J. exp. Biol. **196**, 483–491 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

HEXOSE/H+ SYMPORTERS IN LOWER AND HIGHER PLANTS

THOMAS CASPARI[1], ANDREAS WILL[1], MIROSLAVA OPEKAROVÁ[2], NORBERT SAUER[1] AND WIDMAR TANNER[1]

[1]*Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, 93040 Regensburg, Germany* and [2]*Czech Academy of Science, Institute of Microbiology, Videnska 1083, 14220 Prague 4, Czech Republic*

Summary

A well-studied transporter of plant cells is the hexose/H^+symporter of the unicellular alga *Chlorella kessleri*. Its properties, studied *in vivo*, are briefly summarized. In part, they are atypical and it has been suggested that this porter acts in an asymmetric way. Three genes coding for *Chlorella* hexose transport activity have been identified (*HUP1*, *HUP2* and *HUP3*). *HUP1* cDNA expressed in a mutant of *Schizosaccharomyces pombe* not transporting any D-glucose has been studied in detail. Several mutants with changed K_m values for substrate were obtained, some by random polymerase chain reaction mutation and selection for decreased sensitivity towards the toxic sugar 2-deoxyglucose, some by site-directed mutagenesis. The amino acids affected clustered in the centre of the putative transmembrane helices V, VII and XI. Large families of hexose transporter genes are found in higher plants (*Arabidopsis, Chenopodium, Ricinus*). Their functional role is discussed. Finally, the progress made in studying plant transporters in a vesicle system energized by cytochrome *c* oxidase is summarized.

Introduction

Although green plants are carbon autotrophic organisms, synthesizing their sugars as well as all other organic compounds photosynthetically, plant cells nevertheless require sugar uptake mechanisms. Higher plants are physiological mosaics of autotrophic green and heterotrophic non-green cells and tissues. The roots, stems and reproductive organs are supplied with organic compounds and the leaves deliver sucrose and other oligosaccharides to them. Green algae, in contrast, can frequently grow autotrophically but also mixotrophically, making use of sugars, amino acids and organic acids (Droop, 1974).

Typical and atypical properties of the *Chlorella* hexose/H+ symporter

Chlorella kessleri, a unicellular green algae, can be grown under autotrophic as well as under heterotrophic conditions; in the latter case, glucose serves as sole carbon source. During the shift from carbon autotrophy to heterotrophy, hexose transport activity increases more than 200-fold (Tanner, 1969; Haaß and Tanner, 1973). The transport

Key words: sugar transporters, invertase, *Chlorella, Arabidopsis*, hexose transport.

system induced by sugars or non-metabolizable sugar analogues has been characterized as an H^+ symporter system (Komor, 1973; Komor and Tanner, 1974*a*, 1976), which transports sugars and protons with a stoichiometry of 1:1 and is able to accumulate analogues such as 6-deoxyglucose more than 1000-fold (Komor *et al.* 1973*a*). This accumulation has partly been explained by distinctly different K_m values for sugar influx and efflux and partly by differences in the estimated velocity constants contributing to influx compared with those contributing to efflux (Komor *et al.* 1973*a*; Komor and Tanner, 1974*b*).

Sugar transport and accumulation in this lower eukaryote differ in a number of aspects from those in bacteria. For example, when uncouplers are added to cells that have reached the steady state of sugar analogue accumulation, they lose the sugar only very slowly. The same phenomenon is observed under anaerobic conditions when sugar accumulation is energized by light and this light is subsequently switched off (Komor *et al.* 1972, 1973*b*). Sugar efflux is also 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; Komor *et al.* 1972). Finally, 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 (Komor *et al.* 1979). These observations led to the conclusion that the *Chlorella* hexose transport protein acts in an asymmetric way. Although this asymmetric action is not understood in any mechanistic detail, the gene for the corresponding transport protein has been cloned (Sauer and Tanner, 1989) and an *in vitro* transport test has been established (Opekarová *et al.* 1994), so the questions of how sugars are transported through membranes, how the carriers sense and make use of energy and how the transport step may be regulated may eventually be answered.

Cloning and characterizing plant sugar transporter genes and their products

As discussed above, hexose uptake in *Chlorella kessleri* is highly inducible, which suggested that the gene coding for the transport protein could be cloned by differential screening of cDNA from induced *versus* that from non-induced cells. The *HUP1* cDNA (*h*exose *u*ptake *p*rotein) obtained in this way (Sauer and Tanner, 1989) was shown by heterologous expression in *Schizosaccharomyces pombe* to code for a transport protein responsible for hexose uptake, for sensing a pH gradient and for the accumulation of sugar analogues such as 3-*O*-methylglucose (Sauer *et al.* 1990*a*). The mRNA of the *HUP1* gene, absent in photosynthetically grown cells, appears within 5 min after addition of sugars (Hilgarth *et al.* 1991; R. Stadler, K. Wolf, C. Hilgarth, W. Tanner and N. Sauer, in preparation). The hydropathy plot suggested a structure with 12 transmembrane domains, which has been postulated for a large family of sugar transporters (Marger and Saier, 1993); the model for the *Chlorella* transporter is shown in Fig. 1. Heterologous expression in a *S. pombe* mutant not able to grow on, or to take up, D-glucose (M. Höfer and B. Milbradt, unpublished results) allowed a first investigation of the structure/function relationship using site-directed mutagenesis (Caspari *et al.* 1994).

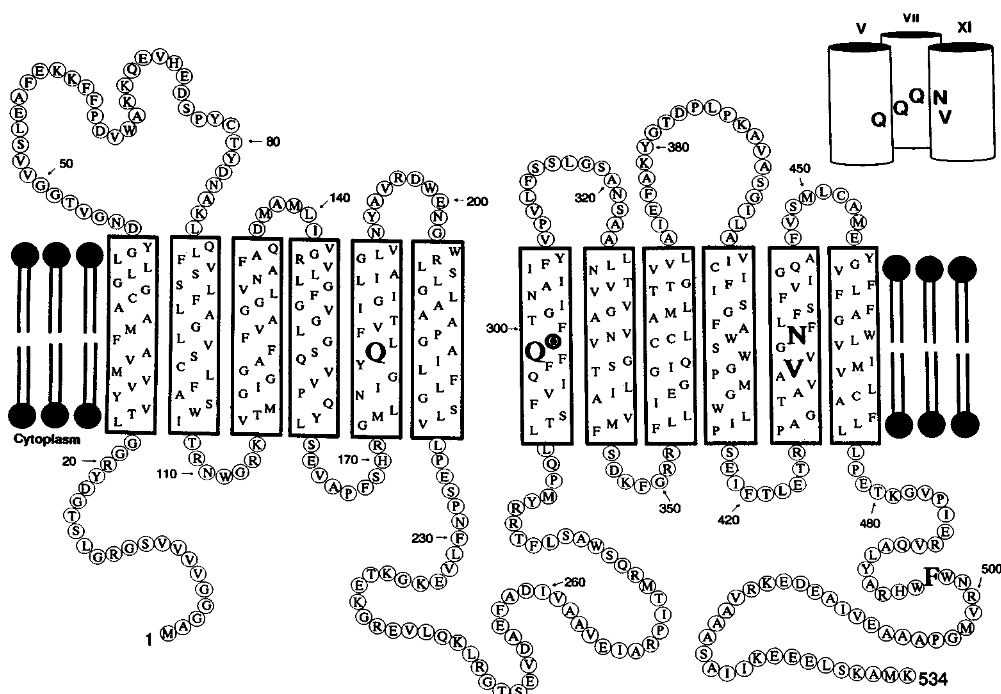

Fig. 1. Sequence and putative topology of the *Chlorella* HUP1 transporter protein. Amino acids replaced in the four K_m mutants randomly generated by the polymerase chain reaction technique are in bold type. F497S was found additionally in one mutant, but was shown not to be responsible for the increased K_m value. Q299N (black) was obtained by site-directed mutagenesis. Inset (top right): model of the three transmembrane helices forming the putative D-glucose recognition site.

Among other observations, it was shown that the three histidyl residues (His-73, His-170 and His-495) could be replaced by arginine without a marked decrease in activity. Moreover, the 27 C-terminal amino acids, but not the 43 C-terminal ones, could be removed without affecting transporter activity. Interestingly, the amino acids Gln-179 and Gln-299, conserved in all hexose transporters sequenced so far, when replaced by asparagines resulted in K_m changes for D-glucose by a factor of 10 (1×10^{-4} to $2\times10^{-4}\,\mathrm{mol\,l^{-1}}$ instead of $1.5\times10^{-5}\,\mathrm{mol\,l^{-1}}$).

To look for other amino acids that may be responsible for substrate binding and specificity, random mutants of *HUP1* cDNA were prepared using the polymerase chain reaction (PCR) technique. *S. pombe* cells grown on gluconate were than transformed with a pool of mutated *HUP1* cDNA and transformants were selected which showed decreased sensitivity towards the toxic sugar analogue 2-deoxyglucose (Will *et al.* 1994). Whereas most transformants showed a drastically decreased rate of glucose uptake, four mutants were obtained which possessed a clearly increased K_m value for D-glucose: Q179E (helix V), Q298R (helix VII) and V433L and N436Y (both in helix XI). Since the two mutants Q298R and N436Y showed an increased K_m value of 1000-fold

(although reasonable V_{max} values), whether more conservative amino acid exchanges in these two positions would still give rise to increased K_m values was investigated. This was indeed the case, as demonstrated with the directed mutations Q298N and N436Q; the K_m values increased 10- to 20-fold (Will *et al.* 1994). It is proposed, therefore, that helices V, VII and XI of the *Chlorella* HUP1 transporter line the sugar translocation path (see inset of Fig. 1) and that the hexose specificity may, in part, be determined by a number of amino acids, four amides (Q179, Q298, Q299 and N436) and one valine (V433), which are located in the putative centre of these helices. The observation that yeast hexokinase B crystallized with a derivatized glucose forms hydrogen bonds to five amino acids, four of which are amides (Anderson *et al.* 1978), may give some support to this hypothesis.

When *HUP1* cDNA was expressed in frog oocytes and substrate specificity was determined by following sugar-dependent membrane depolarization (Aoshima *et al.* 1993), it was noticed that D-galactose was not transported by the product of *HUP1* cDNA, although induced *Chlorella kessleri* does take up D-galactose (Tanner *et al.* 1969). *Chlorella*, however, possesses two more hexose transporter genes (*HUP2* and *HUP3*), which are co-induced by D-glucose (R. Stadler, K. Wolf, C. Hilgarth, W. Tanner and N. Sauer, in preparation). Recently, it was shown that *HUP2* cDNA expressed in *S. pombe* is clearly a D-galactose transporter (relative rates were galactose 100 and glucose 71), whereas the *HUP1* cDNA product transports galactose at less than 10 % of the rate of D-glucose transport (R. Stadler, K. Wolf, C. Hilgarth, W. Tanner and N. Sauer, in preparation).

With the help of probes derived from *HUP1* cDNA, it has been possible to clone and characterize the first hexose transporter STP1 (*s*ugar *t*ransport *p*rotein) of a higher plant, *Arabidopsis thaliana* (Sauer *et al.* 1990*b*). In the meantime, it has become clear from PCR studies that *Arabidopsis* contains at least 12 genes highly related to STP1 (K. Baier and N. Sauer, unpublished results). Moreover, a large family of hexose transporter genes has been described from *Ricinus communis* (Weig *et al.* 1994).

Why does *Arabidopsis* require 12 hexose transporters?

Although it is not yet clear whether all the highly related transporter genes of *Arabidopsis* really transport hexoses, it has at least been shown for four (STP1–STP4) and may well be true for all of them, with possible differences in substrate specificities. The sucrose transporters cloned and sequenced so far (Riesmeier *et al.* 1992; Sauer and Stolz, 1994) are very different from the hexose transporter family (Sauer and Tanner, 1993) and, therefore, are not expected to be present among STP5–STP12. Why would the small *Arabidopsis* plant possess 12 hexose transporters when, in humans, as far as we know, seven seem to be sufficient (Bell *et al.* 1993)? And even more puzzling: why do plants require hexose transporters at all, if the main sugars sent from photosynthesizing leaves (source tissue) to carbon consuming or storing organs (sink tissues) are either sucrose or, less frequently, galactosides of sucrose (sugars of the raffinose family)? Answers to the first question are rapidly coming into sight. The various transporters are expressed in a highly tissue-specific manner: STP1 in the ovary, STP2 in the anthers,

STP3 in the sepals and the stigma, STP4 in the anthers but also in the root tips (E. Truernit and N. Sauer, unpublished results). In addition, it seems likely that some of the transporters are not constitutively expressed, but that their genes may be switched on in response to external (e.g. stress) or internal (hormonal) signals. Thus, a set of hexose transporter genes, each with a specific promotor, may be responsible for constitutive, but tissue-specific, expression or for developmentally or environmentally regulated expression. These transporters may, therefore, constitute the link between developmental information and metabolism and may thereby determine the formation of the various plant cells, tissues and organs in time and space.

What about the second question? Why do plants possess hexose transporters at all? It is true that the main sugars for long-distance translocation within plants are either sucrose or galactosides of sucrose (Zimmermann and Ziegler, 1975). However, it has been speculated for a long time (Glasziou and Gayler, 1972; Eschrich, 1980) that sucrose, after leaving the long-distance translocation system, may be split extracellularly by cell wall invertase and that the monosaccharides arising may be the actual substrates to be taken up by the cells to be fed. This suggestion has recently obtained support from the observation that overexpression of cell wall invertase in leaves of transgenic plants leads to an inhibition of sucrose export from these leaves and an increased re-use of monosaccharides by them (van Schaewen *et al.* 1990). In addition, a maize mutant, defective in kernel filling, was shown to lack a specific cell wall invertase (Miller and Chourey, 1992). Finally, a *Chenopodium* tissue culture, which can be grown photoautotrophically as well as mixo- or heterotrophically (Hüsemann and Barz, 1977), expresses three hexose transporter genes (out of at least seven found in this plant), but the degree of expression does not differ under the three widely differing growth conditions (Roitsch and Tanner, 1994), whereas an extracellular invertase is expressed considerably more extensively when these cells depend on extracellular sugars for growth (T. Roitsch, M. Bittner and D. Godt, in preparation). It seems likely, therefore, that the cooperation of hexose transporters and extracellular cell wall invertase is important for feeding non-photosynthesizing plant cells with organic compounds (Fig. 2) and it is tempting to speculate that the coupling of these two proteins may determine fully, or in part, what has long been termed ‘sink strength’ in plant physiology (Ho, 1988).

In vitro studies with plant sugar transporters

Transport studies with membrane vesicles were first introduced by Kaback in the 1960s and have been intensively applied to investigate the molecular details of the *Escherichia coli* lactose/H$^+$symport (Kaback, 1989). An *in vitro* system established in Konings’ laboratory (Driessen *et al.* 1993), using right-side-out vesicles energized by cytochrome *c* oxidase, is widely used to study H$^+$ symporters. *In vitro* studies of secondary active transporters of plants have so far been performed with plasma membrane vesicles isolated by the aqueous two-phase partitioning method (Bush, 1989; Buckhout, 1989; Lemoine and Delrot, 1989; Williams *et al.* 1992). This uptake system was energized by the formation of a transient membrane potential difference. Recently, it became possible to measure transport mediated by the *HUP1* gene product of *Chlorella kessleri* (Opekarová

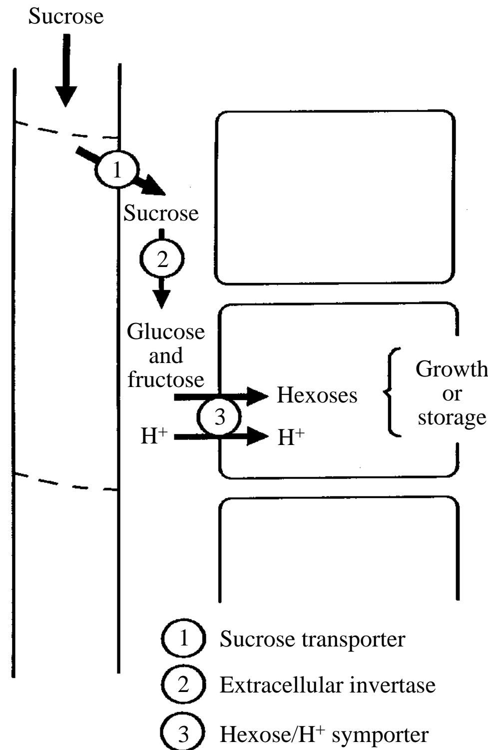

Fig. 2. Model for sugar supply of non-green plant tissues. Long-distance translocation of sucrose proceeds within the phloem in so-called sieve tubes (long stretched cells on the left). Sucrose is unloaded (1) into the cell wall space, where the extracellular invertase (2) hydrolyses it. Hexose transporters (3) are responsible for supplying monosaccharides to the non-photosynthesizing cells.

et al. 1994) in the vesicle system developed by Driessen *et al.* (1993). Plasma membranes of *S. pombe* transformed with the *HUP1* cDNA were fused with proteoliposomes containing cytochrome *c* oxidase. After energization with ascorbate/TMPD/cytochrome *c*, a proton-motive force of more than 130 mV was built up for at least 2 h. D-Glucose was accumulated in such vesicles up to 30-fold; this was not the case in control vesicles prepared from cells transformed with the plasmid not containing the *HUP1* cDNA (Fig. 3). Using this *in vitro* system, it was possible to demonstrate that the degree of accumulation was determined by both kinetic and by thermodynamic parameters. Since the system also allows the manipulation of both components of the proton-motive force individually, the extent to which they contributed to sugar accumulation could be evaluated (Opekarová *et al.* 1994).

In the meantime, this *in vitro* system has also been successfully applied to the study of sucrose/H^+symporters of higher plants (Stolz *et al.* 1994; M. Gahrtz, J. Stolz and N. Sauer, in preparation).

The importance of studying plant transporters in a well-defined *in vitro* system is

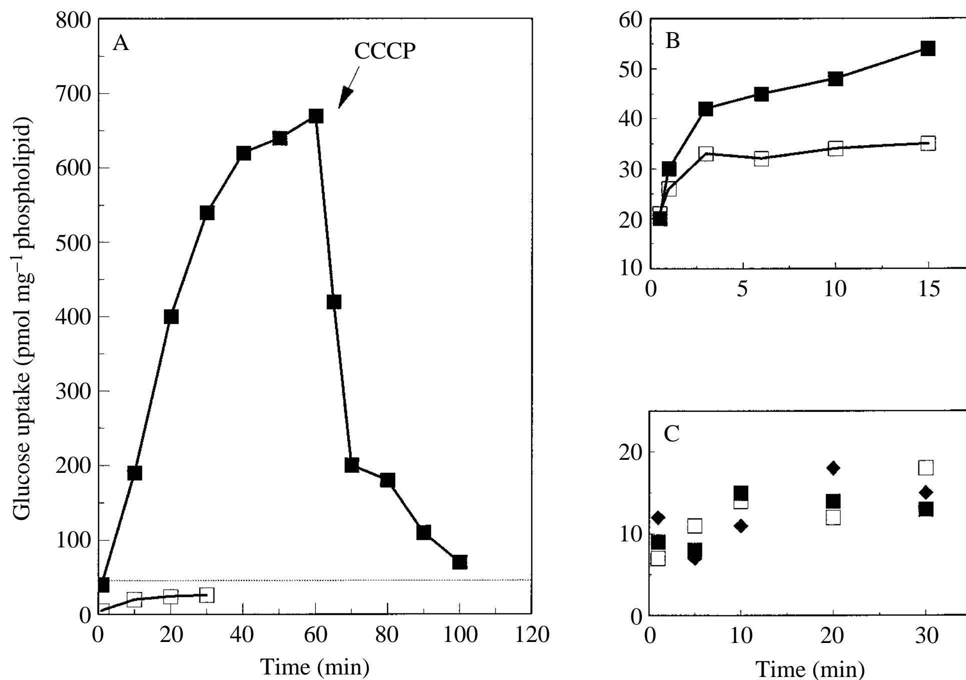

Fig. 3. Uptake of glucose into plasma membrane vesicles of *Saccharomyces pombe*. (A) To the vesicles prepared from *S. pombe* transformed with *HUP1* cDNA, 50 μmol l^{-1} D-glucose, and ascorbate, *N*,*N*,*N*,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) and cytochrome *c* were added at time zero (■) or ascorbate was omitted (□). The dotted line corresponds to the equilibrium concentration. (B) To the same vesicles, 63 μmol l^{-1} D-glucose (■) or L-glucose (□) was added under non-energized conditions. (C) Uptake of 50 μmol l^{-1} glucose into vesicles from plasma membranes of *S. pombe* control transformants (vector without *HUP1* insert): (□) D-glucose, non-energized; (■) D-glucose in the presence of ascorbate, TMPD and cytochrome *c* (energized); (◆) L-glucose in the presence of ascorbate, TMPD and cytochrome *c* (energized).

obvious. First, any purification of fully active transporter proteins requires reconstitution and activity measurement *in vitro*. Purification of hexose and sucrose transporters has been successfully started (Stolz *et al.* 1994). Second, to resolve and to understand some of the unusual properties of the *Chlorella* hexose transporter mentioned above, *in vitro* studies are essential. The 20-year-old observation that the *Chlorella* glucose transporter *in vivo* never acts as a facilitator except in the presence of polyene antibiotics, which interact with sterols (Komor *et al.* 1974), can now be reinvestigated *in vitro* by changing the lipid composition of the vesicles (Opekarová and Tanner, 1994).

The original work reported in this review was supported by the Deutsche Forschungsgemeinschaft (Normalverfahren and SFB 43) and by the Fonds der Chemischen Industrie.

References

ANDERSON, C. M., STENKAMP, R. E., MCDONALD, R. C. AND STEITZ, T. A. (1978). A refined model of the sugar binding site of yeast hexokinase B. *J. molec. Biol.* **123**, 207–219.

AOSHIMA, H., YAMADA, M., SAUER, N., KOMOR, E. AND SCHOBERT, C. (1993). Heterologous expression of the H^+/hexose cotransporter from *Chlorella* in *Xenopus* oocytes and its characterization with respect to sugar specificity, pH and membrane potential. *J. Plant Physiol.* **141**, 293–297.

BELL, G. I., BURANT, C. F., TAKEDA, J. AND GOULD, G. W. (1993). Structure and function of mammalian facilitative sugar transporters. *J. biol. Chem.* **268**, 19161–19164.

BUCKHOUT, T. (1989). Sucrose transport in isolated plasma membrane vesicles isolated from sugar beet (*Beta vulgaris* L.). Evidence for a sucrose proton symport. *Planta* **178**, 393–399.

BUSH, D. R. (1989). Proton-coupled sucrose transport in plasmalemma vesicles isolated from sugar beet (*Beta vulgaris* L. c. v. Great Western) leaves. *Plant Physiol.* **89**, 1318–1323.

CASPARI, T., STADLER, R., SAUER, N. AND TANNER, W. (1994). Structure/function relationship of the *Chlorella* glucose/H^+ symporter. *J. biol. Chem.* **269**, 3498–3502.

DRIESSEN, A. J. M., DE VRIJ, W. AND KONINGS, W. N. (1993). Insertion of lipids and proteins into bacterial membranes by fusion with liposomes. *Meth. Enzymol.* **221**, 394–408.

DROOP, M. R. (1974). Heterotrophy of carbon. In *Algal Physiology and Biochemistry* (ed. W. D. P. Stewart), pp. 530–559. Oxford: Blackwell Scientific Publications.

ESCHRICH, W. (1980). Free space invertase, its possible role in phloem unloading. *Ber. dt. Bot. Ges.* **93**, 363–378.

GLASZIOU, K. T. AND GAYLER, K. R. (1972). Storage of sugars in stalks of sugar cane. *Bot. Rev.* **38**, 471–490.

HAAß, D. AND TANNER, W. (1973). Regulation of hexose transport in *Chlorella vulgaris*. *Plant Physiol.* **53**, 14–20.

HILGARTH, C., SAUER, N. AND TANNER, W. (1991). Glucose increases the expression of the ATP/ADP translocator and the glyceraldehyde-3-phosphate dehydrogenase genes in *Chlorella*. *J. biol. Chem.* **266**, 24044–24407.

HO, L. C. (1988). Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *A. Rev. Plant Physiol. Plant molec. Biol.* **39**, 355–378.

HÜSEMANN, W. AND BARZ, W. (1977). Photoautotrophic growth and photosynthesis in cell suspension cultures of *Chenopodium rubrum. Plant Physiol.* **40**, 77–88.

KABACK, H. R. (1989). Molecular biology of active transport: from membrane to molecule to mechanism. *The Harvey Lectures* **83**, 77–105.

KOMOR, B., KOMOR, E. AND TANNER, W. (1974). Transformation of a strictly coupled active transport system into a facilitated diffusion system by nystatin. *J. Membr. Biol.* **17**, 231–238.

KOMOR, E. (1973). Proton-coupled hexose transport in *Chlorella vulgaris. FEBS Lett.* **38**, 16–18.

KOMOR, E., HAAß, D., KOMOR, B. AND TANNER, W. (1973*a*). The active hexose-uptake system of *Chlorella vulgaris*. K_m-values for 6-deoxyglucose influx and efflux and their contribution to sugar accumulation. *Eur. J. Biochem.* **39**, 193–200.

KOMOR, E., HAAß, D. AND TANNER, W. (1972) Unusual features of the active hexose uptake system of *Chlorella vulgaris*. *Biochim. biophys. Acta* **266**, 649–660.

KOMOR, E., LOOS, E. AND TANNER, W. (1973*b*). A confirmation of the proposed model for the hexose uptake system of *Chlorella vulgaris.* Anaerobic studies in the light and in the dark. *J. Membr. Biol.* **12**, 89–99.

KOMOR, E., SCHWAB, W. G. W. AND TANNER, W. (1979). The effect of intracellular pH on the role of hexose uptake in *Chlorella*. *Biochim. biophys. Acta* **555**, 524–530.

KOMOR, E. AND TANNER, W. (1974*a*). The hexose–proton symport system of *Chlorella vulgaris*. Specificity, stoichiometry and energetics of sugar-induced proton uptake. *Eur. J. Biochem.* **44**, 219–233.

KOMOR, E. AND TANNER, W. (1974*b*). The hexose–proton cotransport system of *Chlorella*. pH-dependent change in K_m-values and translocation constants of the uptake system. *J. gen. Physiol.* **64**, 568–581.

KOMOR, E. AND TANNER, W. (1976). The determination of the membrane potential of *Chlorella vulgaris.* Evidence for electrogenic sugar-transport. *Eur. J. Biochem.* **70**, 197–204.

LEMOINE, R. AND DELROT, S. (1989). Proton-motive-force-driven sucrose uptake in sugar beet plasma membrane vesicles. *FEBS Lett.* **249**, 129–133.

MARGER, M. D. AND SAIER, M. H., JR (1993). A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. *Trends biol. Sci.* **18**, 13–20.
MILLER, M. E. AND CHOUREY, P. S. (1992). The maize invertase-deficient miniature-1 seed mutation is associated with aberrant pedical and endosperm development. *Plant Cell* **4**, 297–305.
OPEKAROVÁ, M., CASPARI, T. AND TANNER, W. (1994). The HUP1 gene product of *Chlorella kessleri*: H^+/glucose symport studied *in vitro*. *Biochim. biophys. Acta* (in press).
OPEKAROVÁ, M. AND TANNER, W. (1994). Nystatin changes the properties of transporters for arginine and sugars: an *in vitro* study. *FEBS Lett.* (in press).
RIESMEIER, J. W., WILLMITZER, L. AND FROMMER, W. B. (1992). Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. *EMBO J.* **11**, 4705–4713.
ROITSCH, T. AND TANNER, W. (1994). Expression of a sugar-transporter gene family in a photoautotrophic suspension culture of *Chenopodium rubrum* L. *Planta* **193**, 365–371.
SAUER, N., CASPARI, T., KLEBL, F. AND TANNER, W. (1990*a*). Functional expression of the *Chlorella* hexose transporter in *Schizosaccharomyces pombe*. *Proc. natn. Acad. Sci. U.S.A.* **87**, 7949–7952.
SAUER, N., FRIEDLÄNDER, K. AND GRÄML-WICKE, U. (1990*b*). Primary structure, genomic organization and heterologous expression of a glucose transporter from *Arabidopsis thaliana*. *EMBO J.* **9**, 3045–3050.
SAUER, N. AND STOLZ, J. (1994). SUC1 and SUC2: two sucrose transporters from *Arabidopsis thaliana*; expression and characterization in baker's yeast and identification of the histidine-tagged protein. *Plant J.* (in press).
SAUER, N. AND TANNER, W. (1989). The hexose carrier from *Chlorella*. cDNA cloning of a eucaryotic H^+-cotransporter. *FEBS Lett.* **259**, 43–46.
SAUER, N. AND TANNER, W. (1993). Molecular biology of sugar transporters in plants. *Bot. Acta* **106**, 277–286.
STOLZ, J., STADLER, R., OPEKAROVÁ, M. AND SAUER, N. (1994). Functional reconstitution of the solubilized *Arabidopsis thaliana* STP1 monosaccharide–H^+ symporter in lipid vesicles and purification of the histidine tagged protein from transgenic *Saccharomyces cerevisiae*. *Plant. J.* (in press).
TANNER, W. (1969). Light-driven active uptake of 3-*O*-methylglucose *via* an inducible hexose uptake system of *Chlorella*. *Biochem. biophys. Res. Commun.* **36**, 278–283.
TANNER, W., GRÜNES, R. AND KANDLER, O. (1969). Spezifität und Turnover des induzierbaren Hexoseaufnahmesystems von *Chlorella*. *Z. Pflanzenphysiol.* **62**, 376–386.
VAN SCHAEWEN, A., STITT, M., SCHMIDT, R., SONNEWALD, U. AND WILLMITZER, L. (1990). Expression of yeast-derived invertase in the cell wall of tobacco and *Arabidopsis* plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. *EMBO J.* **9**, 3033–3044.
WEIG, A., FRANZ, J., SAUER, N. AND KOMOR, E. (1994). Isolation of a family of cDNA clones from *Ricinus communis* L. with close homology to the hexose carriers. *J. Plant Physiol.* **143**, 178–183.
WILL, A., CASPARI, T. AND TANNER, W. (1994). K_m mutants of the *Chlorella* monosaccharide H^+ cotransporter randomly generated by PCR. *Proc. natn. Acad. Sci. USA. (in press)*.
WILLIAMS, L. E., NELSON, S. J. AND HALL, J. C. (1992). Characterization of solute/proton cotransport in plasma membrane vesicles from *Ricinus* cotyledons and a comparison with other tissues. *Planta* **186**, 541–550.
ZIMMERMANN, M. H. AND ZIEGLER, H. (1975). List of sugars and sugar alcohols in sieve tube exudates. In *Encyclopedia of Plant Physiology* , new series, vol. I (ed. A. Pirson and M. H. Zimmermann), pp. 480–503. Berlin, Heidelberg, New York: Springer.