Characteristics of Fps1-Dependent and -Independent Glycerol Transport in *Saccharomyces cerevisiae*

F. C. W. SUTHERLAND,† F. LAGES,‡ C. LUCAS,§ K. LUYTEN,¶ J. ALBERTYN,* S. HOHMANN,§‡
B. A. PRIOR,§ AND S. G. KILIAN†

**Department of Microbiology and Biochemistry, University of the Free State, P.O. Box 339, Department of Biology, University of Minho, 4709 Braga Codex, Portugal; Laboratorium voor Moleculaire Celbiologie, Katholieke Universiteit Leuven, B-3001 Leuven, Belgium; and Department of General and Marine Microbiology, Göteborg University, S-41390 Göteborg, Sweden.**

Received 10 January 1997/Accepted 9 October 1997

Eadie-Hofstee plots of glycerol uptake in wild-type *Saccharomyces cerevisiae* W303-1A grown on glucose showed the presence of both saturable transport and simple diffusion, whereas an fps1 Δ mutant displayed only simple diffusion. Transformation of the fps1 Δ mutant with the glpF gene, which encodes glycerol transport in *Escherichia coli*, restored biphasic transport kinetics. Yeast extract–peptone–dextrose-grown wild-type cells had a higher passive diffusion constant than the fps1 Δ mutant, and ethanol enhanced the rate of proton diffusion to a greater extent in the wild type than in the fps1 Δ mutant. In addition, the lipid fraction of the fps1 Δ mutant contained a lower percentage of phospholipids and a higher percentage of glycolipids than that of the wild type. Fps1p, therefore, may be involved in the regulation of lipid metabolism in *S. cerevisiae*, affecting membrane permeability in addition to fulfilling its specific role in glycerol transport. Simultaneous uptake of glycerol and protons occurred in both glycerol- and ethanol-grown wild-type and fps1 Δ cells and resulted in the accumulation of glycerol at an inside-to-outside ratio of 12:1 to 15:1. Carbonyl cyanide m-chlorophenyldihydrozone prevented glycerol accumulation in both strains and abolished transport in the fps1 Δ mutant grown on ethanol. Likewise, 2,4-dinitrophenol inhibited transport in glycerol-grown wild-type cells. These results indicate the presence of an Fps1p-dependent facilitated diffusion system in glucose-grown cells and an Fps1p-independent proton symport system in derepressed cells.

Glycerol crosses all biological membranes by passive diffusion due to its lipophilic nature. In addition, specific transport proteins are frequently produced by microorganisms, resulting in more rapid transport of glycerol across the membrane. Active glycerol transport systems requiring the expenditure of metabolic energy have been identified in *Zygosaccharomyces rouxii*, *Debaryomyces hansenii*, and *Pichia sorbitophila* (21, 23, 36), whereas glycerol crosses the *Escherichia coli* cytoplasmic membrane via a proteinaceous pore mechanism which is encoded by *glpF* (15).

It has been assumed that glycerol is taken up by *Saccharomyces cerevisiae* by passive diffusion only. Recently *FPS1*, which encodes a protein belonging to the MIP family, has been shown to affect the movement of glycerol across the membrane of *S. cerevisiae* (24). The *FPS1* gene was isolated as a multicopy plasmid, which expresses the *GLP1* gene from the strong yeast phosphoglycerate kinase (*PGK1*) promoter, has been described by Luyten et al. (24).

In this work, glycerol transport in *S. cerevisiae* has been characterized in terms of kinetics and the number and nature of transport systems present. In addition, the effect of deletion of *FPS1* on lipid composition was also investigated. The results show that both an Fps1p-dependent facilitated transport mechanism and an active transport system independent of Fps1p are operative in *S. cerevisiae*. Observations that suggest a role for Fps1p in the control of passive diffusion are also discussed.

### MATERIALS AND METHODS

**Yeast strains.** Congenic strains of *S. cerevisiae* W303-1A were used (31). The construction of the fps1 Δ mutant and the YEpFPS1 multicopy plasmid were previously described by Van Aelst et al. (33). The YEpGLP1 multicopy plasmid, which expresses the *E. coli* glycerol facilitator gene *glpF* under the control of the strong yeast phosphoglycerate kinase (*PGK1*) promoter, has been described by Luyten et al. (24).

**Cultivation.** Strains were grown at 30°C for 12 h on a rotary shaker at 180 rpm in 500-ml Erlenmeyer shake flasks containing 100 or 200 ml of the appropriate medium. In assays for active uptake determination, cells were collected in mid-exponential phase (A650 of 0.3 to 0.4). YEPE medium consisted of (per liter) 20 g of peptone, 10 g of yeast extract and 20 g of glucose. YEPD and YEPE were similarly constituted but contained 20 g of glycerol or ethanol liter⁻¹, respectively, as a carbon source. Mineral medium (MM)-glucose, MM-glycerol-glucose,
and MM-ethanol liquid media contained minerals, as previously described (35), and 20 g of glucose, glycerol, or ethanol·liter⁻¹, respectively, as the main substrate. MM-glycerol-glucose contained, in addition to glycerol, 2 g of glucose·liter⁻¹ as a “starter” substrate.

**Glycerol accumulation.** Accumulation of glycerol in ethanol- and glycerol-grown cells was determined as previously described (21) by using [14C]glycerol (156 mCi·mmol⁻¹, 50% ethanolic solution; Amersham). Intracellular and extracellular glycerol concentrations were measured with a Gilson high-performance liquid chromatograph with a Merck Polyspher OA KC (catalog no. 51270) column maintained at 30°C. Sulfuric acid (0.05 N in ultrapure water) served as the mobile phase at a flow rate of 0.5 ml·min⁻¹. The intracellular volume was determined by using tritiated water and [14C]inulin, as previously described (12, 20).

**Glycerol and proton transport rates.** For determination of initial transport rates, exponential-phase cells were harvested by centrifugation, washed twice with cold distilled water, and resuspended in ice-cold distilled water or morpholineethanesulfonic acid buffer. Changes in the extracellular pH upon glycerol addition were measured as previously described (23). Uptake of [14C]glycerol by YEPD-grown cells was measured by exposing cell suspensions to [14C]glycerol (with variable specific activities ranging from 30 to 750 mCi·mmol⁻¹; Amersham) for 10 s, stopping the reaction by dilution with 5 ml of ice-cold water, filtering, and counting the radioactivity on the filter in a scintillation counter. All other assays were performed as described before (22). Kinetic constants were derived by Eadie-Hofstee plots and confirmed with Lineweaver-Burk plots. Data displaying biphasic kinetics were further analyzed by computer-assisted iteration (SigmaPlot; Jandel Scientific, San Rafael, Calif.) using the following equation for passive diffusion and mediated uptake components acting simultaneously:

\[ v = -K_m v_{\text{pass}} + v_{\text{fac}} = D K_m (S + S) \]

where \( D \) is the passive diffusion coefficient. Enhancement of the passive proton diffusion constant by ethanol was calculated from the slopes of plots of final pH (pH) versus ethanol concentration, according to the following relationship: \( \text{pH}_{\text{r}} = -\log K_m + \log k_p + 2.303 k_b c_p \), where \( k_p \) is the rate of active proton extrusion (mol·s⁻¹), \( c_p \) is the ethanol concentration, \( K_m \) is the value of the proton diffusion constant in the absence of ethanol, and \( k_b \) is the proton diffusion enhancement constant (17).

**Analytical methods.** Dry mass was determined gravimetrically in triplicate. Lipid analysis was done on YEPD-grown cells harvested in the late exponential phase (dry biomass = 1.3 g·liter⁻¹), as previously described (19).

**RESULTS**

**FPS1P involvement in glycerol transport.** Eadie-Hofstee plots of glycerol uptake in the wild type grown on YEPD showed biphasic kinetics (Fig. 1A). Kinetics at low glycerol concentrations were compatible with a saturable uptake system, and those at higher glycerol concentrations were compatible with passive diffusion. Similar results were obtained with glycerol transport in *E. coli*, in which the *glpF* gene encodes glycerol transport (2). Passive diffusion only was evident in the intracellular pH upon glycerol addition were measured as previously described (23). Uptake of [14C]glycerol by YEPD-grown cells was measured by exposing cell suspensions to [14C]glycerol (with variable specific activities ranging from 30 to 750 mCi·mmol⁻¹; Amersham) for 10 s, stopping the reaction by dilution with 5 ml of ice-cold water, filtering, and counting the radioactivity on the filter in a scintillation counter. All other assays were performed as described before (22). Kinetic constants were derived by Eadie-Hofstee plots and confirmed with Lineweaver-Burk plots. Data displaying biphasic kinetics were further analyzed by computer-assisted iteration (SigmaPlot; Jandel Scientific, San Rafael, Calif.) using the following equation for passive diffusion and mediated uptake components acting simultaneously:

\[ v = -K_m v_{\text{pass}} + v_{\text{fac}} = D K_m (S + S) \]

where \( D \) is the passive diffusion coefficient. Enhancement of the passive proton diffusion constant by ethanol was calculated from the slopes of plots of final pH (pH) versus ethanol concentration, according to the following relationship: \( \text{pH}_{\text{r}} = -\log K_m + \log k_p + 2.303 k_b c_p \), where \( k_p \) is the rate of active proton extrusion (mol·s⁻¹), \( c_p \) is the ethanol concentration, \( K_m \) is the value of the proton diffusion constant in the absence of ethanol, and \( k_b \) is the proton diffusion enhancement constant (17).

**Analytical methods.** Dry mass was determined gravimetrically in triplicate. Lipid analysis was done on YEPD-grown cells harvested in the late exponential phase (dry biomass = 1.3 g·liter⁻¹), as previously described (19).

**Expression of GlpF in *S. cerevisiae*.** Strong molecular homology exists between Fps1p and the GlpF glycerol facilitator of *E. coli* (24). In addition, transformation of the *fps1Δ* mutant with *glpF* partially substitutes for yeast Fps1p functions (24). It was of interest, therefore, to determine the transport kinetics of the *E. coli* facilitator expressed in *S. cerevisiae*. Eadie-Hofstee plots of transport kinetics of the *YEpGlpF* transformants showed, like the wild type, biphasic transport (Fig. 1), reflecting the simultaneous operation of passive diffusion and facilitated transport. It can be concluded, therefore, that *glpF* is functionally expressed in *S. cerevisiae*. The *glpF* transformant, however, had a \( K_m \) of 0.04 mM, which is about 100-fold lower than that of the wild type (Table 1). Similarly, glycerol transport in *E. coli* has a 1,000-fold-lower \( K_m \) than in wild-type *S. cerevisiae* (37) (Table 1).

**Energetics of Fps1p-dependent glycerol transport.** The effect of various metabolic inhibitors on the uptake of 20 mM radioactively labeled glycerol in YEPD-grown wild-type cells was determined in investigating the possibility that Fps1p-facilitated transport was dependent upon metabolic energy. Micconazole (5 μM), 2,4-dinitrophenol (5 mM), diethylstilbestrol (5 mM), and vanadyl sulfate (5 μM) had no effect. In addition, measurement of pH changes during uptake showed no evidence of simultaneous uptake of glycerol and protons in glucose-grown cells of either the wild type or the *fps1Δ* mutant, and the intracellular glycerol concentration did not exceed the diffusion equilibrium. It was concluded, therefore, that Fps1p-dependent transport in glucose-grown exponential-phase cells is facilitated diffusion and not active transport.

**Active transport in glycerol- and ethanol-grown cells.** In contrast to glucose-grown cells, glycerol uptake in either glycerol- or ethanol-grown cells of both the wild type and the *fps1Δ* mutant was accompanied by the simultaneous uptake of pro-
tons (Table 1). The $K_m$ of proton uptake could not be determined accurately due to the strong alkalinization of cell suspensions before glycerol addition. $V_{\text{max}}$ values of 148.8 and 77.4 mmol·g$^{-1}$·h$^{-1}$ were obtained for proton uptake in the wild type and the fps1$\Delta$ mutant, respectively. Eadie-Hofstee plots of initial uptake rates in glycerol- and ethanol-grown cells of the fps1$\Delta$ mutant displayed saturable kinetics (Fig. 2).

[$^{14}$C]glycerol was accumulated to inside-to-outside ratios of approximately 12 and 15 in the wild type and the fps1$\Delta$ mutant, respectively, when grown on either ethanol or glycerol. This accumulation was prevented by the presence of 50 μM carbonyl cyanide $m$-chlorophenylhydrazone (CCCP) (results not shown). In addition, 40 μM CCCP severely inhibited glycerol transport in the fps1$\Delta$ mutant grown on ethanol (Fig. 2B). In the wild type, the extent of the inhibitory effect was dependent upon the glycerol concentration used in the transport assay (Fig. 3). At higher concentrations, at which the contribution of simple diffusion to uptake was significant, CCCP had little effect on the uptake rate. In contrast, at lower substrate concentrations, at which the saturable system was dominant, CCCP inhibition of glycerol transport was as high as 50%. 2,4-Dinitrophenol (5 mM) also inhibited glycerol transport in wild-type glycerol-grown cells by 35% (results not shown).

These results clearly indicate the production of an Fps1p-independent proton symport system in S. cerevisiae W303-1A cells grown on ethanol or glycerol that is repressed by growth on glucose. This system was detected throughout the exponential growth phase with ethanol as the substrate, but in glycerol-grown cells its induction seemed to be stringently controlled by the physiological condition of the cells. The system was detected only during an “induction window” defined by the time interval between glucose starter depletion and the start of glycerol consumption. The differences in the CCCP inhibition pattern in the wild type and the fps1$\Delta$ mutant are consistent with the coexistence of the Fps1p-dependent facilitated diffusion and active systems under these conditions. These results fully agree with observations of a different strain of S. cerevisiae, which also indicated the presence of an active system subject to gluconeogenic induction (22), and show that this system is independent of Fps1p.

### Table 1. Kinetics constants for glycerol transport in S. cerevisiae W303-1A

| Strain/Growth medium | $K_m$ (mM) | $V_{\text{max}}$ (μmol·g$^{-1}$·h$^{-1}$) | Passive diffusion coefficient (liters·g$^{-1}$·h$^{-1}$) | Proton uptake upon glycerol addition |
|----------------------|------------|----------------------------------|---------------------------------|----------------------------------|
| Wild type MM-glucose | ND*        | ND*                             | 0.004 (2)                       | -                               |
| Wild type MM-glycerol-glucose | 2.0 ± 0.3 (3) | 186.4 ± 41.2 (3) | 0.005 (1)                       | +                               |
| Wild type YEPE       | 5.0 ± 0.3 (5) | 100.0 ± 5.0 (5) | 0.013 ± (6 × 10$^{-4}$) (5) | -                               |
| Wild type YEPE       | 2.5 ± 1.1 (3) | 160.9 ± 4.6 (3) | ND                             | +                               |
| Wild type YEPE       | 2.1 ± 0.4 (3) | 253.1 ± 46.3 (3) | 0.006 (2)                       | +                               |
| fps1$\Delta$ MM-glucose | ND        | ND                              | 0.005 (2)                       | -                               |
| fps1$\Delta$ MM-glycerol-glucose | 2.7 ± 0.9 (3) | 312.4 ± 34.4 (3) | 0.005 (1)                       | +                               |
| fps1$\Delta$ YEPE   | ND         | ND                              | 0.004 ± (2 × 10$^{-4}$) (5) | -                               |
| fps1$\Delta$ YEPE   | 3.0 (2)    | 221.5 (2)                       | 0.006 (2)                       | +                               |
| fps1$\Delta$ YEPE   | 2.7 ± 0.4 (3) | 298.0 (3)                       | ND                             | +                               |

* Numbers of independent experiments given in parentheses.

* ND, no saturable transport or no simple diffusion detected; ND*, not detected in standard assay with mid-exponential-phase cells.

![FIG. 2. Eadie-Hofstee plots of initial uptake rates of [$^{14}$C]glycerol at pH 5.0 and 30°C by the W303-1A fps1$\Delta$ mutant grown in YEPG (A) and YEPE (B). Assays were performed in the absence (●) and presence (○) of 40 μM CCCP.](http://jb.asm.org/ on May 1, 2019 by guest)
Other effects of Fps1p on membrane functions and on lipid composition. The observation that YEPD-grown wild-type cells had a higher passive diffusion coefficient (0.013 liters · g⁻¹ · h⁻¹) for glycerol uptake than the fps1Δ mutant (0.004 liters · g⁻¹ · h⁻¹) (Table 1) prompted us to further investigate plasma membrane permeability in both strains. To investigate possible differences in general permeability, we determined the ethanol-enhanced proton diffusion constants in both strains. The plots of final pH (pHf) as a function of ethanol concentration were linear but biphasic in both strains, displaying different slopes above and below 1.36 M ethanol (results not shown). Similar results were previously obtained for S. cerevisiae strains as well as a number of other yeasts (18). Below the transition concentration both strains had similar enhancement constants (wild type, 0.23 M⁻¹; fps1Δ mutant, 0.30 M⁻¹), but above this concentration substantial differences were observed (wild type, 1.12 M⁻¹; fps1Δ mutant, 0.54 M⁻¹). These results indicated FPS1-dependent differences in membrane properties between the two strains, with the wild type having greater permeability towards ethanol-enhanced proton diffusion than the fps1Δ mutant. This is in agreement with the data on diffusion of glycerol and indicates that Fps1p probably affects membrane structure or composition.

To investigate the possibility that the observed effect of FPS1 deletion on membrane permeability reflects a role for Fps1p in controlling membrane composition, we determined the effect of FPS1 deletion on cell lipid composition. The phospholipid and glycolipid fractions of the total lipid of the fps1Δ mutant were 25% lower and 62% higher, respectively, than in the wild type (Table 2). There was no significant difference in the sterol composition of these strains (data not shown). These results strengthen the conclusion that Fps1p has, in addition to its function in glycerol transport, a role in determining membrane composition.

TABLE 2. Lipid composition of exponential-phase YEPD-grown wild-type S. cerevisiae W303-1A and fps1Δ mutant cells

| Strain   | Lipids in biomass (%) | Lipid composition (%) |
|----------|-----------------------|-----------------------|
|          |                       | Neutral lipid | Glycolipid | Phospholipid |
| Wild type| 3.3 (0.2)              | 64.1 (3.8)     | 9.2 (0.5)  | 26.7 (1.6)  |
| fps1Δ    | 4.8 (0.3)              | 65.2 (3.9)     | 14.9 (0.9) | 19.9 (1.2)  |

a Standard deviations of three independent experiments given in parentheses.

DISCUSSION

The data presented here provide evidence that S. cerevisiae produces a constitutively expressed glycerol transport protein which operates by facilitated diffusion. Deletion of FPS1 abolishes this transport system. These results and the high degree of homology between Fps1p and other members of the MIP family of transport proteins, including several glycerol facilitators, strongly suggest that Fps1p itself is the transport protein. This notion is further supported by the observation that E. coli GlpF, expressed in an fps1Δ mutant, can partially replace Fps1p for glycerol transport. The possibility that Fps1p acts as a regulator of an unidentified glycerol transport protein, however, cannot be excluded. In this regard, the role of Snf3p in glucose transport in S. cerevisiae is worth noting. Early data indicating the dependence of high-affinity glucose transport upon SNF3 and the high degree of homology between the SNF3 gene product and known hexose transport proteins led to the conclusion that SNF3 encoded the high-affinity glucose transport protein (6, 9). Subsequent observations, however, showed that it probably functions as a regulator of glucose transport rather than as a structural transport protein (5). Recently it has been shown that the actual role of Snf3p is in the sensing of low glucose concentrations (11, 26). This may be followed by the initiation of a signaling cascade involving the C terminus, since this domain has been shown to regulate the ability of the yeast to grow at low glucose concentrations (11, 26). It is interesting that Snf3p, like Fps1p, is distinguished from most membrane proteins in its class by exceptionally long amino- and carboxyl-terminal domains (5, 28).

Nonlinear transport kinetics such as those reported here for glycerol transport in wild-type cells are open to widely differing opinions, as illustrated by the controversy surrounding the interpretation of glucose transport kinetics in S. cerevisiae. These kinetics have been interpreted as indicative of high- and low-affinity systems by some authors (10), whereas others have suggested that it represents the simultaneous action of a facilitated diffusion system and passive diffusion (13). Yet another possibility is that one system is constitutively expressed but its affinity is regulated by the prevailing glucose concentration (38). We have proposed the biphasic kinetics of glycerol transport to be a model for a facilitated diffusion system and a passive diffusion component for several reasons. Firstly, unlike glucose, glycerol is known to cross biological membranes by passive diffusion (4, 21), making it likely that one of the transport modes in the case of biphasic kinetics reflects passive diffusion. Secondly, the kinetics could not be fitted to a Michaelis-Menton model for two saturable systems (data not shown), but it did satisfy the model for one passive diffusion component and one saturable system. Lastly, the near-vertical nature of the low-affinity component of the Eadie-Hofstee plot strongly suggests that a protein is not involved (21, 30).

A role in glycerol transport may not be the only function of FPS1, since deletion of the gene affects cellular lipid composition and results in lower apparent membrane permeability than in the wild type. Likewise, multiple functions for other MIP proteins have been suggested (20). It has also been reported that interaction between the E. coli GlpF glycerol transport system and glycerol kinase results in increased activity of the kinase (37), and there are indications that a similar mechanism may be operative in Xenopus oocytes expressing the frog lens protein, another MIP family member (20). It may be
possible, therefore, that Fps1p interacts similarly with other proteins.

In addition to the Fps1p-dependent facilitated diffusion system, a distinctive glucose-repressible proton symport for glycerol operates in derepressed cells. The facilitated diffusion system seems to have a function in osmotic regulation (24), whereas the active system probably has a metabolic role since it is induced by a shift from glycolysis to gluconeogenesis (22).

Recently, other MIP family members with unknown functions have been identified in *S. cerevisiae* (3, 27). Although no members of the MIP family are known to act as secondary active transport proteins, the possibility that one of these encodes the active system cannot be excluded, and this is presently being investigated. Alternatively, one or more of these MIP proteins may catalyze glycerol transport under conditions not yet kinetically investigated.

Glycerol transport proteins in microorganisms include both facilitated diffusion and active systems. *Fusarium oxysporum* (8) and *E. coli* (2) transport glycerol by facilitated diffusion, whereas active transport occurs in *Z. rouxii*, *D. hansenii* and *P. sorbitophila* (1, 21, 36). Since *E. coli* GlpF complements the loss of glycerol transport in the *S. cerevisiae* fps1Δ mutant, a comparison between these two systems is of interest. Equilibration of glycerol across the membrane in *E. coli* is very rapid (15), typical of a channel protein, whereas that of the facilitated diffusion system in *S. cerevisiae* has a lower rate of transport. This seems to indicate that the *S. cerevisiae* system may not act like a classic channel protein in all respects. Not all transport proteins conform to rigid classification, however, as not all act like a classic channel protein in all respects. Not all transport proteins may catalyze glycerol transport under conditions not yet kinetically investigated.

Glycerol transport proteins in microorganisms include both facilitated diffusion and active systems. *Fusarium oxysporum* (8) and *E. coli* (2) transport glycerol by facilitated diffusion, whereas active transport occurs in *Z. rouxii* (1), *D. hansenii* and *P. sorbitophila* (1, 21, 36). Since *E. coli* GlpF complements the loss of glycerol transport in the *S. cerevisiae* fps1Δ mutant, a comparison between these two systems is of interest. Equilibration of glycerol across the membrane in *E. coli* is very rapid (15), typical of a channel protein, whereas that of the facilitated diffusion system in *S. cerevisiae* has a lower rate of transport. This seems to indicate that the *S. cerevisiae* system may not act like a classic channel protein in all respects. Not all transport proteins conform to rigid classification, however, as not all act like a classic channel protein in all respects. Not all transport proteins may catalyze glycerol transport under conditions not yet kinetically investigated.

Glycerol transport proteins in microorganisms include both facilitated diffusion and active systems. *Fusarium oxysporum* (8) and *E. coli* (2) transport glycerol by facilitated diffusion, whereas active transport occurs in *Z. rouxii* (1), *D. hansenii* and *P. sorbitophila* (1, 21, 36). Since *E. coli* GlpF complements the loss of glycerol transport in the *S. cerevisiae* fps1Δ mutant, a comparison between these two systems is of interest. Equilibration of glycerol across the membrane in *E. coli* is very rapid (15), typical of a channel protein, whereas that of the facilitated diffusion system in *S. cerevisiae* has a lower rate of transport. This seems to indicate that the *S. cerevisiae* system may not act like a classic channel protein in all respects. Not all transport proteins conform to rigid classification, however, as not all act like a classic channel protein in all respects. Not all transport proteins may catalyze glycerol transport under conditions not yet kinetically investigated.

Glycerol transport proteins in microorganisms include both facilitated diffusion and active systems. *Fusarium oxysporum* (8) and *E. coli* (2) transport glycerol by facilitated diffusion, whereas active transport occurs in *Z. rouxii* (1), *D. hansenii* and *P. sorbitophila* (1, 21, 36). Since *E. coli* GlpF complements the loss of glycerol transport in the *S. cerevisiae* fps1Δ mutant, a comparison between these two systems is of interest. Equilibration of glycerol across the membrane in *E. coli* is very rapid (15), typical of a channel protein, whereas that of the facilitated diffusion system in *S. cerevisiae* has a lower rate of transport. This seems to indicate that the *S. cerevisiae* system may not act like a classic channel protein in all respects. Not all transport proteins conform to rigid classification, however, as not all act like a classic channel protein in all respects. Not all transport proteins may catalyze glycerol transport under conditions not yet kinetically investigated.

Glycerol transport proteins in microorganisms include both facilitated diffusion and active systems. *Fusarium oxysporum* (8) and *E. coli* (2) transport glycerol by facilitated diffusion, whereas active transport occurs in *Z. rouxii* (1), *D. hansenii* and *P. sorbitophila* (1, 21, 36). Since *E. coli* GlpF complements the loss of glycerol transport in the *S. cerevisiae* fps1Δ mutant, a comparison between these two systems is of interest. Equilibration of glycerol across the membrane in *E. coli* is very rapid (15), typical of a channel protein, whereas that of the facilitated diffusion system in *S. cerevisiae* has a lower rate of transport. This seems to indicate that the *S. cerevisiae* system may not act like a classic channel protein in all respects. Not all transport proteins conform to rigid classification, however, as not all act like a classic channel protein in all respects. Not all transport proteins may catalyze glycerol transport under conditions not yet kinetically investigated.

Glycerol transport proteins in microorganisms include both facilitated diffusion and active systems. *Fusarium oxysporum* (8) and *E. coli* (2) transport glycerol by facilitated diffusion, whereas active transport occurs in *Z. rouxii* (1), *D. hansenii* and *P. sorbitophila* (1, 21, 36). Since *E. coli* GlpF complements the loss of glycerol transport in the *S. cerevisiae* fps1Δ mutant, a comparison between these two systems is of interest. Equilibration of glycerol across the membrane in *E. coli* is very rapid (15), typical of a channel protein, whereas that of the facilitated diffusion system in *S. cerevisiae* has a lower rate of transport. This seems to indicate that the *S. cerevisiae* system may not act like a classic channel protein in all respects. Not all transport proteins conform to rigid classification, however, as not all act like a classic channel protein in all respects. Not all transport proteins may catalyze glycerol transport under conditions not yet kinetically investigated.

Glycerol transport proteins in microorganisms include both facilitated diffusion and active systems. *Fusarium oxysporum* (8) and *E. coli* (2) transport glycerol by facilitated diffusion, whereas active transport occurs in *Z. rouxii* (1), *D. hansenii* and *P. sorbitophila* (1, 21, 36). Since *E. coli* GlpF complements the loss of glycerol transport in the *S. cerevisiae* fps1Δ mutant, a comparison between these two systems is of interest. Equilibration of glycerol across the membrane in *E. coli* is very rapid (15), typical of a channel protein, whereas that of the facilitated diffusion system in *S. cerevisiae* has a lower rate of transport. This seems to indicate that the *S. cerevisiae* system may not act like a classic channel protein in all respects. Not all transport proteins conform to rigid classification, however, as not all act like a classic channel protein in all respects. Not all transport proteins may catalyze glycerol transport under conditions not yet kinetically investigated.
glucose transporters in *Saccharomyces cerevisiae* are glucose sensors that generate a signal for induction of gene expression. Proc. Natl. Acad. Sci. USA 93:12428–12432.

27. Park, J. H., and M. H. Saier. 1996. Phylogenetic characterization of the MIP family of transmembrane channel proteins. J. Membr. Biol. 153:171–180.

28. Reizer, J., A. Reizer, and M. H. Saier. 1993. The MIP-family of integral membrane channel proteins: sequence comparisons, evolutionary relationships, reconstructed pathway of evolution, and proposed functional differentiation of the two repeated halves of the proteins. Crit. Rev. Biochem. Mol. Biol. 28:235–257.

29. Rottenberg, H. 1979. The measurement of membrane potential and pH in cells, organelles and vesicles. Methods Enzymol. 55:547–569.

30. Stratford, M., and A. H. Rose. 1986. Transport of sulphur dioxide by *Saccharomyces cerevisiae*. J. Gen. Microbiol. 132:1–6.

31. Thomas, B. J., and R. J. Rothstein. 1989. Elevated recombination rates in transcriptionally active DNA. Cell 56:619–630.

32. Truninger, V., and W. Boos. 1993. Glycerol uptake in *Escherichia coli* is sensitive to membrane lipid composition. Res. Microbiol. 144:565–574.

33. Van Aelst, L., S. Hohmann, F. K. Zimmermann, F. K. Jans, and J. M. Thevelein. 1991. A yeast homologue of the bovine lens fibre MIP gene family complements the growth defect of a *Saccharomyces cerevisiae* mutant on fermentable sugars but not its defect in glucose-induced RAS-mediated c-AMP signaling. EMBO J. 10:2095–2104.

34. Van den Bosch, H., and L. L. M. van Deenen. 1965. Chemical structure and biological significance of lysolecithins from rat liver. Biochim. Biophys. Acta 106:326–333.

35. Van Uden, N. 1967. Transport-limited fermentation and growth of *Saccharomyces cerevisiae* and its competitive inhibition. Arch. Microbiol. 58:155–168.

36. Van Zyl, P. J., S. G. Kilian, and B. A. Prior. 1990. The role of an active transport mechanism in glycerol accumulation during osmoregulation by *Zygosaccharomyces rouxii*. Appl. Microbiol. Biotechnol. 34:231–235.

37. Vogegele, R. T., G. D. Sweet, and W. Boos. 1993. Glycerol kinase of *Escherichia coli* is activated by interaction with the glycerol facilitator. J. Bacteriol. 175:1087–1094.

38. Walsh, M. C., H. P. Smits, M. Scholte, and K. van Dam. 1994. Affinity of glucose transport in *Saccharomyces cerevisiae* is modulated during growth on glucose. J. Bacteriol. 176:953–958.

39. Walther, P., M. Müller, and M. E. Schweingruber. 1984. The ultrastructure of the cell surface and plasma membrane of exponential and stationary phase cells of *Schizosaccharomyces pombe*, grown in different media. Arch. Microbiol. 137:128–134.