concentration in the leaf after infiltration has not been measured and the physiological relevance of these data is uncertain so far. High concentrations (150 mM) of either sucrose or glucose also decreased *VfSUT1* transcript levels in sugar-fed cotyledons of developing broad bean seeds, but had no significant effect on the amounts of *VfSTP1* [36]. Such concentrations are not unrealistic

since the apoplastic sucrose concentration at the interface between cotyledons and surrounding seed coats of developing soybean cotyledons (25 days after flowering) was found to be in the range of 150 to 200 mM [48]. The data suggest that both *VfSUT1* expression and transfer cell differentiation are possible only at low sugar concentration.

In conclusion, it is likely that the sugar sensitivity of sugar transporter expression depends on the clone investigated, since the literature contains examples of transporters not sensitive [45], repressed [36,47] or stimulated ([41]; Atanassova et al., in preparation) by the presence of sugars.

In addition to their possible effect on sugar transporters, sugars may also affect the activity of the enzyme which energises these transporters. Sucrose induces the accumulation of the plasma membrane H^+-ATPase in tomato. This effect, which requires the metabolisation of the sugar taken up, would promote cell growth when the sugar supply is abundant [49].

The transition from quiescence to cell division cycle exhibits a characteristic pattern of gene expression. Three groups of genes are specific for the G_0-to-S transition, namely the immediate early, delayed early and late genes [50]. In animals, several metabolic genes, including one encoding a glucose transporter, belong to the group of delayed early genes which are specifically activated by mitogens during the G_0-to-S transition [51]. In plants, cytokinins stimulate the expression of a G1-type cyclin gene that encodes the D3 cyclin. Sucrose stimulates the expression of another *Arabidopsis* G1 cyclin gene encoding the protein D2. Thus quiescent tissue may be induced to divide in culture through the synergistic action of auxin, cytokinins, and carbon source, leading to the formation of an active CDK–G1 cyclin complex and the entry of these cells into the S phase of the cell cycle [52,53]. Our preliminary results suggest a differential regulation of a hexose transporter gene expression (i.e. *VvHT1*) in G1 and G2 phases of cell cycle of partially synchronised tobacco cells (Atanassova et al., unpublished).

Several sugar responsive elements have been described in the promoters of various genes induced [54,55] or repressed [56] by sugars and involved in sugar or protein metabolism. The existence of *trans*-acting factors for sugar repressible genes in plants was recently shown in the promoter of a rice α-amylase gene [56]. Both the transcription rate and mRNA stability of α-amylase gene increase in response to sucrose depletion. Sugar repression of α-amylase gene expression involves transcriptional regulation and three essential motifs are identified as components of the sugar response sequence. One of them, the TATCCA element is also known as an important part of the gibberellin response complex [57], thereby suggesting that sugar and hormone may share common steps in their signal transduction pathways. Analysis of glucose-insensitive mutants of *Arabidopsis* also revealed an unexpected convergence between the glucose and the ethylene transduction pathways, with the gene *GIN1*, conferring insensitivity to glucose, being placed downstream of the ethylene receptor [58]. Interestingly, the existence of several sugar boxes, including the TATCCA element shared with the gibberellin response complex has been described in the promoter of a hexose transporter whose expression is stimulated by glucose ([44]; Atanassova et al., in preparation).

In *C. rubrum* suspension cells, glucose and the fungal elicitor chitosan induce the expression of phenyl alanine ammonia lyase, extracellular invertase and repress the expression of RubisCO [59]. The same stimuli trigger the activation of protein kinase and the protein kinase inhibitor staurosporine enhances the induction of the various genes by glucose, whereas it inhibits their induction by chitosan. These data show that the sugar-sensing and the stress signalisation pathways may affect the expression of common targets, via a cascade of events which differ, but both involve phosphorylation/dephosphorylation processes [59].

2.2. Sugar sensing and translational control

Sucrose repression also involves translational control, as demonstrated for the *Arabidopsis* b-ZIP transcription factor gene *ATB2*. This leucine zipper protein is associated with processes of transport or utilisation of metabolites. The repression of *ATB2* mRNA translation involves a complex leader sequence with four small open reading frames and is specific for sucrose. As the expression of this transcription factor is also light-regulated, a model has been proposed for light and sucrose synergistic con-

trol of *ATB2* gene expression, which could balance carbohydrate availability and demand [60].

2.3. Sugar-sensing and post-transcriptional control

Rodriguez-Concepcion et al. [61] recently demonstrated a different subcellular localisation of a calmodulin (CaM53) in dark-incubated leaf explants in the absence and presence of sucrose. These modifications of localisation are induced by the prenylation of the C-terminus of CaM53. They suggest that CaM53 has a role in sugar sensing and signaling and that the Ca^{2+}-activated pathways are involved in sugar sensing signalisation. This hypothesis is strengthened by the observation that the expression of a CaM-like protein is induced in transgenic tobacco plants accumulating assimilates [62]. How the sugar sensing and transport are related to the prenylation of this protein is still unknown.

Finally, it should be noted that a direct involvement of sugar transport in sugar sensing would imply that sugars entering the cell via plasmodesmata (symplastic transfer) might be perceived differently from sugars absorbed from the apoplast [30].

In conclusion, all the data described above underline that the regulation of the proteins involved in the uptake of organic compounds, specially sugars, is an important issue for cell nutrition and gene expression. This regulation is detailed below.

3. Transcriptional control of transporters

The amounts and the nature of transporters present in a cell, which control in part its nutritional status, is primarily controlled by transcription of the corresponding genes, which allow tissue and cell specific expression.

3.1. Multigenicity, tissue and cell specific expression

Recent years have revealed that plants encode a myriad of plasma membrane transporters mediating fluxes of sucrose, hexoses, amino acids and peptides, not to mention numerous ion channels and ion transporters.

The sucrose transporters are much more substrate specific than the hexose transporters and they are encoded by a much smaller number of genes than the hexose transporters. Although cloning and identification of these proteins are not completed, the attempts made so far indicate that one (spinach [4]; potato [63]; rice [64]) or two (*Arabidopsis* [65]; *Plantago major* [66,67]; carrot [68]) sucrose transporters seem to be expressed, depending on the species. This does not exclude the existence of other sucrose transporters expressed in a very restricted number of cells for a short time (see the case of pollen below). The EST data base indicates that at least an additional gene exists in the *Arabidopsis* genome and, in tomato, three different genes have been identified [14]. In contrast, hexose transporters are encoded by a multigenic family comprising at least 12 members in *Arabidopsis*, eight members in *Ricinus communis* and seven members in *C. rubrum*. [1]. Both amino acid and peptide transporters exhibit a wide substrate specificity, but in *Arabidopsis* the amino acid transporter family includes more than 13 members, sorted in four groups [12], whereas two peptide transporters have been described in the same plant [7,69–71]. Evidence for a multigenic family encoding amino acid transporters also exists in broad bean (*Vicia faba*) [72].

The promoters of these genes allow a more or less tissue- and cell-specific expression, which has been studied through the amount (Northern analysis, RNase protection experiments) and localisation (in situ hybridisation) of transcripts, or more rarely by promoter–reporter gene fusion. Sucrose transporter transcripts are readily detected in Northern blots from total RNA whereas in many instances hexose transporter transcripts can be detected only by Northern analysis with mRNA or by RNase protection assays or RT-PCR ([43,44,73]. This suggests that sucrose transporters are usually expressed at higher levels than hexose transporters. However, in most cases, it is not known whether the low abundance of the transcripts in one organ or one tissue is due to a general low expression in all cells, or to a normal expression level in a few specialised cells of the sample. Precise localisation of the transporters at the cell level has been described only in a few cases.

3.1.1. Sucrose transporters

In potato, a single sucrose transporter cDNA seems to be present and its expression is higher in

the vascular tissues of mature leaves and roots, whereas stem and sink tissues (young leaves) show little expression (*StSUT1* [63]). RNA in situ hybridisation suggests that *StSUT*1 is expressed specifically in the phloem [63], more precisely at high levels in the sieve elements, where the labeling is associated with the branched plasmodesmata connecting the sieve tube and the companion cell and at lower levels in the companion cells [74]. These data, together with the immunocytochemical localisation of the StSUT1 protein in the sieve elements, suggest that *StSUT1* is transcribed in the companion cell and may be transported for translation in the sieve tube across the branched plasmodesmata. The possible regulation of transporter activity associated with this trafficking is still unknown. Although *StSUT1* is preferentially expressed in the phloem, the analysis of transgenic plants expressing antisense *StSUT1* under the control of either the constitutive CaM35S promoter or the companion cell specific promoter rolC indicates that the sucrose transporter is expressed not only in the companion cells, but also in other leaf cells [75]. Indeed, plants expressing antisense *StSUT1* under the control of rolC were not affected in proton-driven sucrose uptake measured with purified PMV. In contrast, this uptake is decreased in plants expressing antisense *StSUT1* under the control of CaM35S. Because the PMV used originate from all leaf cells, while the surface of the conducting complex accounts for less than 3% of the surface of all cells inside a leaf [76], this indicates that the bulk of StSUT1 protein is probably present in mesophyll cells, even if the expression in these cells, considered at the cell level, is less active than in the conducting complex. RolC-controlled antisense expression is sufficient to impair phloem loading and export, underlining the importance of phloem expressed transporters [75], but it is not known whether localised repression in non-phloem cells would not have the same impact. In the mesophyll, SUT1 transporters might be involved in the leakage and in the retrieval of sucrose from the apoplast.

In *Arabidopsis*, Northern analysis indicate that both *AtSUC1* and *AtSUC2* are expressed in young and mature leaves, and at a reduced level in flowers, but only *AtSUC1* is expressed in the roots [65]. The *SUC2* promoter directs expression of GUS activity to the phloem of all green tissues, such as the rosette leaves, the stems and sepals, and the developing pods [77]. Microscopic immunolocalisation suggests that AtSUC2 (homologous to StSUT1) is expressed specifically in companion cells. Antibodies to AtSUC1 do not label the conducting complex [45]. Moreover, these antibodies cross-react with a single 42 kDa band in PMV from sugar beet, which mainly originate from mesophyll cells, whereas no reaction was found with AtSUC2 antibodies [78]. This may suggest that *AtSUC1*, unlike *AtSUC2*, is mainly expressed in non-phloem cells of the leaf. In *Plantago major*, *PmSUC2* is expressed in all parts of the plants, except the fruits, whereas *PmSUC1* transcripts are most strongly and exclusively found in the flowers [67]. Like *AtSUC2*, *PmSUC2* seems specifically expressed in the companion cells [79]. Except in the flowers, PmSUC1 presents an even more vascular bundle-specific expression than PmSUC2 [67], and the protein has been localised in sieve elements. PmSUC1 may be responsible for the retrieval of sucrose being lost from the sieve element–companion cell complex on its way to the sink organs [80]. In carrot, one sucrose transporter (*DcSUT1*) is mostly expressed in the leaf lamina of source leaves and the other one (*DcSUT2*) in various sink organs including the flowers, tap roots and seeds [68]. In monocotyledons, the only sucrose transporter cloned so far (*OsSUT1*) is expressed in the leaf sheath, leaf blade and germinating seed of rice and no expression was found in sinks as the roots and the pedicles before heading [64].

3.1.2. Hexose transporters

Among the hexose transporters, although *AtSTP1* transcripts are most strongly expressed in source leaves [3], most of the other transporters cloned so far exhibit a higher expression in sink organs: roots, flowers and young leaves of tobacco for *NtMST1* [81], anthers and root tips for *AtSTP4* [82], pollen for *AtSTP2* [83] and *Pmt1* [84]. In castor bean (*R. communis*), *HEX3* is predominantly expressed in roots and sink leaves, whereas *HEX1* is mostly expressed in roots, hypocotyls and source leaves. Both clones show either poor or hardly any expression in the cotyledons [73]. The existence of a multigenic family encoding several homologous proteins makes it more difficult to prepare specific immunological probes against one given hexose transporter.

3.1.3. Amino acid transporters

The expression of the various amino acid transporters has been mostly studied in *Arabidopsis* and has been reviewed recently [12]. Most of the transporters are expressed in source and sink organs, but some of them exhibit more specific expression: *AtAAP1* in the seed, *AtAAP3* in roots and seedlings, *AtAAP6* in root and sink leaves. At the tissue level within the seed, histochemical analysis with promoter–GUS fusions indicate that *AtAAP1* is expressed in the endosperm and cotyledons whereas *AtAAP2* is expressed in the phloem of stems and in the vascular strands of siliques and in funiculi [85]. *AtLHT1*, a lysine histidine transporter is present in all tissues, but more strongly expressed in sink organs such as young leaves, flowers and siliques. In situ hybridisation also localised its expression on the surface of roots in young seedlings and in pollen [86]. *AtProT1*, a proline transporter is expressed in all organs, but highest levels are found in roots, stems and flowers. In the flowers, the transcripts were detected mainly in the floral stalk phloem that enters the carpels. *AtProT2* is expressed ubiquitously in the plant [70]. In potato, *StAAP1* and *StAAP2* are expressed only in the source leaf and in the stem [12]. In *Ricinus*, Northern analysis indicate that *RcAAP1* and *RcAAP2* are expressed abundantly in the cotyledon and roots and at a lower level in the endosperm and hypocotyl. *RcAAP1* expression was localised in the stele cells adjacent to the xylem poles by in situ hybridisation [87]. Several clones encoding amino acid transporters have been recently characterised in broad bean, a legume species [72]. *VfAAP2* was most strongly expressed in stems and at a lower level in sink leaves and pods. Three other clones (*VfAAPa*, *VfAAPb* and *VfAAPc*) were expressed at a high level in the flowers.

3.1.4. Peptide transporters

The *AtNTR1* peptide transporter clone is strongly expressed in developing pods, at intermediate levels in source leaves and at low levels in sink leaves, stem and roots [69]. *AtPTR2* cannot be detected by RNA gel blot hybridisation and after RT-PCR, it was detected only in the roots of *Arabidopsis* [7]. These transporters mediate the transport of various di- and tetrapeptides, with the highest affinity found for Leu–Leu. Peptide transport activity in leaf tissues, whose physiological function is still poorly understood, strongly decreases with leaf age [88].

Leaf cells also possess a plasma membrane H^+/glutathione transporter preferring oxidised glutathione and glutathione conjugates over reduced glutathione [89]. Glutathione is an important compound involved in sulfur transport, detoxication and the balance of redox conditions in the cell. Although the glutathione transporter has never been cloned in any prokaryotic or eukaryotic organism, recent work has allowed the identification of the glutathione transporter gene in yeasts and the preparation of yeast mutants that might be helpful to clone the plant gene and study its regulation (Bourbouloux et al., in preparation).

An ABC transporter localised on the vacuole is able to transfer glutathione conjugates from the cytoplasm to the vacuole of barley leaf cells [90]. This transporter, which has a poor affinity for reduced or oxidised glutathione, is energised directly by ATP hydrolysis, is involved in xenobiotic detoxication and is induced by treatment with specific safeners [91]. A similar, but different transport system, also induced by safener treatment exists for the glucoside conjugates of some herbicides [91].

3.2. Some examples of developmental control

In addition to cell specific expression, a tight control of expression in development has been described in a few cases including the sink/source transition of the leaves, pollen maturation and seed development.

3.2.1. Sink/source transition of the leaf

The young leaves import their assimilates symplastically and, during the sink/source transition, a number of events take place that allow apoplastic transport and phloem export. This transition occurs from the tip to the base of the leaf [27]. Microscopic observations show a general decrease of plasmodesmatal density between all cell types and this decrease is even more apparent between specialised interfaces. This results in the progressive symplastic isolation of the sieve tube/companion (transfer) cell complex [92]. Recent studies using GFP as a tracer of symplastic communications also show that the size exclusion limit of plasmodesmata can change during leaf development. GFP may be unloaded from class

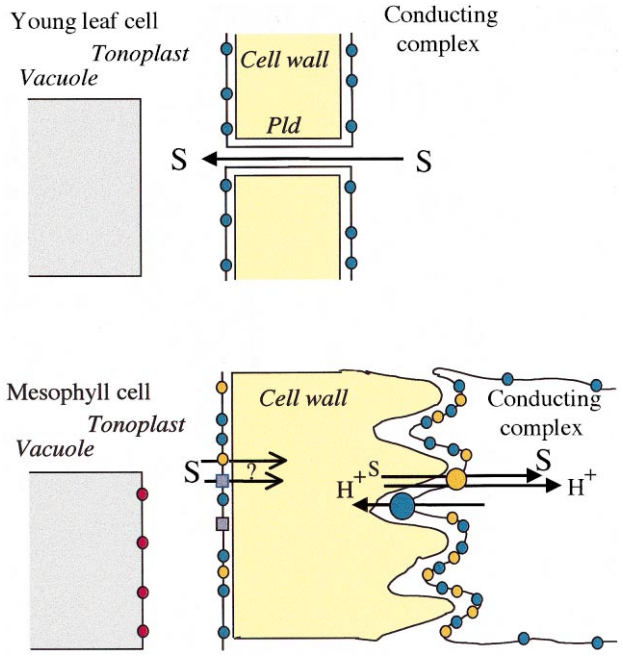

Fig. 2. Cell wall and membrane modifications accompanying the sink/source transition of the leaf. The sink/source transition is characterised by a closing of plasmodesmata (devoid of H^+-ATPase), the polarised incorporation of H^+-ATPase (blue circle) and sucrose transporters (yellow circle, purple square) and in the membranes, particularly the membrane bordering the conducting complex, as well as incorporation of a putative hexose transporter (red circle) in the tonoplast of parenchyma cells. It is not known if the transporter mediating sucrose efflux from mesophyll cells is the same as the influx transporter (yellow circle), or is a different one (purple square). Redrawn from data given in [63,77,94–98]. Further details and discussion are given in the text.

I, II and III veins, but not from minor veins [93]. In parallel with the decrease in plasmodesmatal density and permeability, the density of the H^+-ATPase allowing the functioning of proton-coupled transport strongly increases in the transfer cells [94]. It is interesting to note that the plasma membrane located in the plasmodesmata, involved in symplastic transport, is devoid of H^+-ATPase [95]. The polypeptide composition of the plasma membrane undergoes dramatic changes during the sink/source transition, which are accompanied by the incorporation of a sucrose transporter and an increase in the ability to take up sucrose [96,97]. During the sink–source transition, *StSUT1* expression is up regulated in potato [63] and likewise, *AtSUC2* expression proceeds from the tips to the base of *Arabidopsis* leaves, showing that it is coupled to their source strength [77]. In contrast, the expression of the monosaccharide transporter *NtMST1* is down-regulated during the sink/source transition [81], but in *Arabidopsis*, *AtSTP1* is expressed both in sink and source leaves [3]. There is convincing evidence for the tonoplast localisation of a putative hexose transporter which is upregulated during the sink/source transition of sugar beet leaves, but whose function was not successfully tested in yeast [98]. Contrary to many other hexose transporters, this clone is easily detected by Northern blots and its expression product is clearly seen on Western blots, which suggests a mesophyll localisation. The signals coordinating the decrease in plasmodesmatal frequency, plasmodesmatal permeability and the appearance of sugar transporters are still unknown. The data relative to the sink/source transition are summarised in Fig. 2.

3.2.2. The pollen grain

The male gametophyte or pollen grain is symplastically isolated from the sporophytic tissue and uptake of sugars and amino acids is required to support pollen maturation, germination and growth of the pollen tube. During their dehydration, the pollen grains accumulate high levels of proline and sugars. After germination, the growth of the pollen tube in the stylar fluids of the pistil over several millimetres or centimetres requires a considerable amount of energy and material for the constant de novo synthesis of the cell wall. Various transporters involved in pollen maturation and germination have been described. *AtSTP2* expression is confined to the early stages of gametophyte development, during which it would allow the uptake of glucose units resulting from callose degradation [83]. *AtSTP4* is present in the plasma membrane of the pollen tube, but absent in the plasma membrane of pollen grain before germination [83]. The expression of *PMT1* gene, whose sequence is related to *STP4*, is activated after the first pollen mitosis and high levels of transcripts accumulate in mature and germinating pollen of tobacco [84]. In the same species, a pollen specific putative sucrose transporter (*NtSUT3*) is also expressed during a very short time of the maturation [99], suggesting that the pollen is able to use both hexoses and sucrose to support its high metabolic activity. Proton-sucrose cotransport was also described in germinat-

ing pollen of lily (*Lilium longiflorum*) [100]. NsAAP1, a putative amino acid transporter related to AtLHT1 is expressed from first pollen mitosis and dramatically increase in mature pollen shortly before anthesis in *Nicotiana sylvestris* [101]. The expression of *LePtroT1*, which encodes a transporter mediating the uptake of proline, glycine betaine and γ-aminobutyric acid (*LeProT1*) is localised in mature and germinating pollen of tomato [102]. It would serve for osmotic adjustment during pollen dehydration and mediate the uptake of material needed for the synthesis of OH-Pro rich proteins during pollen elongation [102]. The functioning of all these proton-coupled transporters is allowed by an active H^+-ATPase [103]. Concerning the ovules, the only transporters described so far having a specific role in their nutrition are *PmSUC1* in young ovules of *Plantago* [67], and *AtProT1*, whose expression in the flowers is down regulated after fertilisation [70].

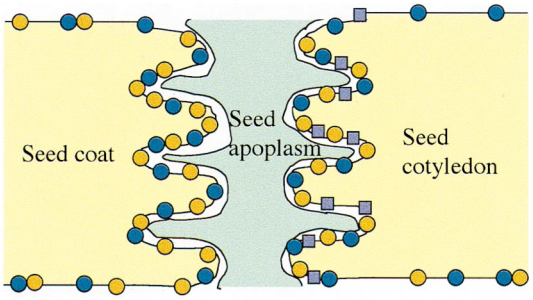

Fig. 3. The embryo/seed coat interface of broad bean seed. The transfer cells bordering this interface possess plasma membrane invaginations bordering the cell wall protuberances, which ensure an increased surface available for exchanges. The invaginations exhibit a high density of H^+-ATPase (orange circle), sucrose transporters (SUT, blue square) and sucrose binding proteins (SBP, blue circle) in transfer cells bordering both the embryo and the seed coat. Redrawn from data given in [36,105–107].

3.2.3. Legume seeds

The data concerning the distribution of the H^+-ATPase and of sucrose transporters at the seed coat/embryo interface are summarised in Fig. 3. In developing broad bean seeds, sucrose efflux occurs primarily from the thin-walled parenchyma transfer cells bordering the inner surface of the seed coat [104]. The released sucrose is retrieved from the seed apoplasm by the epidermal transfer cell complex located at the cotyledon surface [105]. Immunocytochemical localisations indicate that a H^+-ATPase is located in relatively high densities within the wall ingrowth regions of the epidermal transfer cells of developing cotyledons [106,107], as well as in the thin-walled parenchyme transfer cells of the seed coat [107]. The H^+-ATPase is expressed at decreasing levels with increasing distance from the transfer cell layers. In situ hybridisation localised a member of the *SUT1* family in the epidermal cell complexes of the cotyledons, whereas SBP, a sucrose binding protein involved in non-saturable sucrose uptake (see below), was immunolocalised exclusively to the plasma membranes located in the wall ingrowth regions of the transfer cells [107]. The cloning of *VfSUT1* and the localisation of its transcript confirm that a sucrose transporter is strongly expressed in the transfer cells of the cotyledons during their differentiation after the heart stage. Transfer cells and *VfSUT1* expression develop in the epidermis at the seed coat contact zone, which suggests a possible control by signals coming from the maternal seed coat [36]. The hexose transporter clone *VfSTP1* is expressed mainly in *Vicia faba* roots and at a lower level in pods and sink leaves. In the seed, *VfSTP1* strongly accumulated during the midcotyledon stage in epidermal cells covering the mitotically active parenchyma [36]. The location and the timing of *VfSTP1* expression are, therefore, different from that of VvSUT1. In germinating seedlings from castor bean, *RcSUT1*, a partial clone homologous to sucrose transporters, is strongly expressed in the lower epidermal layer which contains transfer cells and in the phloem [108]. This transporter would be involved in active loading by the lower epidermis, followed by symplastic transfer to the parenchyma cells, via the numerous plasmodesmata connecting the cells of the lower epidermis and the adjacent parenchyma cells.

A 62 kDa sucrose binding protein (SBP) originally identified by photoaffinity labeling of soybean cotyledonary microsomal membrane preparation [109] also seems to play a role in sucrose transport. Immunolocalisation indicates that SBP is associated with the plasma membrane of cells particularly active in sucrose transport, including the companion cells and sieve elements of spinach leaf phloem [110] and cotyledonary cells in soybean seed [111] and the transfer cells of the seed coat in broad bean [107].

Interestingly, the paraveinal mesophyll cells of soybean leaf, which are actively engaged in assimilate transfer between the mesophyll and the phloem, are also enriched in SBP [112]. Molecular cloning and sequence analysis of the deduced SBP protein indicates that it shares no homology with other known transport protein and that it does not span the membrane. The protein would be a tightly bound peripheral protein, with the bulk of the protein exposed to the extracellular environment [113] and may be present under oligomeric form in the membrane [114]. When expressed in yeast cells, *SBP* mediates non-saturable, protonophore insensitive sucrose uptake [115]. Whether SBP mediates sucrose transport on its own [114] or associated with a sucrose transporter as a regulatory protein [115] is not yet clear, as is its exact function (influx or efflux) in vivo. This protein, whose expression is tightly controlled at the cell level may be responsible for sucrose efflux. It is possible that this efflux system has been described as apparent non-saturable sucrose uptake in various materials [116,117], due to the fact that at high external sucrose concentration, the sucrose gradient favours passive uptake, and not efflux. Unfortunately, no SBP homologue has been described so far in other plants, although the cross reactivity of the soybean 62 kDa antiserum with spinach and broad bean suggests that the protein is present in other species.

These examples indicate that transporter expression is strictly controlled at the cellular and subcellular level and this raises the question of their targeting and degradation (see below). Overall, the data indicate a quite complex regulation of transporter expression throughout various tissues and cells in the plant. This diversity ensures the integration of solute transport at the whole plant level, by allowing the differential expression of sets of transport systems tailored to the changing requirements of various organs and tissues.

3.3. Environmental control

3.3.1. Light

Light may control the expression and activity of transporters either directly as a physical signal involving specific receptors and/or because it affects the nutrient status of the cells, in particular the sugar content, through photosynthesis. Sugar control of transporter activity has been studied in more detail than the light effect itself. SUT1 transcripts levels in tomato and protein amount in potato decrease during the dark phase and increase at light [74]. These data fit nicely with the nycthemeral changes in sugar export [20], phloem composition [118], apoplastic and intracellular sucrose concentrations [119]. A diurnal rhythm for the amounts of *DcSUT1* transcripts in the aerial parts of carrot has also been described, contrasting with the stable levels found for *DcSUT2*, expressed mainly in storage parenchyma tissues of the carrot root [68]. In contrast to these data which show a strong expression of *SUT1* at light, *OsSUT1* transcripts were higher in aetiolated rice seedlings than in light grown seedlings. Switching dark grown seedlings to the light induced an expression of *OsSUT1* which peaked at 3–6 h after the onset of illumination and strongly decreased thereafter, during the accumulation of chlorophyll and sucrose phosphate synthase transcripts [64]. In contrast to *SUT1*, the amount of *PmSUC1* and *PmSUC2* transcripts do not show a significant variation of expression along the diurnal cycle [80].

3.3.2. Water stress and salt stress

In *Arabidopsis*, water stress and salt stress induce a strong expression of *ProT2*, while the expression of the broad specificity amino acid transporters *AAP4* and *AAP6* is repressed [70]. This coordinate response may result in a better transport of proline relative to the other amino acids, since under these conditions, proline content increases in the sieve sap [120], whereas the export of other amino acids via the phloem is decreased [121]. *LeProT3*, another proline transporter, is expressed during the desiccation of the pollen grain in tomato [102]. Both dehydration and cold treatment induce the expression of *ERD6*, a putative tonoplast hexose transporter, in *Arabidopsis* plants, but the tissue and cell specificity of expression have not been studied [122]. In celery, water and salt stress decrease the expression of a sucrose transporter (Noiraud et al., submitted for publication). In parallel with what has been described above for proline and amino acid transport, this decrease in sucrose transport might favour the accumulation and transport of mannitol, which is transported in the phloem of this species and may act as an osmoprotectant.

3.3.3. Mechanical stress and phytopathogen attack

Reporter gene assays and RNase protection experiments indicate that the hexose transporter *AtSTP4* is rapidly induced in cells adjacent to mechanical lesions in *Arabidopsis*. Furthermore, *STP4* expression is also induced rapidly in suspension-cultured *Arabidopsis* cells treated with a bacterial or a fungal elicitor, as well as in *Arabidopsis* plants exposed to fungal attacks [82]. One of the functions of *STP4* would be, therefore, to meet the increased carbohydrate demand of cells responding to environmental stress and to recover hexoses resulting from cell wall degradation. A *STP4* homologue is also induced during the interaction between wheat and *Erysiphe graminis* (Lappartient et al., personal communication).

A detailed analysis of the effects of ageing and mechanical stresses on the regulation of the H^+-ATPase and sugar and amino acid transporters at the transcriptional and post-transcriptional level has been conducted in sugar beet leaves [78,123–125]. The tissues were submitted either to ageing (peeling of the lower epidermis and floating of leaf discs) or cutting (excision of the leaf and infiltration of water in the leaf blade through the cut petiole). Transport activities were measured both in leaf tissues, which gives an uptake capacity resulting from the activity of the ATPase and of the transporters, and with purified PMV energised by an artificial proton-motive force, which gives a picture resulting from the intrinsic activity of the transporters only. Proton-pumping activity was also measured in vitro and in vivo. The amounts of transcripts for the ATPase and the transporters were monitored, as well as the amounts of the ATPase and sucrose transporter by ELISA with specific antibodies. In leaf discs, ageing induced a general and strong increase (three- to four-fold) of sucrose, hexose and valine transport within 12 h; cutting only stimulated sucrose transport (2.5-fold) [123]. Although both cutting and ageing induced an increased amount of ATPase transcripts and protein, ATPase activity and proton-pumping were stimulated only after ageing, which suggests post-translational regulation of the ATPase, in addition to a transcriptional control by mechanical treatments [124]. In this experimental system, salicylic acid inhibited valine and sucrose uptake by decreasing the energy charge of the tissues and, thus, the amount of ATP available for proton pumping by the H^+-ATPase [126]. Cutting resulted in enhanced amounts of sucrose transporter transcripts and protein and enhanced transport both in PMV and leaf discs [78]. The transcript amount was stimulated more strongly than the protein amount, which may suggest a translational regulation. There are other examples, at least in heterologous expression systems, that plant membranes transporters are transcribed but not translated [73]. The stimulation of proton-driven sucrose uptake measured in PMV (65%) was higher than the stimulation of sucrose transporter amount (20%), which may also suggest post-translational control (see also regulation by phosphorylation, below). Overall, the stimulating effect of cutting in vivo may be explained by different controls on sucrose transporter activity, with the ATPase activity remaining unchanged. In contrast, ageing, which also increased the sucrose transporter transcripts and the amount of protein, did not induce a higher activity of the transporter in the PMV. Taken together, the data gave evidence for a complex control of sucrose transporter activity by mechanical treatments at different levels: transcriptional, post-transcriptional and post-translational [78]. Similar but less detailed conclusions could be drawn for the hexose transporter and valine transporters, whose amounts in the PMV were not studied. In slices of red beet storage tissues, the enhancement of sugar transport occurring during washing seems to be due only to an increase in plasma membrane H^+-ATPase activity rather than to changes in transporter activity [127].

Although these data clearly show that the plant cell possesses the mechanisms allowing a multi-step control of its uptake capacity, the treatments used may generate several perturbations, among which it is not clear what is the active signal: leakage of cell wall fragments, modification of the water, hormonal and sugar status of the cells, and others. A similar experimental system was used by Chiou and Bush [47] who showed that the activation of the sucrose transporter induced by ageing was suppressed by addition of sucrose in the infiltrating medium. The data therefore suggest that one of the signals involved in the phenomena described above could be sucrose itself (but see Section 2).

3.3.4. Hormonal control

Although the hormonal control of assimilate transport has been the subject of numerous physiological studies in the 1980s (see for review [128]), neither detailed nor systematic study at the molecular level has been published on plant membrane transporters. SUT1 transcripts and protein can be induced by the addition of auxins and cytokinins to detached leaves [15,46]. In *C. rubrum* suspension cells, there is a coordinate induction of one hexose transporter (among the three present in the cells) and of one extracellular invertase, whereas intracellular invertases are not increased. This induction results in enhanced uptake of sucrose (after hydrolysis) and of hexoses [129]. The VvHT1 hexose transporter from grapevine is also induced by kinetin (Atanassova et al., unpublished results). These effects of cytokinin may be involved in their stimulation of cell division.

3.4. Promoter analysis

In spite of the complex patterns of expression described above, so far very little is known about the *cis* elements and the transcription factors involved in the control of cell specific expression.

In some instances, analysis of plants expressing promoter–reporter gene fusion has shown cell specific expression (*AtSUC2* [77]; *AtSTP4* [82]; *AtSTP2* [83]; *AtAAT1(CAT1)* [130]; *AtAAP1* and *AtAAP2* [85]), but to our knowledge, no detailed functional analysis of the promoters is available, and very few promoter sequences of genes encoding the plant plasma membrane transporters are found in the data banks. No box nor sequence responsible for tissue specific expression of plant membrane transporter has yet been described. When a transcript is found in various cell types, for example *StSUT1* in sieve tubes and in non-phloem cells, it is not known whether the same promoter is involved in both types of cells.

Despite the lack of striking sequence homology, the comparison of several sugar transporter promoters reveals the presence of some common regulatory motifs (Table 1). The promoter regions are arbitrary presented as a proximal promoter to −100 bp upstream of the TATA box and a distal promoter up to 2 kb. The proximal promoter region is usually characterised by the classical boxes, TATA and CAAT, but also encompasses E-boxes and I-boxes.

Table 1
Essential motifs potentially involved in expression regulation of some sugar transporter promoters

Promoter	pVvHT1	pSTP3	pSTP4	pSUC1	pSUC2	pVvSUC1
Length (bp)	2438	1777	2541	2584	2166	2186
Proximal	TATA	TATA	TATA	TATA	TATA	TATA
	CAAT	CAAT		CAAT		
		E box	E box	E box		E box
			I box		I box	I box
Distal					CAAT	
	ERELEE4	ERELEE4	ERELEE4	ERELEE4	ERELEE4	
	E box	E box			E box	E box
	I box					I box
	AMY box		AMY box	AMY box		AMY box
	SURE1		SURE2			

Hexose transporters from *Vitis* (VvHT1) and *Arabidopsis* (STP3, STP4);
Sucrose transporters from *Vitis* (VvSUC1) and *Arabidopsis* (SUC1, SUC2)

TATA box	TATABOX	(CTATAAATAC,TATAAAT,TATTAAT,TATATAA,TTATTT)	
CAAT box	CAATBOX	(CAAT)	
ERELEE4-		(AWTTCAAA)	ethylene responsive element
E box	EBOXBNNAPA	(CANNTG)	ABRE, abscisic acid responsive element of storage protein genes
I box	IBOXCORE	(GATAA)	conserved sequence upstream of light regulated genes
AMY box	AMYBOX1	(TAACARA)	conserved sequence upstream of alpha amylase genes
	AMYBOX2	(TATCCAT)	conserved sequence upstream of alpha amylase genes
SURE1	SURE1STPAT21	(AATAGAAAA)	sucrose responsive element
SURE2	SURE2STPPPPAT21	(AATACTAAT)	sucrose responsive element

The most regularly found *cis*-elements in distal promoters are ERELEE4 motif, E-boxes and I-boxes, thus supporting a possible modulation of gene expression in response to physiological (ethylene, ABA) and environmental signals (low temperature, cold, drought, light). Surprisingly, sugar responsive sequences are almost absent in promoters studied, except in *pVvHT1* et *pAtSTP4* that may be regulated by source–sink interactions.

In carrot, the screening of a leaf library resulted in the isolation of two *DcSUT1* clones of different lengths (*DcSUT1a*, 1861 bp; *DcSUT1b*, 2132 bp). These clones did not differ in their ORF, but sequence deviations were found in the 5′ upstream non-coding sequences and were completely different in their 3′ non-coding domains [68].

Sequence analysis of the promoters of *AtAAP1* and *AtAAP2* indicates that in *AtAAP2*, an intron is located directly upstream of the ATG, whereas *AtAAP1* contains no intron in the untranslated leader. Although both genes are expressed at the same time, no striking homologies could be found in their promoters. Four ACGT-core-motifs supposed to be involved in many environmental responses were repeated around position −123 in *AtAAP1*. An E-box (also described in the β-phaseolin promoter) and a SEF3 motif were also found in this promoter. Since the cell specificity of *AtAAP1* and storage protein genes are similar, these elements might be involved in developmental regulation of expression [85]. The promoter of *AtAAP1* contains several potential motifs putatively induced in nitrate control of expression and the expression of *AtAAP1* in leaf tissues is both nitrate and light inducible, as well as regulated by the diurnal cycle [131]. The 5′ flanking region of *NsAAP1* (expressed in the pollen) contains long regions homologous to the promoter region of the tobacco pollen specific eIF-4A8 translation factor [101].

4. Post-transcriptional control of the transporters

Once they has been transcribed, translated and targeted to the membrane, the activity of the transporters will depend on the thermodynamic environment across the membrane and on post-translational regulation by various processes including phosphorylation/dephosphorylation, redox regulation and allosteric control.

4.1. Turnover, targeting and degradation

4.1.1. Turnover and targeting

There is evidence that the plasma membrane proteins may be rapidly internalised by endocytosis [132]. Turnover of the plant plasma membrane ATPase has a half-life of 12 min after auxin treatment [133]. In yeasts, sugars may affect the stability of the sugar transporters [134]. In potato, expression of SUT1 is diurnally regulated at both the mRNA and protein levels, and the half-time of protein turnover is in the range of a few hours [74]. The amount of transporter present in the membranes depends both on its synthesis and targeting, and on its degradation. Cell specific expression of the sucrose transporter has also been described (SUT1 in the sieve tube [74], SUC2 in the companion cells [45,79]) and processes allowing the transport of transcripts and/or proteins from the companion cell to the sieve tube are necessary to account for the localisation of SUT1. However, very little is known on the targeting and degradation of plant membrane transporters.

In animals, epithelial cells also present an asymmetry of sugar transporter distribution. In renal epithelial cells, the sodium-glucose cotransporter of the plasma membrane (SGLT) is localised at the apical side of the cell, whereas the facilitated transporter (GLUT) is localised in the basolateral portion of the cell [135]. Redistribution of these transporters and cell polarity may be mediated by a cytoskeleton-dependent pathway or by lateral redistribution of the transporters in the membrane [136]. Insulin stimulates glucose uptake by adipose and muscle tissue by recruiting intracellular GLUT4 molecules to the cell surface. In unstimulated adipocytes, GLUT4 appears to recycle continuously between the cell surface and the intracellular storage compartment with which it is associated and insulin increases the cell surface GLUT4 levels by increasing the rate at which it is externalised and reducing the rate of internalisation [137]. No experimental model is presently available to develop similar studies on the plant conducting complex. Factors responsible for the distribution of asymmetrically distributed mRNAs (in-

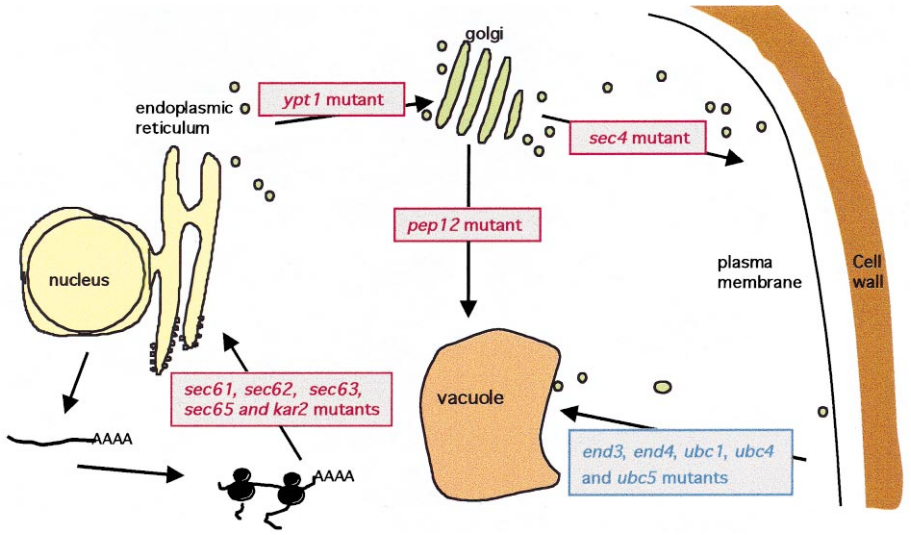

Fig. 4. An example of some yeast mutants affected in targeting and degradation of membrane proteins. Redrawn from [139–144].

cluding *cis*-elements in the 3′ ends of the mRNA) and the interacting transfactors have also been identified in animals. Similar mechanisms might be involved in the asymmetric distribution of membrane proteins in the conducting complex [138].

In yeasts, the half time of turnover is much shorter for plasma membrane transporters (1 h) than for bulk proteins [1]. Many yeasts mutants affected in membrane protein targeting [139–142] and degradation [143–145] have been described, which may be useful for deciphering these processes in plant cells (Fig. 4). The *sec61-3* mutant is affected in the normal secretion pathway [142]; the *sec65-1*, *sec62-1* and *sec62-2* mutants are affected in the signal recognition particle system allowing transport to the reticulum. The *shr3* mutant is affected in a reticulum membrane protein required for the early stage of secretion of the yeast amino acid permeases [146]. These permeases also require COPII components for packaging into transport vesicles in vitro [147]. The *shr3* mutant has been used in an attempt to clone plant homologues by complementation in yeasts [70]. Although no homologue was found, this work allowed the identification of new amino acid transporters from *Arabidopsis*.

4.1.2. Degradation

Transporter degradation has not yet been studied in plants. In yeasts, the degradation of the GAL2 galactose transporter is quickly induced by glucose addition (0.5 min in the presence of glucose versus 90 min in its absence) [145]. The degradation of this protein and of other membrane proteins such as the PDR5 ABC transporter or the α factor receptor involves the phosphorylation of the protein, its ubiquination, its targeting to the tonoplast and vacuolar proteolysis [144,148,149]. Yeast amino acid permeases are also degraded after ubiquitination [150]. Mutants have been described for each one of these steps. The *end3* and *end4* mutants are affected in GAL2 [145] and PDR5 [143] internalisation. The *ubc1*, *ubc4* and *ubc5* mutants are deficient in protein ubiquination [150]. In this context, it should be noted that ubiquitin is one of the major proteins of phloem sap [151]. Alternate pathways for membrane transporter degradation are possible, which involve recycling of the internalised protein to the plasma membrane, or its degradation without endocytosis [152,153]. Even though the plant secretory and degradation pathways may possess specific features, several basic components have been identified by yeast complementation [154–157] and even more in mammals [158]. Yeasts, therefore, provide a promising tool for the study of plant plasma membrane transporters targeting and degradation.

4.2. Lipidic environment

It has been established that the activity of membrane proteins is influenced by their lipidic environ-

ment. So far, most studies have concentrated on the role of phospholipid and sphingolipid composition. The existence of microdomains enriched in specific lipids in which specific proteins are embedded has also been demonstrated. The lipidic composition of the plasma membrane of various cells (mesophyll/companion cell/sieve tube) might, therefore, be important for the targeting and the activity of various transporters. Unfortunately, no detailed analysis has been made in plants. Yeast mutants affected in the lipid composition of membranes have been described, which may be used to express plant membrane transporters and study their dependence on lipid environment [159]. Sterols also represent major components of the plasma membrane and in vitro studies have shown that the activity of plasma membrane H^+-ATPase from corn is modulated by the sterol composition of the reconstituted vesicles [160]. Likewise, the animal Na^+/K^+-ATPase is sensitive to the sterol composition of the membrane [161]. Studies on the reconstituted hexose transporter from *Chlorella* have shown that its interaction with ergosterol is important in energy coupling [162]. An in vivo study is under way in our laboratory to evaluate the influence of the sterol environment on the activity of PmSUC2. *PmSUC2* was expressed in a *S. cerevisiae* strain whose growth depends on an exogenous source of sterol in the medium. Sucrose uptake was measured when the transformed yeast strain was grown either on ergosterol, the major yeast sterol or on two different commercial sources of phytosterols in which sitosterol represents the major sterol. The yeast does not discriminate between the various phytosterols since their relative composition recovered reflects exactly the sterol composition provided in the medium. Although the growth of the strain was significantly lowered upon addition of sitosterol, sucrose uptake was reproducibly enhanced five times as compared to the control strain which accommodated exogenous ergosterol. Therefore, reconstitution of a phytosterol environment in the vicinity of the plant sucrose transporter seems to increase its activity (Rocher et al., unpublished). Another approach to study the effect of sterol composition on the activity of the transporters may be grounded on the use of PMV prepared from plant mutants affected in their sterol content [163]. Comparative studies of the lipidic composition of the plasma membranes of phloem enriched tissues (and ideally of conducting complex) and of non-phloem tissues are needed.

4.3. Thermodynamic and kinetic control

In vitro at least, the degree of accumulation of the substrates is determined both by kinetic and thermodynamic parameters [164]. Sucrose uptake across the tonoplast may be mediated by facilitated transporters [165,166] or by a H^+/sucrose antiport [167–169]. The existence of a H^+/glucose antiport is still debated [13]. In contrast, experiments with purified PMV energised by an artificial proton motive force clearly show that the sucrose [170–172], the hexose [173] and the amino acid [174–176] plasma membrane transporters cotransport protons and that they are able to use both components of the proton motive force, ΔpH and $\Delta\psi$. Similar conclusions were reached with reconstitution systems using cytochrome *c* oxidase and the HUP1 *Chlorella* hexose transporter [164]. The proton-motive force available for one given transporter will depend (a) on the number and on the activity of the H^+-ATPase in the cell, which generates the two components of the proton motive force that may be used by the transporter, i.e. the pH gradient and the electrical gradient, and (b) on the concurrent and partial dissipation of these gradients by other transporters and channels present in the same cell. For a given proton-motive force, the activity of the transporter may also depend on the relative part played by the chemical (ΔpH) and electrical component ($\Delta\psi$), and by the external and internal concentrations of the mineral and organic substrate(s) of the transporter.

4.3.1. Thermodynamic control

The transcription of the plasma membrane H^+-ATPase may be controlled by development [177], auxin [133] and NaCl [178]. Post-translational control may include interactions between the C-terminal part of the enzyme and its active site [179] and 14-3-3 proteins [180], regulation by the redox potential and phosphorylation [181]. The ATPase is more strongly expressed in the phloem cells [182] and more precisely in the companion cell ([183]; *Arabidopsis*) or in the transfer cell ([94,106,107]; broad bean). Both in *Arabidopsis* and in broad bean, the H^+-ATPase and the sucrose transporter colocalise in the same

cell (companion cell or transfer cell). Further, within the transfer cell of broad bean, the asymmetrical distribution of the ATPase [106] and of the sucrose transporter [107], which are concentrated in the plasma membrane regions bordering the wall ingrowths favours a maximal efficiency for the uptake and retrieval of assimilates. The mechanisms allowing this asymmetrical distribution are not known. In Solanaceae where the sucrose transporter is localised in the sieve tube, it has to be assumed that an electrical coupling exists between the companion cell and the sieve tube, or that there is a sucrose gradient between these two types of cells [73].

Many transporters may be multifunctional. In animal cells, facilitative glucose transporters (GLUTs) may transport water and large ring compounds in addition to glucose [184]. Whether and to what extent plant membrane sugar transporters are able to transport water is not known, but it may be interesting to study this possibility in terms of coupling between sugar and water fluxes and mass flow. Even the most specific of the plant membrane transporters studied here, the sucrose transporter, may recognise at least two substrates, sucrose and maltose, although this may depend on the species ([29]; Lemoine, this issue). The hexose transporters, the amino acid transporters and the peptide transporters all recognise a wide range of substrates. The in vivo function of all these transporters will depend on their K_m and on the transmembrane gradients of their substrates. A striking example in this regard is the rapeseed *BnNRT1,2* transporter that is able to transport two substrates differing strongly in size and charge, nitrate and histidine [185]. The pH dependence of the activity of the transporter differs for the substrates, with histidine transport favoured at alkaline and nitrate at acidic external pH. The K_m for both substrates, measured after expression of *BnNRT1,2* in *Xenopus* oocyte depended on the voltage, but was always above 1 mM. In roots, where the transporter is mainly expressed, this transporter does not face external concentrations of His and would, therefore, mainly serve for nitrate transport. However, this does not exclude a function in histidine transport in the internal cells of the roots or in other organs. Perhaps some other peptide and amino acid transporters of the BnNRT1,2 family already characterised also have anion substrates not yet characterised. This observation also makes it more difficult to name the transporters according to their substrate [185].

Another nitrate transporter, CHL1, presents remarkable properties, since it may mediate high affinity or low affinity nitrate uptake, depending on the source of nitrate and on the external pH [186]. Similar studies may be worth undertaking with the sugar transporters owing to the complex kinetics frequently observed.

4.3.2. Kinetic control

The activity of many transporters is pH-dependent. Although this simple pH dependence has often been taken as an argument for a proton-cotransport mechanism, it must be stressed that the external pH may modify the global charge of proteins, lipids and substrates in the case of amino acids and peptides and the surface charge of the membrane. The simple observation that uptake is pH-dependent is therefore not sufficient for concluding that a proton cotransport mechanism is operating and electrical depolarisation and proton fluxes associated with substrate transport should be demonstrated. The acidification of the cell wall due to the H^+-ATPase may affect the uptake rate of the transporters. For example, both *AtSUC1* and *PmSUC1* are rather insensitive to external pH ('neutral' sucrose transporters), whereas *AtSUC2* and *PmSUC2* show a steep pH dependence and increasing transport rates with decreasing extracellular pH ('acid' sucrose transporters) [65,67]. The physiological significance of this difference is not yet clear. Although extracellular pH may affect the apparent K_m of the sucrose transporter [187], detailed kinetic analysis in leaf discs and PMV suggest that the proton binding site of the transporter is always occupied under physiological conditions [187–190]. However, experiments with *Xenopus* suggest that proton external concentration may be limiting under some conditions ([191], see below). pH dependence is also important for multifunctional transporters, for which the uptake of different substrates may be affected differently by the external pH (see below). Finally, it is not clear how exactly the pH dependence studies of uptake reflect the in vivo situation. Experimentally, the pH is usually controlled in the bulk phase of the medium and the buffering capacity of the cell wall and local variations of its composition

may affect the pH sensed by the transporter. The application of micromethods allowing local measurements of ΔpH and $\Delta\psi$ would be helpful for understanding the functioning of the conducting complex. For example, it is not known how the asymmetrical distribution of the ATPase in the transfer cells affects the proton motive force developed on the different faces of these cells and in the sieve tube.

Use of electrophysiological and radiotracer flux methods on *Xenopus* oocytes expressing various plant membrane transporters resulted in the development of kinetic models that indicated some of the potential limiting steps of transporter functioning. Kinetic information may also be gained in detailed uptake/efflux studies with cells [192] and purified PMV (see above, and [8] for review). The kinetic properties of StSUT1 can be explained by an eight-state ordered simultaneous model with H^+ binding to the transporter before sucrose, with both ligands transported simultaneously across the membrane [191]. StSUT1 is negatively charged with voltage driving protons into their binding site (but see [189]). Comparison of the kinetic properties of the transporter with the probable in vivo environment led to the conclusion that sucrose transport in vivo is probably not limited by membrane potential, but rather by apoplastic sucrose and proton concentrations. Also, the slow rate of H^+ dissociation may be a rate-limiting step in the transport cycle [191]. Interestingly, StSUT1 also mediates uncoupled H^+ and sucrose transport. Uncouplers only marginally affect the counterexchange properties of the sucrose transporter and of the hexose transporter of broad bean leaf discs [193]. All these data may be related to the existence of proton-independent saturable uptake system described in broad bean [116] or to the proton-independent linear system described both in broad bean and sugar beet leaf discs [116,117,187] and in soybean cotyledon protoplasts [194]. The possibility exists that the saturable proton-independent phase [116,187] or the linear proton-independent uptake phase demonstrated with leaf discs represent normal functioning in the direction of efflux in vivo.

The expression of STP1 in oocytes suggests that H^+/hexose cotransport mediated by this transporter occurs via a sequential mechanism (i.e. H^+ and glucose are transported separately) [195], unlike the simultaneous mechanism operating for SUT1. In this system, the transporter density was estimated at 10^{10} per oocyte (compared to about 10^{11} for SGLT1) and the turnover number was 59 s^{-1}, compared to 20–57 s^{-1} for mammalian sodium-coupled transporters. Detailed kinetic studies with the HUP1 hexose transporter from *Chlorella* demonstrated the importance of internal pH for this transporter, which acts in an asymmetric way. The transporter is completely inactive for all fluxes (influx, efflux and exchange flux) when the intracellular pH is 6.0 or below, although it is optimally active at an extracellular pH of 6.0 [196]. Sugar efflux is very slow when cells with a high internal sugar analogue concentration are resuspended in sugar-free medium, but it can be stimulated by external sugar (a positive *trans* effect for efflux of more than 50-fold) [197]. In reconstituted vesicles, it was also concluded that the internal pH may be a limiting factor for D-glucose accumulation mediated by HUP1 [164]. In this system, the accumulation of glucose is not simply defined by the magnitude of the proton motive force, but also by carrier activity and by substrate leakage.

Expression of *AAP1/NAT2* in *Xenopus* oocytes led to the conclusion that H^+/amino acid cotransport mediated by this protein occurs via a random simultaneous mechanism and that the transport mechanism does not depend on the amino acid [198]. Membrane voltage enhances the maximal transport rate and the affinities for H^+ and amino acid. The transport velocity depends on the amino acid, and this may be due to differences in a rate-limiting step in the transport cycle, possibly the translocation rate of the fully loaded transporter. The data also suggest that the transporter has more than one binding site for H^+ and amino acid. A turnover number of 350–800 s^{-1} was calculated [198]. Stoichiometry experiments enabled Boorer and Fischer [199] to determine the charge on the transported amino acid species after expression of AAP5 in oocytes. They showed that AAP5 transports anionic, cationic and neutral amino acids via the same mechanism, i.e. with a fixed amino acid coupling stoichiometry. Thus, in planta, the energy consumption for amino acid transport is independent of the net charge of the amino acid.

4.4. Post-translational covalent modifications

4.4.1. Phosphorylation

The activity of major membrane proteins such as the plasma membrane H^+-ATPase [181], plasma membrane aquaporins [200,201] and the tonoplast aquaporins [202] is regulated by phosphorylation. This also holds true for less abundant plasma membrane proteins such as the tobacco suspension anion channel [203] and the major cytoplasmic enzymes of sugar [204] and nitrogen metabolism [205]. Catabolite inactivation of the yeast amino acid permease may also involve phosphorylation/dephosphorylation processes [1]. The possibility of a control of sugar, amino acid and peptide transporters by phosphorylation thus deserves some attention. Computer analysis of the deduced peptide sequences of various sucrose transporters revealed the presence of serine and threonine residues located in cytoplasmic loops and in consensus sequences for potential phosphorylation CK2 sequence: [ST]xx[DE] and PKC sequence: [ST]x[RK] which are highly conserved (Thr^{103}) or more or less conserved (Ser^{38}, Ser^{99}, referring to BvSUT1 sequence, accession number AC83850). Infiltration of the phosphatase inhibitor okadaic acid into sugar beet leaves rapidly inhibited proton-driven sucrose uptake measured in plasma membrane vesicles [125]. Okadaic acid did not affect the amount of sucrose transporter present in PMV, and methyl okadaic acid, a biologically inactive analogue of okadaic acid, had only a marginal effect on uptake. The data suggest that the phosphorylation of the transporter decreases its activity. In this context, it is noteworthy that cutting of plant tissues may activate kinases [206] and that sugars have been shown to increase kinase activities associated with the plasma membrane [207]. However, more detailed investigations involving the use of site-directed mutagenesis are required to confirm the physiological significance of sucrose transporter regulation by phosphorylation.

Alignment of the deduced peptide sequences from various *AtSTP* indicates that Thr^{104} and Ser^{228}, located in the cytoplasmic loops, are conserved in a consensus sequence for phosphorylation [208]. Both sites are also conserved in *VvHT1*, encoding a hexose transporter in *Vitis vinifera*, a phylogenetically distant species.

The AtAAPs also contain two conserved potential sites for phosphorylation (Thr^{116} and Thr^{248}), referring to AtAAP2, and okadaic acid also inhibits proton-driven amino acid transport in purified PMV (Roblin et al., submitted for publication). AtProT1 and AtProT2 contain one potential phosphorylatable site (Thr^{247} in AtProt1). LeProT2 and LeProT3 both contain a putative 14-3-3 recognition site ($RSx_{1,2}SxP$) where the second site can be phosphorylated. This sequence is absent in Le ProT1 and AtProT1 and AtProT2. Introduction of this sequence by site-directed mutagenesis into LeProT1 and its modification in LeProT2 did not change the activity of these proteins [102].

There are only a few peptide transporter sequences available, which makes it difficult to search for conserved residues; however, several potential phosphorylation sites are present in AtPTR1 and AtPTR2.

4.4.2. N-Glycosylation

N-Glycosylation is restricted to the luminal side of the endoplasmic reticulum, only to those parts of a membrane protein which face the ER lumen, which later on end up facing the cell exterior [1]. Sucrose transporters contain several potential *N*-glycosylation sites (N[~P][ST] [~P]) which are more or less conserved (Asn3 and 92 in StSUT1 [63]; Asn154 and 402 in AtSUC1 and AtSUC2 [65]), but only a few of these sites are located in extracellular loops (Asn402 in AtSUC).

Neither the *Chlorella* HUP1 nor the *Arabidopsis* STP1 peptide sequence contain potential *N*-glycosylation sites, which agrees well with the lack of inhibition of the inducible transporter HUP1 by tunicamycin [9,209]. Asn^{316} in AtSTP2 is located in a consensus sequence for glycosylation, but there is no functional evidence that the protein is actually glycosylated in vivo [83]. The cDNA encoding a putative tonoplast hexose transporter in sugar beet leaves has been expressed both in vitro and in vivo, in yeasts and tobacco cells. The migration pattern found for the protein was the same as in sugar beet. It was concluded that the protein does not undergo significant post-translational modification such as the cleavage of a signal peptide or protein glycosylation [98]. Thus, it is likely that in agreement with the passive hexose transporter from mammalian cells [210], but unlike the hexose transporter from

yeasts [211], the plant hexose transporter does not require glycosylation for its expression and functioning.

After expression of *AtAAP1(NAT2)* in rabbit reticulocyte lysate in the presence or absence of microsomal membranes, the protein has the same mobility on SDS–PAGE, which suggests that it is not glycosylated [208].

4.5. Allosteric control

In plant cells, examples of a control of transporter activity by a mechanism which may be allosteric have been described only for the tonoplast amino acid and peptide transporters. In the absence of ATP, the uptake of most amino acids across the tonoplast is slow. In some instances, addition of ATP may stimulate amino acid uptake by a direct effect on the amino acid transporter, since non-hydrolyzable ATP analogues do not stimulate uptake of several neutral and charged amino acids (Gly, Arg, Asp) by the vacuole. This shows that ATP hydrolysis by the V-type ATPase or the amino acid transporter itself are not involved in transport activation of these amino acids, unlike what is observed for Phe [212–214]. The activation is specific for ATP, since other nucleotides are without effect and it exhibits a sigmoidal response as a function of ATP concentration. Depending on the amino acids, stimulation of transport is obtained with ATP only, or with either ATP or Mg-ATP. Interestingly, the efflux of amino acids from barley vacuoles exhibit features similar to the uptake, i.e. stimulation by ATP and inhibition by hydrophobic amino acids. This suggests that influx and efflux of amino acids across the tonoplast may be mediated by the same permease [215].

A similar stimulation of peptide uptake by barley vacuoles in the presence of ATP, but without ATP hydrolysis, has been described [216].

A putative cAMP binding exists in AtAAP2, AtAAP3 and AtAAP5, but it is located in an extracellular loop (position 287 in AtAAP2).

The Na^+-glucose cotransporter active in intestine and kidney (SGLT1) is allosterically modulated by RS1, a 70 kDa protein. Radiation inactivation experiments and co-injection of SGLT1 and RS1 in *Xenopus* indicated a functional molecular mass of around 300 000 for Na^+glucose transport and to show that transport activity depended on SGLT1/RS1 stoichiometry [217]. The functional molecular mass of plant membrane transporters is still unknown and possible allosteric control by associated proteins has not yet been investigated. Association between SBP and a sucrose transporter has been suggested [107], but SBP mediates sucrose transport in yeast devoid of plant sucrose transporter [115].

4.6. Environmental control

4.6.1. Temperature

Temperature may affect the activity of the transporters indirectly via the fluidity of the membrane, conformational changes of the protein and the viscosity of the surrounding medium. The variations of membrane fluidity in response to temperature will depend on the lipid and sterol composition, which differ between organs [218] and possibly between different cell types. Conformational changes induced by temperature on the sucrose transporter may be deduced from its sensitivity to *N*-ethylmaleimide (NEM) [219]. A pretreatment of purified PMV from sugar beet leaves at 23°C with NEM inhibited subsequent proton-driven sucrose uptake; the same pretreatment at 12°C had no effect. The effects of temperature on NEM were specific to the sucrose carrier, since valine uptake was inhibited similarly by NEM at 12 and 23°C. A differential effect of NEM at various temperatures has also been described for the tonoplast pyrophosphatase [220]. It has been shown that the activity of the H-ATPase is controlled not only by the viscosity of the aqueous phase, but also by the viscosity of the aqueous phase in the vicinity of the enzymes [221]. Although similar effects on proton-cotransporters have not been investigated, they may deserve attention, given the high viscosity of the phloem sap compared to the apoplastic fluid surrounding the conducting complex.

4.6.2. Pollutants

External parameters that might also affect the activity of plant membrane transporters are pollutants. Sulfur dioxide is one of the major gaseous air pollutant that causes damage to agricultural crops and natural vegetation. After penetration in the plant, sulfur dioxide rapidly dissolves as sulfite in the aqueous extracellular and intracellular fluids. In PMV

prepared from broad bean leaves, sulfite was shown to directly inhibit the sucrose transporter but had no effect on the H^+-ATPase [222]. Sulfite inhibition of sucrose uptake was not detected in vesicles stored in the presence of dithiothreitol, suggesting a possible reaction of sulfite with the thiol groups present in (or close to) the binding site of the sucrose transporter.

5. Conclusions

The recent years have yielded an exponential amount of exciting knowledge on proton-coupled cotransporters of the plasma membrane. They have revealed the complexity and the high degree of integration of these transporters at the subcellular level (cell polarity of the companion/transfer cells), at the cell level (pollen grain), at the tissue level (sieve tube versus companion cell) and at the organ level (leaves, roots, flowers, fruits, etc.). Much of this work has consisted in a necessary phase of description and listing of new transporter clones, but the biochemical and physiological work, especially in terms of regulation, is still ahead.

There are relatively few detailed functional investigations and where this has been done, it concerns a limited number of species. Not very many transporters have been successfully expressed in yeasts and *Xenopus* to test their substrate specificity and their kinetic and thermodynamic control. The identification of new transporters needs to be continued in species not yet investigated, which present a particular economical interest (cereals, fruit species) or interesting features in terms of sugar transport (Cucurbitaceae, Apiaceae). The identification of new transporters is relevant for transporter regulation. For example, are the sucrose or raffinose transporters from Cucurbitaceae (where phloem loading may be symplastic) regulated in the same way as in species where phloem loading is apoplastic? What is the regulation of sucrose and polyol transporters in species which translocate polyols in addition to sucrose? Identification of new transporters may also lead to the cloning of proteins involved in sugar sensing. Major emphasis should be put on the cloning of the genes encoding tonoplast transporters, which are so far unknown, with one possible exception [98]. Although the transporters cloned so far possess a common structure with 11–12 transmembrane domains, it is not excluded that other types of proteins (porins, ABC-transporters) are involved in exchanges of sugars, amino acids and peptides across the tonoplast and the plasma membrane.

At the gene level, identification of *cis* elements controlling tissue-specific expression and/or sensitivity to external (light, pathogens, water status) and internal (hormones, sugars and substrates in general) signals should be undertaken and will serve as a basis for the identification of transcription factors binding to these elements.

Immunolocalisation studies have been restricted so far almost only to the sucrose transporters of a few species and families (*Arabidopsis*, *Plantago*, Solanaceae). The precise role and localisation of SUC1/SUC2 transporters are important for regulation of sugar transport and their study should be extended to other species in addition to *Plantago* and *Arabidopsis*. Similar studies should be developed on other species and with other transporters (hexose, amino acids, peptides). Obtaining specific antibodies may be more difficult given that the hexose and amino acid transporters belong to multigenic families with many related members. An important question which is still open concerns the efflux transporter mediating sugar efflux from the mesophyll cell to the apoplast or from the conducting complex to the receiving cells in the sinks. Although preliminary biochemical studies suggest the existence of a specific transporter for sucrose efflux from leaf mesophyll cells [223], this has not yet been identified clearly. Efflux may just correspond to an uncoupled functioning of one of the transporter identified so far as involved in uptake or may imply a different class of protein mediating transport on its own or by association with an uptake transporter [107]. The characterisation of SBP deserves also to be extended to other species.

The use of two hybrid systems adapted to membrane proteins may be envisaged to isolate proteins interacting with the transporters (kinases, phosphatases or allosteric regulators of the RS1 type). Allosteric regulation by small molecules (ATP) has been shown in the case of tonoplast amino acid transporters, but should be investigated with other transporters.

Yeasts have been revealed as an extraordinarily powerful tool for the identification of plant mem-

brane transporters. The existence of various mutants and their convenience for expression studies also make them attractive to decipher several potential modes of regulation of these transporters: targeting, degradation, phosphorylation and lipid environment.

All these approaches should be developed in concert with whole plant physiology, thanks to non-invasive methods which become more and more precise and versatile at their resolution, such as NMR [224], GFP imaging [93,225] or sugar localisation by luminescence [35]. The sequencing projects and the analysis of tagged mutants will also be helpful for the identification of the components involved in transporter regulation.

As pointed out by Tanner and Caspari [1], the decade to come in membrane transport studies will be concerned with transporter regulation and this looks as a very promising and exciting field to explore, given the techniques and tools now available and the complexity of this research area. With regard to the numerous transporters already identified, the different levels of regulation and the different species to be investigated, development of this research will require difficult choices and should also be coordinated.

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