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Identification of an M_r 75 000 component of the H^+/D-glucose cotransporter from *Zea mays* with monoclonal antibodies directed against the mammalian Na^+/D-glucose cotransporter

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Monoclonal antibodies which interact with the mammalian Na^+/D-glucose cotransporter and bind to M_r 75 000 and M_r 47 000 polypeptide components of this transporter have been described (Koepsell, H., Korn, K., Raszeja-Specht, A., Bernotat-Danielowski, S. and Ollig, D. (1988) J. Biol. Chem., 263, 18419–18429). The interaction of these antibodies with plasma membranes from *Zea mays* L. coleoptiles containing an H^+/D-glucose cotransporter was studied. Four monoclonal antibodies cross-reacted with M_r 75 000 and M_r 33 000 polypeptides. One of these antibodies, which inhibits Na^+/D-glucose cotransport in the kidney and stimulates Na^+/D-glucose cotransport in intestine, stimulates electrogenic uptake of 3-*O*-methyl-D-[^{14}C]glucose in plant membrane vesicles. The data indicate common epitopes in the mammalian Na^+/D-glucose cotransporter and the H^+/D-glucose cotransporter of plants and suggest that both transporters contain an M_r 75 000 polypeptide component.

Introduction

Bacteria, algae, plants and animal cells have developed cotransport systems for cations and sugars. These transporters are energized by H^+ or Na^+ gradients which are generated by H^+-ATPases or Na^+/K^+-ATPases. Thus, *Escherichia coli* contains an H^+/arabinose and a H^+/xylose cotransporter [1], whereas in *Chlorella* and in plant cells H^+/D-glucose cotransporters [2–9] have been described. Animals contain Na^+/D-glucose cotransporters in renal and intestinal epithelial cells and facilitated D-glucose-diffusion systems in many different cell types [10–15]. Molecular cloning of sugar transporters revealed amino-acid sequences of (a) facilitated D-glucose-diffusion systems from bacteria and eucaryotic cells [12–16], of (b) the H^+/arabinose and H^+/xylose cotransporter from *E. coli* [1] and of c) an M_r 73 030 polypeptide which is presumed to be a component of the intestinal Na^+/D-glucose cotransporter from rabbit [17]. Significant homology exists between the H^+/arabinose or H^+/xylose cotransporter from *E. coli* and the facilitated D-glucose transporters from yeast, human HepG2 hepatoma cells, rat liver, rat brain or human skeletal muscle (Refs. 1, 12–16, and H.K., unpublished data). Conversely, no homology was detected between the presumed component of the intestinal Na^+/D-glucose cotransporter [17] and the cloned facilitated D-glucose transporters or the H^+/arabinose or H^+/xylose cotransporter (Ref. 17, and H.K., unpublished data). Recently, we identified polypeptides with the molecular weights of 75 000 and 47 000 as components of the porcine renal Na^+/D-glucose cotransporter [18]. Monoclonal antibodies directed against these polypeptides inhibited or stimulated Na^+/D-glucose cotransport and/or high-affinity phlorizin binding in pig kidney [19]. These antibodies cross-react with M_r 75 000 and M_r 47 000 polypeptides in kidney and intestine of rabbit and rat and some of these stimulate Na^+/D-glucose cotransport and high-affinity phlorizin binding in rat intestine (Ref. 20, and H.K., unpublished data). In the present study we investigated a plasma-membrane fraction of *Zea mays* L. coleoptiles for cross-reactivity with our antibodies against the mammalian Na^+/D-glucose cotransporter. Several antibodies crossreacted with two polypeptides with the molecular weights of 75 000 and 33 000 and one antibody was able to stimulate electrogenic uptake of *O*-methyl-D-glucose into plant membrane vesicles. Some of the results have been reported in an abstract [21].

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Materials and Methods

3-*O*-Methyl-D-[^{14}C]glucose (12.1 MBq/mmol) was purchased from Amersham, while unlabelled 3-*O*-methyl-D-glucose and mouse myeloma IgG3 (kappa) was from Sigma. Mouse myeloma IgM, rabbit antisera against mouse IgM or IgG, iodine-125, molecular mass marker proteins, proteinase inhibitors and all other chemicals were obtained as described earlier [18,19]. The monoclonal antibodies against the porcine renal Na^+/D-glucose cotransporter used in the study (R5A3, R4A5, R4A6, T4B2) were generated and partially purified as reported recently [19]. The IgM-antibodies R4A5, R4A6 and T4B2 were harvested from clone supernatants containing 10% (v/v) fetal calf serum whereas the antibody R5A3 (IgG3) was harvested from clones grown in serum-free medium.

Preparation of plant membrane vesicles

Coleoptiles from 5-day-old corn seedings of *Z. mays* [22] were plasmolyzed and extracted as reported earlier [23], except that the extraction was performed in the absence of bovine serum albumin and in the presence of the proteinase inhibitors phenylmethylsulfonyl fluoride (1 mM), leupeptin (10 μM), aprotinin (1.7 μM) and benzamidine (1 mM). From the supernatant after a 10 min centrifugation at $10\,000 \times g$ the microsomal membranes were spun down by 60 min centrifugation at $50\,000 \times g$ and were subsequently separated on a sucrose step-gradient [24]. From the 31%/36% interphase of the gradient, plant membrane vesicles were collected. This membrane fraction is enriched in plasma membrane vesicles [25]. The plant membrane vesicles were washed with and suspended in a buffer containing the permeant anion chloride (buffer A: 250 mM sucrose/100 mM Mops/50 mM KCl/10 mM ascorbic acid/2 mM $MgSO_4$, adjusted to pH 7.4 with KOH) or a buffer containing the impermeant anion cyclamate (buffer B: 250 mM sucrose/100 mM Mops/50 mM potassium cyclamate/10 mM ascorbic acid, adjusted to pH 7.4 with KOH). They were frozen in liquid nitrogen and stored at −20 °C until use.

Identification of antigenic polypeptides

Plant membrane vesicles were separated on SDS-polyacrylamide slab gels and subsequently blotted to nitrocellulose [19,26]. To partially renaturate the antigen after blotting, the nitrocellulose-attached antigen was incubated for 16 h at 37 °C before antibody binding was measured [26].

Measurements of antibody effects on the 3-O-methyl-D-glucose-uptake

Plant membrane vesicles containing 0.6 mg of protein/ml were first incubated for 4 h at 4 °C and subsequently for 30 min at 25 °C with monoclonal antibodies, and then for 15 min at 25 °C with or without 20 μg/ml of valinomycin. The incubation was performed in buffer A or buffer B containing the investigated antibodies or control antibodies (IgG3 or IgM from mouse myeloma cells). For transport measurements 50 μl aliquots of the vesicle/antibody mixtures were rapidly mixed with 50 μl of buffer A or buffer B containing 20 μM of radioactivity labelled 3-*O*-methyl-D-glucose (25 °C). At selected time intervals the uptake was stopped by the addition of 2 ml of ice-cold buffer A or B. The vesicles were then applied to 0.45 μm cellulose acetate filters and washed with buffer A or B; the radioactivity on the filters was then determined [24]. Initial transport rates were calculated by subtracting the uptake at time zero from the uptake after incubation for 5 s. All measurements were performed in triplicate.

Results

To investigate antibody interaction with plant plasma membranes, a microsomal fraction from *Z. mays* L. coleoptiles was prepared by density gradient centrifugation [24,25] which was enriched in plasma membrane marker enzymes [27,28]. This fraction contained inside-out and right-side-out plant plasma membrane vesicles [24,27] which exhibit H^+/D-glucose cotransport activity: Firstly, H^+/*O*-methyl-D-glucose efflux from the inside-out oriented vesicles, containing an ATP-driven proton pump, was demonstrated [24]. Thus, *O*-methyl-D-glucose efflux from *O*-methyl-D-glucose-loaded vesicles was increased when MgATP was added to the buffer. The proton pump increases the proton concentration in the vesicles [29]. Secondly, at ionic equilibrium with potassium as the only cation, saturable *O*-methyl-D-glucose uptake (into outside-out and possibly also into inside-out vesicles) was measured and apparent K_m values between 2 and 4 mM *O*-methyl-D-glucose were determined (T.R., unpublished data). The initial rates of *O*-methyl-D-glucose uptake were increased significantly when the measurements were performed in the presence of a permeant anion or in the presence of valinomycin (Table I, and T.R., unpublished data). Thus, the analysed transport of *O*-methyl-D-glucose includes cotransport of cations or countertransport of anions. Since anion–sugar countertransport or K^+/sugar cotransport has not been detected in pro- and eucaryotes [7,10] these data provide further evidence that in the plasma membrane vesicles from *Z. mays* *O*-methyl-D-glucose is cotransported with H^+.

In Western blots of porcine renal brush-border membranes the monoclonal antibodies R5A3, R4A5, R4A6, T4B2 bind to M_r 75 000 and/or M_r 47 000 polypeptides which are components of the porcine renal Na^+/D-glucose cotransporter [18,19]. The antibodies alter function and structure of this transporter. Binding of the monoclonal antibodies to M_r 75 000 and/or M_r 47 000 poly-

TABLE I

Demonstration that antibody R5A3 stimulates electrogenic O-methyl-D-glucose uptake into plant membrane vesicles

Plasma membrane vesicles from *Z. mays* L. coleoptiles (0.6 mg of protein/ml) containing potassium cyclamate were preincubated without and with 0.08 mg/ml of antibody R5A3. Thereafter, initial uptake rates of 10 μM *O*-methyl-D-glucose were measured in the absence and presence of 20 μg/ml of valinomycin and of 50 mM D-glucose (see Materials and Methods). The measurements were performed in the presence of 50 mM potassium cyclamate inside and outside the vesicles (absence of permeant anions). Mean ± S.D. from three measurements are presented.

Presence of R5A3	Presence of 50 mM D-glucose and/or valinomycin during transport		Initial rates of *O*-methyl-D-glucose uptake (pmol/mg of protein per min)
	D-glucose	valinomycin	
0	D-glucose	0	9 ± 2
0	D-glucose	valinomycin	11 ± 2
0	0	0	30 ± 4
R5A3	0	0	39 ± 4
0	0	valinomycin	84 ± 7
R5A3	0	valinomycin	123 ± 10

peptides was also demonstrated in Western blots of brush-border membranes from rat kidney, rabbit kidney, porcine intestine, rat intestine and rabbit intestine (Ref. 20, and H.K., unpublished data). The antibodies are thought to be directed against conserved epitopes of the mammalian Na^+/D-glucose cotransporter. In Fig. 1, binding of the monoclonal antibodies to plasma membrane polypeptides from *Z. mays* L. coleoptiles was investigated in Western blots. While a mixture of non-specific IgG and IgM did not show significant binding (Fig. 1f), the four monoclonal antibodies react with two plant polypeptides with the molecular weights of 75 000 and 33 000 (Fig. 1b–d).

Preliminary experiments showed that antibody R5A3 stimulated and antibody R4A5 inhibited H^+/D-glucose cotransport in plant membrane vesicles (data not shown). To study the effect of R5A3 in detail the influence of R5A3 on the time-course of *O*-methyl-D-glucose uptake into plant plasma membrane vesicles was investigated (Fig. 2). These measurements were performed in the presence of 250 mM sucrose, 100 mM Mops (pH 7.4) and 50 mM KCl on both membrane sides and were started by addition of 10 μM *O*-methyl-D-glucose to the outside of the vesicles. The uptake rate of *O*-methyl-D-glucose was increased when the vesicles were preincubated with R5A3, while the equilibrium concentration of *O*-methyl-D-glucose in the vesicles after 2 min incubation was not changed. In the presence of 50 mM D-glucose the initial uptake rate of *O*-methyl-D-glucose was reduced to less than 15% (Fig. 2), while 50 mM L-glucose had no effect, indicating that the transporter is stereospecific for D-glucose (data not shown).

Since the nonspecific *O*-methyl-D-glucose uptake measured in the presence of 50 mM D-glucose was not altered by the antibody R5A3 (Fig. 2), the antibody does not alter the passive *O*-methyl-D-glucose-permeability of the vesicles but interacts specifically with the D-glucose transporter. The *O*-methyl-D-glucose uptake in Fig. 2 predominantly represents electrogenic H^+/*O*-methyl-D-glucose cotransport, since the initial *O*-methyl-D-glucose uptake rates were reduced more than 2 fold when the measurements were performed in the presence of the impermeant anion, cyclamate, instead of chloride (compare Fig. 2 and Table I). Table I shows that the initial *O*-methyl-D-glucose-uptake measured in the presence of cyclamate was increased significantly when the generation of an vesicle inside-positive mem-

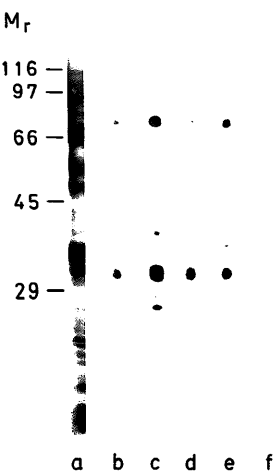

Fig. 1. Crossreaction of monoclonal antibodies against the mammalian Na^+/D-glucose cotransporter with polypeptides from plant plasma membranes. Polypeptides of a plasma membrane enriched microsomal fraction from *Z. mays* L. coleoptiles were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose and cut into strips. The strips were stained with Amido black (a) or reacted with hybridoma supernatants from the cell lines R5A3 (b), R4A5 (c), R4A6 (d) or T4B2 (e). As a control, the reaction was also performed with culture medium containing 50 μg/ml of mouse myeloma IgG plus 50 μg/ml of mouse myeloma IgM (f). The lines indicate the positions of the molecular mass marker proteins at 116 kDa (β-galactosidase), 97 kDa (phosphorylase *b*), 66 kDa (bovine serum albumin), 45 kDa (ovalbumin), and 29 kDa (carbonic anhydrase).

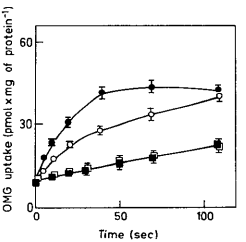

Fig. 2. Effect of a monoclonal antibody against the mammalian Na^+/D-glucose cotransporter on the time-course of 3-*O*-methyl-D-glucose uptake into plant plasma membrane vesicles. Plasma membrane vesicles from *Z. mays* L. coleoptiles containing 0.6 mg of protein/ml were incubated in the absence (open symbols) or presence (closed symbols) of 0.08 mg/ml of antibody R5A3 as described in Materials and Methods. Thereafter, the time-dependent uptake of radioactively labeled *O*-methyl-D-glucose (10 μM) into the vesicles was measured at equilibrium of ions in the presence of 50 mM KCl inside and outside the vesicles (see Materials and Methods). The measurements were performed in the absence (○, ●) or presence (□, ■) of 50 mM D-glucose. Mean ± S.D. of a representative experiment are presented.

brane potential during the electrogenic *O*-methyl-D-glucose transport was prevented by the addition of valinomycin, which allows compensatory efflux of K^+ from the vesicles. Since the initial *O*-methyl-D-glucose uptake rate was also increased by R5A3 when the uptake was measured in the presence of valinomycin,

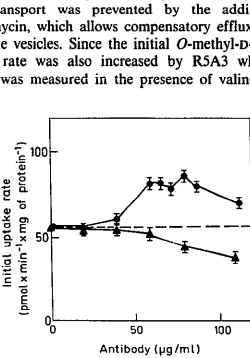

Fig. 3. Concentration dependence of the effect of antibody R5A3 on the initial rates of 3-*O*-methyl-D-glucose uptake into plant membrane vesicles. Plasma membrane vesicles from *Z. mays* L. coleoptiles containing 0.6 mg of protein/ml incubated with different concentrations of the IgG3 antibody R5A3 (●) or of mouse myeloma IgG3 (▲). Thereafter, initial uptake rates of 10 μM radioactively labeled *O*-methyl-D-glucose into the vesicles were measured as described in Materials and Methods. The measurements were performed at equilibrium of ions in the presence of 50 mM KCl inside and outside the vesicles.

R5A3 alters the activity of the electrogenic D-glucose transporter rather than the permeability of cyclamate or potassium. Fig. 3 shows the effect of different R5A3 concentrations on the initial uptake rates of 10 μM *O*-methyl-D-glucose which were measured at equilibrium of potassium and chloride. In this experiment *O*-methyl-D-glucose uptake was maximally stimulated 50–60% by the IgG3-antibody R5A3 over that measured in the presence of nonspecific mouse myeloma IgG3. The half-maximal effect on the membrane vesicles containing 0.6 mg of protein/ml was obtained with an antibody concentration of about $3 \cdot 10^{-7}$ M. Nonspecific myeloma IgG3 did not stimulate *O*-methyl-D-glucose uptake but showed some inhibition at higher concentrations.

Discussion

In the present study four monoclonal antibodies directed against the mammalian Na^+/D-glucose cotransporter were employed to identify components of the H^+/D-glucose cotransporter from plants. In porcine renal brush-border membranes these antibodies inhibit or stimulate Na^+/D-glucose cotransport and/or Na^+-dependent phlorizin binding [19] and bind to M_r 75000 and M_r 47000 polypeptides which have been identified as components of the renal Na^+-D-glucose cotransporter. The monoclonal antibodies (R5A3, R4A5, R4A6, T4B2) are assumed to cross-react with the renal Na^+/D-glucose cotransporter from rabbit and rat as well as with the intestinal Na^+/D-glucose cotransporter from pig, rabbit and rat, since also in these tissues the antibodies bind specifically to polypeptides with the apparent molecular weights of 75000 and 47000 (Ref. 20, and H.K., unpublished data). Furthermore, in membrane vesicles of rat intestine some of the antibodies were able to stimulate Na^+-dependent high-affinity phlorizin binding (R5A3, R4A6) and Na^+-gradient-dependent D-glucose uptake (R5A3). The observation that in porcine kidney, antibody R5A3 inhibited Na^+/D-glucose cotransport as well as Na^+-dependent phlorizin binding whereas in rat intestine the same antibody stimulated Na^+/D-glucose cotransport and Na^+-dependent phlorizin binding suggests that the renal and intestinal Na^+/D-glucose cotransporters are similar but not completely identical. Recent data suggest that there exists also some homology between the mammalian Na^+/D-glucose cotransporter and the mammalian facilitated D-glucose diffusion system. Thus, immunohistochemical studies revealed that some of our monoclonal antibodies reacted mainly at the renal and intestinal brush-border membrane where the Na^+/D-glucose cotransporter has been localized, but also showed some cross-reaction at the basal-lateral membrane where the facilitated diffusion system for D-glucose is present (Refs. 20, 10, and H.K., unpublished data). Moreover,

in Western blots of erythrocyte membranes some of our antibodies showed some cross-reaction with a polypeptide of an apparent molecular weight between 44000 and 50000 which is supposed to represent the facilitated diffusion system for D-glucose (Ref. 30, and H.K., unpublished data).

The present study shows that the above-described four monoclonal antibodies against the mammalian Na^+/D-glucose cotransporter cross-react with M_r 75000 and M_r 33000 polypeptides of a microsomal fraction from *Z. mays* L. coleoptiles, which was enriched in plasma membranes. Since one of the antibodies (R5A3) which inhibits Na^+/D-glucose cotransport in porcine kidney and stimulates Na^+/D-glucose cotransport in rat intestine, was able to stimulate electrogenic H^+/*O*-methyl-D-glucose cotransport in plants, the data suggest that the H^+/D-glucose cotransporter from plants contains polypeptide components with molecular weights of 75000 and 33000. Whether both polypeptides are subunits of the H^+/D-glucose cotransporter with partially homologous amino-acid sequences or whether the antigenic M_r 33000 polypeptide is a proteolytic splitting product of the M_r 75000 polypeptide cannot be decided from our data, since we cannot exclude proteolysis, although proteinase inhibitors were added during the preparation of the plasma membranes (for discussion see Ref. 19).

Since the information on primary structures of sugar transporters is still fragmentary, the evolution of transporters which catalyze facilitated diffusion and cation-cotransport of sugars is an unresolved question. Our data demonstrate the existence of common epitopes on the mammalian Na^+/D-glucose cotransporter and the H^+/D-glucose cotransporter from *Z. mays*. Homology between the H^+/arabinose cotransporter from *E. coli*, the H^+/xylose cotransporter from *E. coli* [1] and several facilitated D-glucose diffusion systems from pro- and eucaryotic cells [12–16] has been demonstrated (Refs. 1, 16, H.K., unpublished data). Moreover, an M_r 73080 polypeptide has been cloned which is considered to be a component of the Na^+/D-glucose cotransporter from rabbit intestine, since the related mRNA was able to stimulate drastically Na^+/D-glucose cotransport in oozytes from *Xenopus laevis* [17]. Surprisingly, no homology of this polypeptide with any of the cloned sugar transporters could be detected (Ref. 17, H.K., unpublished data). These data can be explained in two ways: either (a) the mammalian Na^+/D-glucose cotransporter and the H^+/D-glucose cotransporter belong to one transporter family while the facilitated D-glucose diffusion systems, the H^+/arabinose cotransporter and H^+/xylose cotransporters belong to another, or (b) the facilitated D-glucose diffusion systems, the H^+/sugar cotransporters from *E. coli* and plants and the eucaryotic Na^+/D-glucose cotransporters against which our antibodies are directed all have developed from a common ancestor, whereas the recently cloned M_r 73080 polypeptide from intestine is either part of a different transporter family or represents a membrane protein that stimulates the endogenous Na^+/D-glucose cotransporter which has been detected in oozytes from *X. laevis* [31]. Future cloning experiments have to be performed to elucidate the evolution of sugar transporters. In these, DNA sequences of cloned sugar transporters and antibodies directed against sugar transporters may be employed to screen lambda gt10 and lambda gt11 libraries of animals, bacteria and plants.

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References

1 Maiden, M.C.J., Davis, E.O., Baldwin, S.A., Moore, D.C.M. and Henderson, P.J.F. (1987) Nature 325, 641–643.
2 Gogarten, J.P., Bentrup, F.-W. (1984) in Membrane Transport in Plants (Cram, W.J., Janáček, K., Rybová, R. and Sigler, K., eds.), pp. 183–188. John Wiley & Sons. Chichester.
3 Guy, M., Reinhold, L. and Rahat, M. (1980) Plant Physiol. 65, 550–553.
4 Lin, W., Schmitt, M.R., Hitz, W.D. and Giaquinta, R.T. (1984) Plant Physiol. 76, 894–897.
5 Martinoia, E., Kaiser, G., Schramm, M.J. and Heber, U. (1987) Plant Physiol. 131, 467–478.
6 Komor, E. (1982) in Plant Carbohydrates I (Loewus, F.A. and Tanner, W., eds.), Encyclopedia of Plant Physiology, Vol. 13a, pp. 635–676, Springer, Heidelberg.
7 Reinhold, L. and Kaplan, A. (1984) Annu. Rev. Plant Physiol. 35, 45–83.
8 Komor, E. and Tanner, W. (1976) Eur. J. Biochem. 70, 197–204.
9 Komor, E., Schobert, Ch. and Cho, B.-H. (1983) FEBS Lett. 156, 6–10.
10 Kinne, R. (1976) Curr. Topics Membr. Transp. 8, 209–267.
11 Semenza, G., Kessler, M., Hosang, M., Weber, J. and Schmidt, U. (1984) Biochim. Biophys. Acta 779, 343–379.
12 Mueckler, M., Caruso, C., Baldwin, S.A., Panico, M., Blench, I., Morris, H.R., Allard, W.J., Lienhard, G.E. and Lodish, H.F. (1985) Science 229, 941–945.
13 Birnbaum, M.J., Haspel, H.C. and Rosen, O.M. (1986) Proc. Natl. Acad. Sci. USA 83, 5784–5788.
14 Thorens, B., Sarkar, H.K., Kaback, H.R. and Lodish, H.F. (1988) Cell 55, 281–290.
15 Kayano, T., Fukumoto, H., Eddy, R.L., Fan, Y.-S., Byers, M.G., Shows, T.B. and Bell, G.I. (1988) J. Biol. Chem. 263, 15245–15248.
16 Celenza, J.L., Marshall-Carlson, L. and Carlson, M. (1988) Proc. Natl. Acad. Sci. USA 85, 2130–2134.
17 Hediger, M.A., Coady, M.J., Ikeda, T.S. and Wright, E.M. (1987) Nature 330, 379–381.
18 Neeb, M., Kunz, U. and Koepsell, H. (1987) J. Biol. Chem. 262, 10718–10727.
19 Koepsell, H., Korn, K., Raszeja-Specht, A., Bernotat-Danielowski, S. and Ollig, D. (1988) J. Biol. Chem. 263, 18419–18429.
20 Haase, W. and Koepsell, H. (1989) Eur. J. Cell. Biol. 48, 360–374.
21 Rausch, T., Raszeja-Specht, A., Verstappen, R. and Koepsell, H. (1988), Plant Physiol. 86, 76.

22 Rausch, T., Butcher, D.N. and Hudd, G. (1988) Plant Sci. 57, 19–25.
23 Rausch, T., Ziemann-Roth, M. and Hilgenberg, W. (1985) Plant Physiol. 77, 881–885.
24 Rausch, T., Butcher, D.N. and Taiz, L. (1987) Plant Physiol. 85, 996–999.
25 Chanson, A., McNaughton, E. and Taiz, L. (1984) Plant Physiol. 76, 498–507.
26 Birk, H.-W. and Koepsell, H. (1987) Anal. Biochem. 164, 12–22.
27 Hager, A. and Biber, W. (1984) Z. Naturforsch. 39c, 927–937.
28 Rausch, T. (1987) in Plant Vacuoles (Marin, B., ed.), pp. 287–289, Plenum, New York.
29 Briskin, D.P., Thornley, W.R. and Wyse, R.E. (1985) Plant Physiol. 78, 871–875.
30 Koepsell, H. (1986) Rev. Physiol. Biochem. Pharmacol. 104, 65–137.
31 Weber, W.-M., Schwarz, W. and Passow, H. (1989) J. Membrane Biol., in press.