Structural analysis of a plant sucrose carrier using monoclonal antibodies and bacteriophage lambda surface display

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Abstract Monoclonal antibodies were raised and selected against recombinant *Plantago major* PmSUC2 sucrose carrier protein. Epitopes of two monoclonal antibodies (PS2-1A2 and PS2-4D4) were mapped using N-terminally truncated PmSUC2 proteins and a lambda library displaying random PmSUC2 peptides. PS2-1A2 recognizes an octapeptide close to the N-terminus of PmSUC2, PS2-4D4 binds to a decapeptide at the very C-terminus. Analyses of antibody binding to yeast protoplasts with functionally active, tagged *PmSUC2* protein revealed that both epitopes are located in cytoplasmic domains of PmSUC2. These results support a model for plant sucrose transporters containing 12 transmembrane helices with the N-terminus and the C-terminus on the cytoplasmic side of the plasma membrane.

Key words: Monoclonal antibody; Phage display; Topology; Sucrose transport; *Plantago*

1. Introduction

Genes encoding plant sucrose-H^+ symporters have been cloned from numerous species [1–7]. The proteins are localized in the plasma membrane and import sucrose into the long distance transport system [8–10] or into developing embryos [4,7]. Mutant plants with reduced expression levels of a sucrose transporter gene show severe changes in their phenotype, such as stunted growth, reduced root development and chlorosis [11–14].

Little is known about the structure of these transporters that share some homology [15] with the *Escherichia coli* melibiose permease [16,17]. At present, all statements on their structure are inferred from results obtained for distantly related, non-plant proteins, such as the mammalian Glut1 glucose facilitator or the lactose permease lacY and the melibiose permease melB from *E. coli* (reviewed in [18,19]). Also for these transporters direct structural information from crytallographic analyses is missing. However, indirect approaches, such as spectroscopic analyses and hydrogen exchange experiments of the Glut1 glucose facilitator, revealed the helix and β-sheet content and that about 80% of the polypeptide backbone are accessible to water [20–22]. Glut1 was also the first transporter for which 12 transmembrane helices were postulated [22] and peptide-specific antibodies revealed the cytoplasmic location of the C-terminus of the Glut1 glucose transporter [23]. Eventually 12 transmembrane helices were confirmed by glycosylation scanning [25]. A similar model was proposed for lacY, the *E. coli* lactose permease [24] and fusions of the *phoA* gene to 3′-deletions of *melB* [26,27] or *lacY* [28,29] allowed predictions on the location of the 12 transmembrane domains.

This paper describes the first topological analyses of a plant sucrose carrier. The epitopes of two mABs directed against PmSUC2 were mapped and used to localize both the N-terminus and the C-terminus of this carrier protein on the cytoplasmic side of the plasma membrane.

2. Materials and methods

2.1. Generation of monoclonal antibodies

Affinity-purified PmSUC2biohis6 [30] was used to immunize mice. Spleen cells were fused to the myeloma cell line P3-X63-Ag8.653 [31] according to standard procedures [32]. The monoclonal hybridoma cell lines 1A2 and 4D4 were obtained by limited dilution. The resulting mABs PS2-1A2 and PS2-4D4 were characterized as IgG_1 kappa light chains (Mouse Typer Kit, Bio-Rad, München, Germany). The reactivity of the hybridoma supernatants with PmSUC2 was screened ELISAs using microtiter plates coated with total membranes from the yeast strains GDY2 (expressing *PmSUC2*), GDY2133 (expressing *PmSUC2biohis6*) or GDY1000 (control strain expressing no plant sucrose transporter) [33]. After incubation with anti-mouse IgG-peroxidase conjugate (Promega W402B, Madison, USA) plates were incubated with the peroxidase substrate 2,2-azino-D-[3-ethylbenzthiazoline-sulfonate(6)]. Color development was quantified photometrically at 405 nm.

2.2. Screening for phages interacting with PS2-1A2 or PS2-4D4

The used phage display library was described previously [34]. Phages interacting with PS2-1A2 or PS2-D4 were enriched by affinity binding to immobilized mABs. Bound phages were released [35] and used to infect *E. coli* Q358 [36]. Phage plaques were lifted to nitrocellulose filters that were incubated with the hybridoma supernatants and with anti-mouse IgG-phosphatase conjugate (Promega S372B, Madison, USA). Inserts of immunopositive phages were sequenced.

2.3. Generation of N-terminally truncated PmSUC2 proteins

A *Bam*HI restriction site was introduced upstream from the *PmSUC2* start ATG codon in the *PmSUC2* cDNA clone pTP18 [2]. This site was used for the insertion of PCR fragments of N-terminally truncated *PmSUC2* cDNAs carrying a start ATG codon followed by the codons for amino acid residues 9 or 27 of the original *PmSUC2* sequence. The truncated clones were expressed in yeast strain SEY2102 [37] using the yeast/*E. coli* shuttle vector NEV-E [3]. The resulting strains produced truncated PmSUC2 proteins lacking amino

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Abbreviations: CCCP, carbonyl cyanide-*m*-chlorophenylhydrazone; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescenin isothiocyanate; mAB, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TRITC, tetramethylrhodamine B isothiocyanate

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acids 2 to 8 (strain RSY1-08) or 2 to 26 (strain RSY1-26). A control strain (RSY1) with the wild-type cDNA was also generated.

2.4. Membrane preparation, SDS-PAGE and Western blotting

SDS-PAGE of yeast total membrane preparations [38] were performed as described [39]. Proteins were transferred to nitrocellulose membranes as described [40] in the presence of 0.02% SDS. Binding of mABs was detected using peroxidase-coupled secondary antibodies (Promega W402B, Madison, USA) and ECL reagents (Amersham-Pharmacia, Freiburg, Germany).

2.5. Transport of ^{14}C-sucrose into proteoliposomes

Dialyzed hybridoma supernatants were mixed 1:11 (vol/vol) with PmSUC2biohis6-containing proteoliposomes [33], frozen, thawed and sonicated to form vesicles with mABs on either side of the membrane. Vesicles were incubated for 1 h on ice and uptake of ^{14}C-sucrose was measured as described [33].

2.6. Preparation, permeabilization and fluorescence labelling of yeast protoplasts

Yeast cells were harvested, washed and resuspended in 10 mM Tris/HCl, pH 7.5, 2 mM EDTA, 0.1% β-mercaptoethanol, 1 M sorbitol. After a 1 h incubation on ice, cells were washed with and resuspended in 50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1 M sorbitol. Cells were protoplasted with β-1,3 glucanase (Quantazyme ylg, Quantum biotechnologies, Montreuil-Sous-Bois, France) and hybridoma supernatants were added in the presence or absence of 0.2% Triton X-100. After removal of unbound antibodies, cells were incubated with avidin-TRITC conjugate (Sigma E3011, Deisenhofen, Germany; diluted 1:100) for labelling of biotin proteins and with anti-mouse IgG-FITC conjugate (Sigma F0257, Deisenhofen, Germany; diluted 1:100) for labelling of bound mABs. TRITC and FITC fluorescence was detected after excitation with light of 547 nm or 495 nm, respectively.

3. Results

3.1. Generation of mABs reacting with PmSUC2

Mice were immunized with affinity-purified PmSUC2biohis6 [33] representing a functionally active, C-terminal fusion of PmSUC2 [2,33] to the biotinylation domain of the *K. pneumoniae* oxaloacetate decarboxylase [41] and to six histidine residues [42]. After the fusion of spleen cells to a myeloma

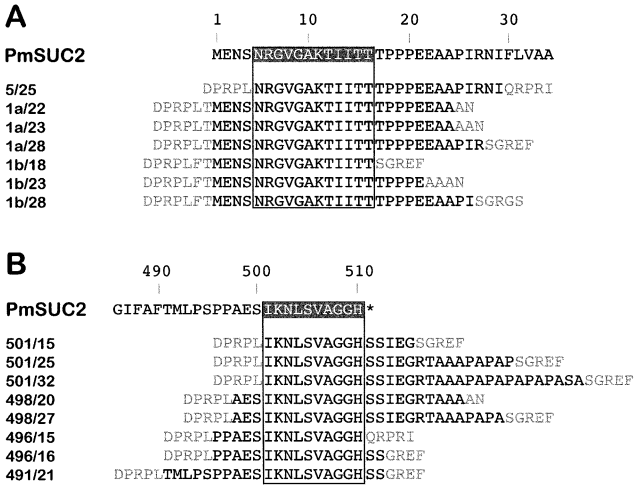

Fig. 1. Amino acid sequences derived from the cDNA inserts of phages reacting with PS2-1A2 and PS2-4D4. The N-terminal (A) and C-terminal (B) sequences of PmSUC2 are given. Numbers to the left represent phage clones yielding positive signals with PS2-1A2 (A) or PS2-4D4 (B). Amino acid residues given in black are derived from PmSUC2, residues in grey from flanking sequences of the vector. Amino acid residues present in all phage inserts are boxed, the resulting minimal recognition sequences are highlighted.

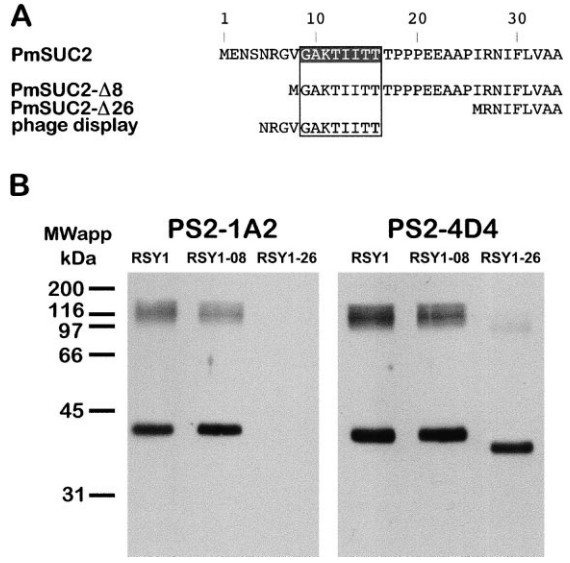

Fig. 2. Alignment of the N-terminal sequences of the PmSUC2 wild-type protein, the N-terminally truncated proteins PmSUC2-Δ8 and PmSUC2-Δ26, and the dodecapeptide determined as recognition site of PS2-1A2 (A). The octapeptide present in all sequences is boxed and highlighted in the wild-type sequence. B: Total membrane proteins (0.5 μg/lane) from yeast cells expressing wild-type *PmSUC2* cDNA, or *PmSUC2-Δ8* or *PmSUC2-Δ26* mutant cDNAs analyzed on Western blots with PS2-1A2 or PS2-4D4.

cell line, the specificities of the hybridoma supernatants were tested on ELISA plates coated with total membranes of wild-type or PmSUC2-expressing yeast cells. Two positive clones were purified by limited dilution and the corresponding mABs, PS2-1A2 and PS2-4D4, were characterized as IgG_1 kappa light chains (data not shown).

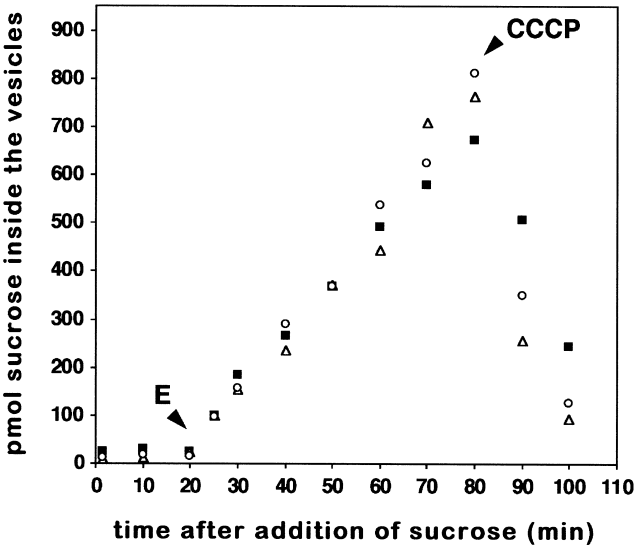

Fig. 3. Analyses of the transport activity of PmSUC2biohis6 in proteoliposomes in the absence or presence of PS2-1A2 or PS2-4D4. Proteoliposomes containing beef heart cytochrome-*c*-oxidase and recombinant PmSUC2biohis6 protein were formed in the absence of mABs (■), in the presence of PS2-1A2 (△) or in the presence of PS2-4D4 (○). After addition of radiolabelled ^{14}C-sucrose proteoliposomes were energized (E) with ascorbate, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine and cytochrome-*c* [30] or deenergized with uncoupler (CCCP) as indicated.

3.2. Identification of the PmSUC2 epitopes recognized by PS2-1A2 and PS2-4D4

From a lambda phage display library expressing random fragments of a *PmSUC2biohis6* cDNA [33] seven independent phages reacted with PS2-1A2, eight with PS2-4D4. In Fig. 1 the translated peptide sequences derived from these identified cDNA fragments were aligned and compared with the sequence of the wild-type PmSUC2 protein. All peptides recognized by PS2-1A2 (Fig. 1A) contained overlapping fragments from the N-terminus of the PmSUC2 protein. The fusion peptides of λ phages 5/25 and 1b/18 defined a dodecapeptide present in all seven sequences. This peptide corresponds to amino acid residues 5 to 16 of the original PmSUC2 protein sequence [2]. All peptides recognized by PS2-4D4 (Fig. 1B) contained overlapping fragments from the C-terminus of the PmSUC2 protein. The fusion peptides of λ phages 501/15, 501/25 and 501/32 and of λ phage 496/15 defined a decapeptide present in all eight peptides. This epitope corresponds to amino acid residues 501 to 510 of the PmSUC2 protein sequence representing the last ten amino acid residues in the wild-type PmSUC2 protein [2].

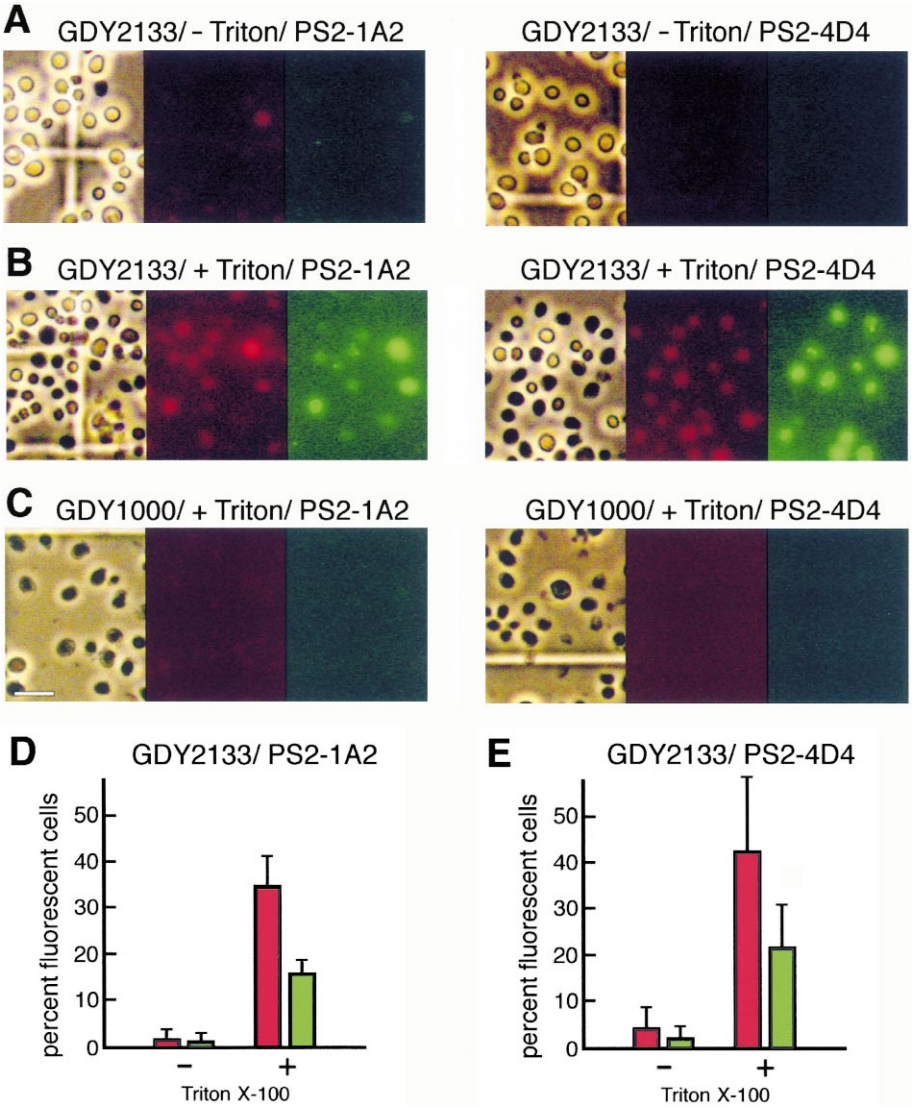

Fig. 4. Analyses of the topology of *PmSUC2*. A: *PmSUC2biohis6*-expressing GDY2133 cells were protoplasted and incubated with PS2-1A2 (left) or PS2-4D4 (right) followed by a simultaneous incubation with avidin-TRITC conjugate and anti-mouse IgG-FITC conjugate. Cells were microscopically analyzed (from left to right) under phase contrast, under TRITC excitation light and under FITC excitation light. Most cells were intact after the protoplasting procedure (no dark cells in phase contrast and no TRITC fluorescence). No green FITC fluorescence is detected. B: GDY2133 cells were treated and analyzed as described in A but permeabilized with 0.2% Triton X-100 prior to the mAB treatment. Many of the cells were permeabilized (dark cells in phase contrast and showing TRITC fluorescence). Green FITC fluorescence indicates antibody binding after permeabilization. C: GCY1000 control cells were treated as described in B and analyzed as described in A. Although most of the cells were permeabilized neither TRITC nor FITC fluorescence was detected. Space bar = 20 μm for A to C. D: Quantitative analyses of the results obtained with PS2-1A2 in not permeabilized (−Triton X-100) and permeabilized (+Triton X-100) GDY2133 cells as shown in A and B, including the standard deviation. The percentage of cells showing TRITC (red bars) or FITC fluorescence (green bars) represents the average of three independent experiments. E: Quantitative analyses of the results obtained with PS2-4D4 in not permeabilized (−Triton X-100) and permeabilized (+Triton X-100) GDY2133 cells as shown in A and B. For additional information see D.

3.3. Analysis of PS2-1A2 and PS2-4D4 binding to wild-type and truncated PmSUC2 proteins

The interaction of PS2-1A2 and PS2-4D4 with PmSUC2 was further analyzed with total membranes from yeast strains expressing cDNAs encoding wild-type PmSUC2 protein (strain RSY1) or PmSUC2 proteins lacking amino acids 2 to 8 (PmSUC2-Δ8; strain RSY1-08) or amino acids 2 to 26 (PmSUC2-Δ26; strain RSY1-26). In PmSUC2-Δ8 only eight amino acids of the dodecapeptide epitope for PS2-1A2 are present, in PmSUC2-Δ26 the epitope is deleted. The decapeptide epitope identified for PS2-4D4 is present in all of these proteins (Fig. 2A).

Membrane preparations of these yeast strains and of a control strain containing no PmSUC2 sequences were analyzed on Western blots with PS2-1A2 and PS2-4D4 (Fig. 2B). Both, wild-type PmSUC2 and PmSUC2-Δ8 were recognized by PS2-1A2, reducing the epitope for PS2-1A2 to the amino acid residues 9 to 16 of wild-type PmSUC2 protein. As expected PS2-1A2 did not react with PmSUC2-Δ26. All three proteins were recognized by PS2-4D4 (Fig. 2B). The deletion in PmSUC2-Δ26 caused a reduction in the apparent molecular weight of this protein.

3.4. Sucrose transport of PmSUC2 is not affected by the mABs

As shown for the Na^+/H^+ antiporter of *E. coli*, antibodies can interfere with the activity of a protein [43]. We determined the transport activity of affinity-purified and reconstituted PmSUC2biohis6 protein in the presence of PS2-1A2 or PS2-4D4 on both sides of the proteoliposome membranes or in the absence of any mAB. Fig. 3 shows that transport and accumulation of ^{14}C-sucrose inside these vesicles were not impaired by added mABs. In all analyses uptake of ^{14}C-sucrose after energization and efflux of ^{14}C-sucrose after addition of CCCP were essentially the same indicating that none of the two mABs interferes with sucrose transport.

3.5. The epitopes of PS2-1A2 and of PS2-4D4 are located on the cytoplasmic side of the plasma membrane

We used PS2-1A2 and PS2-4D4 to study the topology of PmSUC2 in a heterologous system. Protoplasted yeast cells were treated with PS2-1A2 or PS2-4D4 and subsequently with an anti-mouse IgG-FITC conjugate. Binding of mABs to extracellular domains of PmSUC2 should yield green FITC fluorescence when analyzed under the fluorescence microscope. As a control for the integrity of the plasma membranes, avidin-TRITC conjugate was included with the IgG-FITC conjugate treatment. This conjugate binds only to biotinylated proteins in the cytoplasm. Red TRITC fluorescence indicates that the plasma membrane has been disrupted and green FITC fluorescence may also result from mAB binding to a cytoplasmic domain.

The data presented in Fig. 4 show that PS2-1A2 and PS2-4D4 recognize their epitopes only in permeabilized protoplasts, i.e. on the cytoplasmic side of the PmSUC2 protein. No FITC fluorescence was detected in intact protoplasts. As demonstrated in Fig. 4C these reactions are specific for PmSUC2biohis6-expressing GDY2133 cells. No fluorescence is detected in permeabilized GDY1000 control cells carrying the empty vector. Longer exposure of these cells yielded some red TRITC fluorescence but no FITC fluorescence indicating that PmSUC2biohis6 represents the most abundant biotinylated protein in yeast cells, a finding also supported by Western blot analysis (data not shown).

Fig. 4D and E represent the quantitative results of three independent permeabilization analyses performed as in Fig. 4A and B. The reproducibly larger number of cells showing TRITC fluorescence may be explained with the smaller size of the avidin-TRITC conjugate allowing diffusion into poorly permeabilized cells not accessible for the much larger IgG-FITC conjugates.

4. Discussion

Sequencing of phage inserts identified in a *PmSUC2biohis6* display library using the anti-PmSUC2 mABs PS2-1A2 and PS2-4D4 and Western blot analyses of truncated PmSUC2 mutant proteins identified the epitopes of these mABs (Figs. 1 and 2). The epitope peptides were not found in any of the other plant sucrose carriers sequenced so far and PS2-1A2 and PS2-4D4 do not recognize PmSUC1 [4], AtSUC1 [3] or AtSUC2 [3] on Western blots (data not shown). Moreover, screenings of publicly available protein data libraries with these peptides revealed no matches suggesting that both sequences are unique and might be used as epitope tags for other proteins.

Our data show also that N-terminally truncated PmSUC2 proteins (Fig. 2) are still targeted to the plasma membrane. This is deduced from the unchanged sucrose transport rates determined in yeast cells expressing wild-type PmSUC2 protein or the PmSUC2-Δ26 protein (data not shown) and from the almost identical amounts of recombinant protein in these cells (Fig. 2B). This may explain the high sequence variability within the N-termini of plant sucrose transporters.

Hydrophobicity analyses of the PmSUC2 protein [44] and analyses of the orientation and length of putative transmembrane domains with the program TMpred [45] suggest 12 regions sufficiently long to span a biological membrane (data not shown). According to the TMpred prediction both the N-terminus and the C-terminus of PmSUC2 are expected on the cytoplasmic side of the plasma membrane. Our data represent experimental support for these predictions (Fig. 4). Both mABs recognize their epitopes in transgenic yeast only after permeabilization of the plasma membrane, when the avidin-TRITC conjugate was able to bind to biotinylated proteins (Fig. 4B). This confirmed the integrity of the used protoplasts, as protein-biotin ligases are cytoplasmic proteins and the so far identified biotin proteins in yeast have been localized to the cytoplasm [46,47].

Our data suggest a topology for plant sucrose transporters that is in full agreement with the models determined for the mammalian hexose transporters [22], for the lactose [24] and the melibiose permeases [26,27] from *E. coli*. It supports previous, sequence derived suggestions that the *E. coli* melB protein and plant sucrose transporters are homologous [48] and that plant sucrose transporters may be members of the superfamily of transmembrane facilitators [49].

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References

[1] Riesmeier, J.W., Willmitzer, L. and Frommer, W.B. (1992) EMBO J. 11, 4705–4713.
[2] Gahrtz, M., Stolz, J. and Sauer, N. (1994) Plant J. 6, 697–706.
[3] Sauer, N. and Stolz, J. (1994) Plant J. 6, 67–77.
[4] Gahrtz, M., Schmelzer, E., Stolz, J. and Sauer, N. (1996) Plant J. 9, 93–100.
[5] Weig, A. and Komor, E. (1996) J. Plant Physiol. 147, 685–690.
[6] Hirose, T., Imaizumi, N., Scofield, G.N., Furbank, R.T. and Ohsugi, R. (1997) Plant Cell Physiol. 38, 1389–1396.
[7] Weber, H., Borisjuk, L., Heim, U., Sauer, N. and Wobus, U. (1997) Plant Cell 9, 895–908.
[8] Stadler, R., Brandner, J., Schulz, A., Gahrtz, M. and Sauer, N. (1995) Plant Cell 7, 1545–1554.
[9] Stadler, R. and Sauer, N. (1996) Bot. Acta 109, 299–306.
[10] Kühn, C., Franceschi, V.R., Schulz, A., Lemoine, R. and Frommer, W. (1997) Science 275, 1298–1300.
[11] Riesmeier, J.W., Willmitzer, L. and Frommer, W.B. (1994) EMBO J. 13, 1–7.
[12] Kühn, C., Quick, W.P., Schulz, A., Riesmeier, J.W., Sonnewald, U. and Frommer, W.B. (1996) Plant Cell Environ. 19, 1115–1123.
[13] Lemoine, R., Kühn, C., Thiele, N., Delrot, S. and Frommer, W.B. (1996) Plant Cell Environ. 19, 1124–1131.
[14] Bürkle, L., Hibberd, J.M., Quick, W.P., Kühn, C., Hirner, B. and Frommer, W.B. (1998) Plant Physiol. 118, 59–68.
[15] Naderi, S. and Saier, M. (1996) Mol. Microbiol. 22, 390–391.
[16] Tsuchiya, T., Raven, J. and Wilson, T.H. (1977) Biochem. Biophys. Res. Commun. 76, 26–31.
[17] Lopilato, J., Tsuchiya, T. and Wilson, T.H. (1978) J. Bacteriol. 134, 147–156.
[18] Kaback, H.R. and Wu, J. (1997) Q. Rev. Biophys. 30, 333–364.
[19] Walmsley, A., Barrett, M.P., Bringaud, F. and Gould, G.W. (1998) Trends Biochem. Sci. 23, 476–481.
[20] Chin, J.J., Jung, E.K., Chen, V. and Jung, C.Y. (1987) Proc. Natl. Acad. Sci. USA 84, 4113–4116.
[21] Alvarez, J., Lee, D.C., Baldwin, S.A. and Chapman, D. (1987) J. Biol. Chem. 262, 3502.
[22] Mueckler, M., Caruso, C., Baldwin, S.A., Paniew, M., Blench, F., Morris, H.R., Jeffrey, W., Lienhard, G.E. and Lodish, H.F. (1985) Science 229, 941–945.
[23] Davies, A., Meeran, K., Cairns, M.T. and Baldwin, S.A. (1987) J. Biol. Chem. 262, 9347–9352.
[24] Seckler, R., Wright, J.K. and Overath, P. (1983) J. Biol. Chem. 258, 10817–10820.
[25] Hresko, R.C., Kruse, M., Strube, M. and Mueckler, M. (1994) J. Biol. Chem. 269, 20482–20488.
[26] Botfield, M.C., Nuguchi, K., Tsuchiya, T. and Wilson, T.H. (1992) J. Biol. Chem. 267, 1818–1822.
[27] Pourcher, T., Bibi, E., Kaback, H.R. and Leblanc, G. (1996) Biochemistry 35, 4161–4168.
[28] Calamia, C. and Manoil, C. (1990) Proc. Natl. Acad. Sci. USA 87, 4937–4941.
[29] Ujwal, M.L., Jung, H., Bibi, E., Manoil, C., Altenbach, C., Hubbell, W.L. and Kaback, H.R. (1995) Biochemistry 34, 14909–14917.
[30] Stolz, J., Darnhofer-Demar, B. and Sauer, N. (1995) FEBS Lett. 377, 167–171.
[31] Kearney, T., Radbruch, A., Liesegang, B. and Rajewski, K. (1979) J. Immunol. 123, 1548–1551.
[32] Köhler, G. and Milstein, C. (1975) Nature 256, 495–497.
[33] Stolz, J., Darnhofer-Demar, B. and Sauer, N. (1995) FEBS Lett. 377, 167–171.
[34] Stolz, J., Ludwig, A. and Sauer, N. (1998) FEBS Lett. 440, 213–217.
[35] Maruyama, I.N., Murayama, H.I. and Brenner, S. (1994) Proc. Natl. Acad. Sci. USA 91, 8273–8277.
[36] Mikawa, Y.G., Maruyama, I.N. and Brenner, S. (1996) J. Mol. Biol. 262, 21–30.
[37] Emr, S.D., Schekman, R., Flessel, M.C. and Thorner, J. (1983) Proc. Natl. Acad. Sci. USA 80, 1080–1084.
[38] Stolz, J., Stadler, R., Opekarová, M. and Sauer, N. (1994) Plant J. 6, 225–233.
[39] Laemmli, U.K. (1970) Nature 227, 680–685.
[40] Burnett, W.N. (1980) Anal. Biochem. 11, 195–203.
[41] Schwarz, E., Oesterhelt, D., Reinke, H., Beyreuther, K. and Dimroth, P. (1988) J. Biol. Chem. 263, 9640–9645.
[42] Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R. and Stüber, D. (1988) Bio/Technology 6, 1321–1325.
[43] Padan, E., Venturi, M., Michel, H. and Hunte, C. (1998) FEBS Lett. 441, 53–58.
[44] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[45] Hofmann, K. and Stoffel, W. (1993) Biol. Chem. Hoppe-Seyler 347, 166.
[46] Ivessa, A.S., Schneiter, R. and Kohlwein, S.D. (1997) Eur. J. Cell Biol. 74, 399–406.
[47] Rhode, M., Lim, F. and Wallace, J.C. (1998) Arch. Biochem. Biophys. 290, 197–201.
[48] Sauer, N. and Tanner, W. (1993) Bot. Acta 106, 277–286.
[49] Marger, M.D. and Saier, M.H. (1993) Trends Biochem. Sci. 18, 13–20.