Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7949–7952, October 1990
Botany

Functional expression of the *Chlorella* hexose transporter in *Schizosaccharomyces pombe*

(H$^+$-symporter/3-*O*-methylglucose/pH jump)

NORBERT SAUER, THOMAS CASPARI, FRANZ KLEBL, AND WIDMAR TANNER

Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, 8400 Regensburg, Federal Republic of Germany

Communicated by Harry Beevers, July 13, 1990

ABSTRACT **_Schizosaccharomyces pombe_ cells were transformed with an _S. pombe_ expression vector containing a full-length cDNA of the _Chlorella_ hexose transporter. The transformed cells accumulated 3-_O_-methylglucose up to 10-fold, whereas wild-type _S. pombe_ and control transformants could only equilibrate this sugar analogue. In a pH-jump experiment, in which extracellular pH was lowered by 1.9 units, the accumulation ratio was increased in transformed cells but not in control cells. This result indicates that the gene product, _Chlorella_ H$^+$/glucose-symporter protein, and a pH gradient suffice for active sugar uptake. K_m values for glucose, 6-deoxyglucose, and 3-_O_-methylglucose of 1.5×10^{-5} M, 2.7×10^{-4} M, and 1.0×10^{-3} M, respectively, were identical in _Chlorella_ and in _S. pombe_ cells transformed with _Chlorella_ cDNA and ≈100-fold lower than those of the endogenous transport system of _S. pombe_.**

The unicellular green alga *Chlorella kessleri* (this strain was incorrectly classified as *Chlorella vulgaris* in our previous publications) can accumulate hexose analogues several hundred-fold by an inducible hexose-uptake system (1, 2). This active uptake is achieved by an electrogenic H$^+$ cotransport mechanism (3, 4). Recently the *Chlorella HUP1* gene has been cloned by differential hybridization; it is expressed only in induced *Chlorella* cells (5). From its sequence the gene is predicted to code for a membrane protein 533 amino acids in length (5). The gene product shows a high degree of similarity (30% of the amino acids are identical) to bacterial (6), fungal (7), and mammalian (8) sugar transporters. Although the gene was not expressed in a *Chlorella* mutant defective in hexose uptake (5), direct proof that the gene product is the *Chlorella* hexose transporter had not been obtained. To achieve this and, furthermore, to see whether the HUP1 protein is the only membrane protein required for active transport, we tried to express a full-length cDNA clone of the *Chlorella HUP1* gene in *Schizosaccharomyces pombe*. Wild-type *S. pombe* cells are not able to accumulate the hexose analogues 3-*O*-methylglucose and 6-deoxyglucose (9).

The results obtained with transformed *S. pombe* cells clearly prove that the *Chlorella* gene codes for a H$^+$-hexose transporter. The presence of the gene product and a H$^+$-gradient suffices to cause the accumulation of 3-*O*-methylglucose in *S. pombe*.

MATERIALS AND METHODS

Chemicals. All radioactive compounds were purchased from and 6-deoxyglucose was tritiated by Amersham Buchler (Braunschweig, F.R.G.). Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was from Sigma.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Strains. The strain of *C. kessleri* and the growth conditions have been described (5, 10). For transformation and heterologous expression in *S. pombe* we used the strain 1-32 (11), which was grown on minimal medium [0.67% yeast nitrogen base without amino acids/2% (wt/vol) glucose].

RNA Isolation and Separation. Total RNA from *C. kessleri* was isolated from frozen cells that were powdered in a mortar under liquid nitrogen and homogenized in a mixture of equal volumes of phenol and 100 mM Tris·HCl, pH 9.0 (12). RNA was further purified as described by Palmiter (13). RNA isolation from *S. pombe* was performed according to the procedure of Domdey *et al.* (14). RNA was separated on 1% agarose gel in the presence of formaldehyde and transferred to a nitrocellulose filter as described by Maniatis *et al.* (15). Northern (RNA) blots were probed with the radiolabeled insert of pTF201 (see below). Blots were hybridized in 50% formamide/2× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate)/1× Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin)/0.1% SDS/salmon sperm DNA at 100 μg/ml. Washes were done at 42°C at 0.1× SSC/0.1% SDS.

Cloning in *S. pombe*. Transformation of *S. pombe* strain 1-32 was performed according to Ito *et al.* (16) with the following changes: 50 μg of sonified salmon sperm DNA was added together with the DNA to be transformed; the heat shock was omitted.

For transformation we introduced a *Bam*HI site 73 base pairs (bp) downstream of the TAA stop codon of the cDNA clone pTF201 (5), which carries a full-length cDNA of the *Chlorella* hexose carrier. This clone has a unique *Sac* I site 95 bp upstream of the start ATG. The 1770-bp *Sac* I–*Bam*HI fragment was cloned into *Sac* I/*Bam*HI-digested yeast expression vector pEVP11 (17). The resulting construct pSP1 carries the *Chlorella* hexose carrier cDNA downstream of the *S. pombe adh* promoter (18) plus the *Saccharomyces cerevisiae LEU2*$^+$ gene. Control cells were transformed with pEVP11 only. *LEU*$^+$ transformants were detected on 1.8% agar minimal plates (1% glucose/0.65% yeast nitrogen base without amino acids).

Transport Tests. For transport tests the *S. pombe* strains TCY15 (transformed with pEVP11) and TCY12 (transformed with pSP1) were grown in minimal medium to an OD_{578} of 0.7–1.2. For each test, cells were harvested, washed twice with ≈15 ml of 100 mM potassium phosphate buffer, pH 6.0, and resuspended in 1 ml of the same buffer (OD_{578} = 10). Cells were shaken in a rotary shaker at 32°C, and the tests were started by adding radioactive sugar. Samples were withdrawn at given intervals, filtered through nitrocellulose filters (0.8-μm pore size), and washed with excess ice-cold buffer. Incorporation of radioactivity was determined by scintillation counting.

RESULTS AND DISCUSSION

Expression in *S. pombe*. Because *S. pombe* has recently been used successfully for the functional expression of bac-

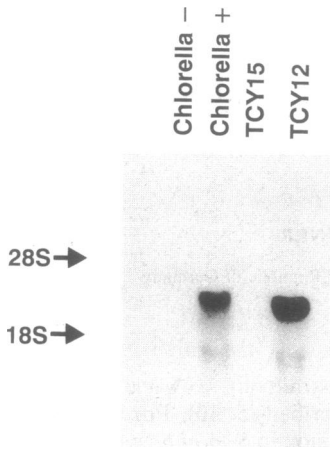

FIG. 1. RNA blot analysis of total RNA from *Chlorella* cells induced (+) and not induced (−) for sugar uptake in comparison with total RNA from *S. pombe* TCY12 and TCY15. Forty micrograms of total RNA was loaded per lane; RNA blots were probed with the labeled insert of the *Chlorella* HUP1 cDNA clone pTF201.

terio-opsin (19), this organism was selected to explore heterologous expression of a *Chlorella* membrane protein. Transformation was done with *S. pombe leu1-32* (h^-). The vector pSP1 contained the *Chlorella* cDNA behind the constitutive *S. pombe* alcohol dehydrogenase promoter. The total RNA of transformed TCY12 cells gave a very strong signal on Northern (RNA) blots (Fig. 1), which was absent in TCY15 cells transformed with vector pEVP11 only. This result shows that the endogenous glucose-transporter gene of *S. pombe* does not hybridize with the *Chlorella* gene under stringent conditions and also that a stable mRNA is produced in TCY12 cells. The slightly lower molecular weight of the transcript expressed in *S. pombe* TCY12 is probably from partial deletion of the 3′-untranslated end of HUP1 cDNA during pSP1 construction. Transformed *S. pombe* cells (TCY12) took up ^{14}C-labeled glucose from 10 μM solution at a rate 10 times higher than the control transformants or untransformed cells (data not shown).

Accumulation of 3-*O*-Methylglucose by Transformed *S. pombe*. Transport in *S. pombe* has been little studied, but Höfer and Nassar (9) reported that *S. pombe* cells accumulate 2-deoxyglucose as well as glucosamine ≈10- to 20-fold by a secondary active glucose-uptake system; these cells are not, however, capable of accumulating 3-*O*-methylglucose as *Chlorella* does (1, 2).

It was important, therefore, to test whether *S. pombe* cells transformed with the *Chlorella* HUP1 cDNA could accumulate 3-*O*-methylglucose. Fig. 2*a* shows that TCY12 cells take up 3-*O*-methylglucose to ≈2 to 3 times the concentration equilibrium, whereas the control cells transport at a considerably lower rate and reach the concentration equilibrium only. Adding an uncoupling agent to transformed cells with accumulated sugar leads to a rapid efflux (Fig. 2*A*). Höfer and Nassar (9) have pointed out that sugar transport in *S. pombe* measured simply in buffer is limited by metabolic energy,

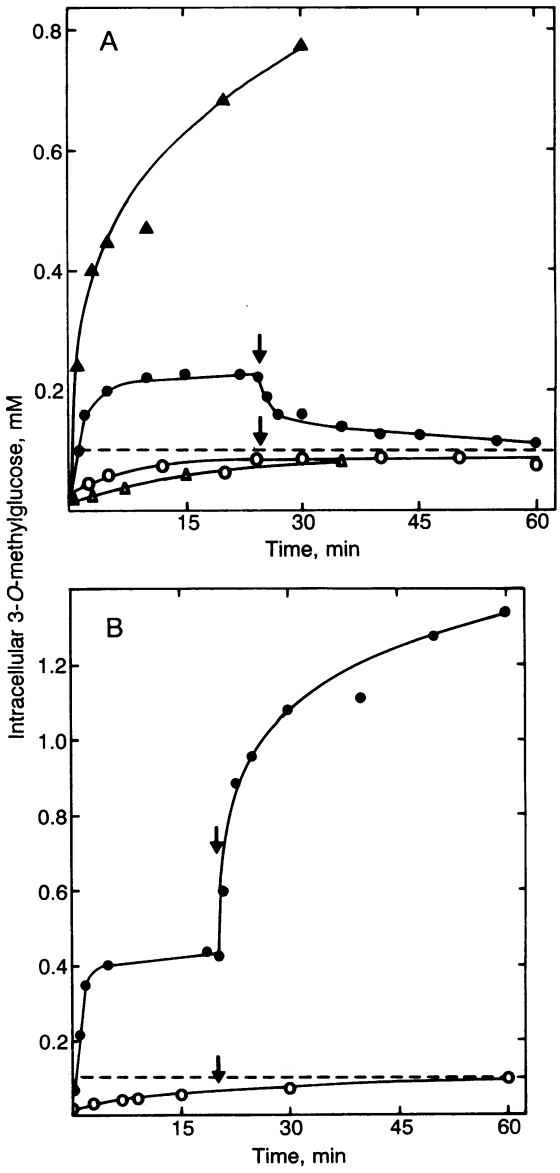

FIG. 2. (*A*) Effect of energization on 3-*O*-methylglucose accumulation. Cells were incubated with 0.1 mM 3-*O*-[*methyl*-^{14}C]glucose (specific activity, 137 kBq/μmol); for other conditions see *Materials and Methods*. Uncoupler carbonyl cyanide *m*-chlorophenylhydrazone was added to *S. pombe* TCY12 (●) and TCY15 (○) cells to a final concentration of 50 μM. 3-*O*-Methylglucose accumulated in the presence of 120 mM ethanol in TCY12 (▲) and TCY15 (△) cells. Broken line shows the concentration equilibrium. (*B*) Influence of H^+ concentration in medium on 3-*O*-methylglucose accumulation. Uptake measurements were done as in *A*. At indicated time (arrows) 29.5 μl of 1 M HCl was added to TCY12 (●) and TCY15 (○) cells, suddenly decreasing pH from 6.0 to 4.1. Broken line gives the concentration equilibrium.

which, however, can be supplied in the form of ethanol. 3-*O*-Methylglucose was, indeed, accumulated ≈8-fold by

Table 1. K_m values for sugar transport in *C. kessleri*, *S. pombe*, and transformed *S. pombe* cells TCY12 and TCY15

	K_m, M			
	TCY12	*C. kessleri**	TCY15	*S. pombe*†
D-Glucose	1.5×10^{-5}	1.5×10^{-5}	7.5×10^{-3}	3×10^{-3}
6-Deoxyglucose	2.7×10^{-4}	2.1×10^{-4}	1.6×10^{-2}	4.5×10^{-2}
3-*O*-Methylglucose	1.0×10^{-3}	1.5×10^{-3}	1.3×10^{-1}	1.8×10^{-1}

*Data were taken from ref. 23.
†Data were taken from ref. 9.

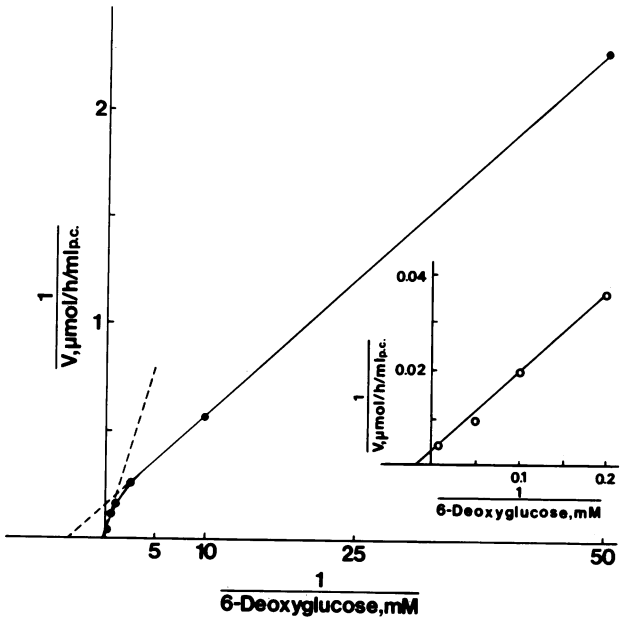

FIG. 3. Lineweaver–Burk diagram for determining K_m values for 6-deoxyglucose in TCY12 (●) and TCY15 control cells (○; *Inset*). (*Inset*) Straight line corresponds to steep dashed line in large figure. V, velocity. p.c., packed cells.

transformed cells in the presence of ethanol (Fig. 2*A*), whereas adding ethanol to control cells was without effect. The difference in the accumulation ratio of 3-*O*-methylglucose of ≈40-fold at an outside concentration of 10^{-4} M in *Chlorella* (1–4) and in ethanol-energized *S. pombe* of ≈10-fold depends on the relative amounts of gene product expressed and integrated per membrane area. In addition *S. pombe* possesses its endogenous facilitator besides the active *Chlorella* transporter; thus the *Chlorella* transporter in a way pumps into a system with specific "holes" for sugar.

As shown by the ethanol effect, the accumulation plateau in *S. pombe* is limited by the degree of energization (Fig. 2*A*), which for H^+-symporting transporters means by the protonmotive force (pmf) or, in the simplest case, by the amount of H^+ in the medium. This fact suggested the experiment shown in Fig. 2*B*. When the accumulation plateau of ≈4-fold was reached in transformed *S. pombe* cells, pH of the medium was lowered from 6.0 to 4.1 by simply adding HCl. Fig. 2*B* shows that the accumulation of 3-*O*-methylglucose increases further to ≈13-fold, indicating that only a pH gradient and the *Chlorella* glucose transporter are required for active sugar transport.

Kinetics and Specificity of the *Chlorella* Transporter in *S. pombe*. The K_m values for glucose and the glucose analogues 3-*O*-methylglucose and 6-deoxyglucose were determined for TCY12 and TCY15 *S. pombe* cells and compared with the corresponding values of *Chlorella*. Table 1 clearly demonstrates that the K_m values for the three sugars are identical in *S. pombe* TCY12 to those previously determined for *Chlorella*. Wild-type *S. pombe* (9) as well as the control TCY15 cells have K_m values differing by approximately a factor of one hundred, which explains that the endogenous transport system did not interfere with determination of the transport properties of TCY12 cells. When sufficiently high sugar concentrations were tested, two transport systems were revealed in TCY12, as indicated by the nonlinear part of the Lineweaver–Burk plot (Fig. 3). As expected, the steeper part of the curve corresponded to the single straight line obtained with control cells.

Table 2. Inhibition of 3-*O*-[*methyl*-^{14}C]glucose uptake by various sugars

Competing sugar	Inhibition, %	
	TCY12	*C. kessleri**
None	0	0
D-Glucose	94	82
D-Fructose	95	87
D-Galactose	55	64
D-Xylose	72	54
D-Arabinose	40	26

*Data were taken from ref. 20.

Sugar specificity of the *Chlorella* transporter expressed in *S. pombe* was determined qualitatively. In *Chlorella* cells induced for sugar uptake, it had been shown previously that many hexoses and pentoses compete with each other, suggesting that only one transporter with a rather broad specificity is responsible for uptake of D-glucose, D-fructose, D-galactose, D-arabinose, and D-xylose (20). In Table 2 the inhibition of 3-*O*-[*methyl*-^{14}C]glucose uptake by various hexoses and pentoses is shown for TCY12 cells and compared with the corresponding values obtained for *Chlorella*. Transport specificities as deduced from the relative degrees of inhibition of transformed *S. pombe* TCY12 cells are very similar to those observed for *Chlorella*. In *S. pombe* TCY15 the uptake of 3-*O*-[*methyl*-^{14}C]glucose at 1×10^{-2} M was not inhibited by 0.1 M of the various hexoses and pentoses tested (data not shown). Taken together the data on kinetics and specificity demonstrate that the *Chlorella* sugar-transport protein integrated into the plasma membrane of *S. pombe* functions indistinguishably from the transporter in *Chlorella*. Thus, yeast cells may be much better suited for heterologous expression of transporters, in general, as compared with *Escherichia coli* or oocytes (21, 22), the only systems thus far used. The latter are more delicate to handle and less stable, and expression occurs only transiently.

We thank Ulrike Stöckl for technical assistance and Drs. A.-M. and M. E. Schweingruber for advice on handling *S. pombe*. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 43) and by Fonds der Chemie.

1. Tanner, W. (1969) *Biochem. Biophys. Res. Commun.* **36,** 278–283.
2. Komor, E., Haass, D., Komor, B. & Tanner, W. (1973) *Eur. J. Biochem.* **39,** 193–200.
3. Komor, E. (1973) *FEBS Lett.* **38,** 16–18.
4. Komor, E. & Tanner, W. (1976) *Eur. J. Biochem.* **70,** 197–204.
5. Sauer, N. & Tanner, W. (1989) *FEBS Lett.* **259,** 43–46.
6. Maiden, M. C. J., Davis, E. D., Baldwin, S. A., Moore, D. C. M. & Henderson, P. J. E. (1987) *Nature (London)* **325,** 641–643.
7. Celenza, J. L., Marshall-Carlson, L. & Carlson, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2130–2134.
8. Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., Jeffrey, W., Lienhard, G. E. & Lodish, H. F. (1985) *Science* **229,** 941–945.
9. Höfer, M. & Nassar, F. R. (1987) *J. Gen. Microbiol.* **133,** 2163–2172.
10. Sauer, N. & Tanner, W. (1984) *Z. Pflanzenphysiol.* **114,** 367–375.
11. Beach, D. & Nurse, P. (1981) *Nature (London)* **290,** 140–142.
12. Haffner, M. H., Chin, M. B. & Lane, B. G. (1978) *Can. J. Biochem.* **56,** 729–733.
13. Palmiter, R. D. (1974) *Biochemistry* **13,** 3606–3615.
14. Domdey, H., Apostol, B., Lin, R.-J., Newman, A., Brody, E. & Abelson, J. (1984) *Cell* **39,** 611–621.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
17. Russel, P. & Nurse, P. (1986) *Cell* **45,** 145–153.
18. Russel, P. & Hall, B. D. (1983) *J. Biol. Chem.* **258,** 143–149.
19. Hildebrandt, V., Ramezani-Rad, M., Swida, U., Wrede, P.,

Grzesiek, S., Primke, M. & Büldt, G. (1989) *FEBS Lett.* **243**, 137–140.
20. Tanner, W., Grünes, R. & Kandler, O. (1970) *Z. Pflanzenphysiol.* **62**, 376–386.
21. Sarkar, H. K., Thorens, B., Lodish, H. F. & Kaback, R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5463–5467.
22. Pzermutt, M. S., Koranyi, L., Keller, K., Lacy, P. E., Scharp, D. & Mueckler, M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8688–8692.
23. Komor, E. & Tanner, W. (1974) *Eur. J. Biochem.* **44**, 219–233.