std1, a Gene Involved in Glucose Transport in *Schizosaccharomyces pombe*

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A wild-type strain, Sp972 h⁻, of *Schizosaccharomyces pombe* was mutagenized with ethylmethanesulfonate (EMS), and 2-deoxyglucose (2-DOG)-resistant mutants were isolated. Out of 300 independent 2-DOG-resistant mutants, 2 failed to grow on glucose and fructose (mutants 3/8 and 3/23); however, their hexokinase activity was normal. They have been characterized as defective in their sugar transport properties, and the mutations have been designated as *std1-8* and *std1-23* (sugar transport defective). The mutations are allelic and segregate as part of a single gene when the mutants carrying them are crossed to a wild-type strain. We confirmed the transport deficiency of these mutants by [^{14}C]glucose uptake. They also fail to grow on other monosaccharides, such as fructose, mannose, and xylulose, as well as disaccharides, such as sucrose and maltose, unlike the wild-type strain. Lack of growth of the glucose transport-deficient mutants on maltose revealed the extracellular breakdown of maltose in *S. pombe*, unlike in *Saccharomyces cerevisiae*. Both of the mutants are unable to grow on low concentrations of glucose (10 to 20 mM), while one of them, 3/23, grows on high concentrations (50 to 100 mM) as if altered in its affinity for glucose. This mutant (3/23) shows a lag period of 12 to 18 h when grown on high concentrations of glucose. The lag disappears when the culture is transferred from the log phase of its growth on high concentrations. These mutants complement phenotypically similar sugar transport mutants (YGS4 and YGS5) reported earlier by Milbradt and Hoefer (Microbiology 140:2617–2623, 1994), and the clone complementing YGS4 and YGS5 was identified as the only glucose transporter in fission yeast having 12 transmembrane domains. These mutants also demonstrate two other defects: lack of induction and repression of shunt pathway enzymes and defective mating.

In recent years, more attention has been directed to the understanding of sugar transport in various systems, including *Escherichia coli* (12), *Saccharomyces cerevisiae* (23), *Schizosaccharomyces pombe* (18, 22), plants (14), *Drosophila* cell lines (30), human erythrocytes (8), and some parasites, such as *Leishmania* spp. (17). Except for *E. coli* and *S. cerevisiae*, we know very little about the mechanism of sugar transport in other systems. Transport of sugar has been studied in a few other yeast species as well (10, 11).

Unlike the multiple (low- and high-affinity) sugar transport systems described in many yeast species (1, 3, 5, 13, 28) and the fungus *Neurospora crassa* (25), fission yeast seems to have a much simpler mode of transport. In many glucose transport systems, the electrochemical proton gradient plays a major role. In *S. pombe* too, the sugar molecules are known to be translocated across the membrane in symport with H⁺ (16). Both aerobic glucose transport and anaerobic glucose transport are catalyzed by the glucose carrier (16). Recently Milbradt and Hoefer (22) reported a sugar transport-defective mutant and its complementing clone, designated *GHT1*, from *S. pombe* (18, 22).

Since *S. pombe* seems to have a single glucose transporter, it would help us dissect out the mechanism underlying regulation of sugar transport. This paper describes the isolation and characterization of two mutants which are sugar transport defective (*std1* gene mutation). The mutations are allelic, and the mutants fail to grow on monosaccharides such as glucose, fructose, and mannose and disaccharides such as sucrose and maltose. One of them is a mutant with an altered affinity and is able to grow on high concentrations of glucose, whereas the other cannot. We also report here the presence of extracellular maltase in fission yeast. Our study clearly revealed that the *std1* mutants are different from the earlier isolated glucose symporter mutants in *S. pombe*.

MATERIALS AND METHODS

Strains and growth conditions. The *S. pombe* and *E. coli* strains used in this study are listed in Table 1. Fission yeast cells were grown in YES medium (0.5% yeast extract and other supplements containing 0.01% [each] uracil, adenine, leucine, lysine, and histidine) with the indicated carbon sources. Synthetic complete medium was prepared as mentioned by Sherman et al. (26) for *S. cerevisiae*. For auxotrophic selection, the respective amino acids were omitted. *E. coli* cells were grown in Luria-Bertani medium (0.5% yeast extract, 0.5% NaCl, plus 1% Bacto tryptone). Yeast extract, Bacto tryptone, malt extract, and agar were obtained from Difco Laboratories, Detroit, Mich. All amino acids were from Sisco Laboratories, Mumbai, India.

Enzyme assay. Hexokinase and glucose-6-P dehydrogenase activities were assayed as described earlier (20). All of the substrates and enzymes were purchased from either Sigma Chemical Company, St. Louis, Mo., or Boehringer, Mannheim, Germany. Routine molecular biological techniques were used as described by Sambrook et al. (24). Restriction enzymes were purchased from New England Biolabs, England; and used according to the manufacturer's instructions.

Genetic analysis. Genetic manipulation was done by tetrad analysis (2) with the appropriate strains in opposite mating types. Approximately equal amounts of freshly growing cells in opposite mating types were mixed on a malt extract agar plate (3% Bacto malt extract, 0.05% amino acid supplements [pH adjusted to 5.5 with NaOH]) and incubated at 30°C overnight. Asci with spores were separated from the mating mixture with a micromanipulator on a thin layer of slab medium containing YES medium with 1.5% agar. This was incubated at 37°C for 5 to 10 h in order to digest the asci walls. Spores were separated by the micromanipulator on the same slab medium and were transferred to a YES agar plate containing a permissive carbon source. Spores were germinated by incubation at 30°C for 3 to 5 days, and the segregants were analyzed.

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TABLE 1. Strains used in this study

Strain	Genotype	Source
Sp972	h⁻	J. Robinson
PN 1	*leu1-32 ura4-D18* h⁺	P. Nurse
20T1B	*leu1-32 ura4-D18* h⁻	This study
3/23	*std1-23* h⁻	This study
3/23-1T1A	*leu1-32std1-23* h⁺	This study
3/23-16T1A	*leu1-32 ura4-D18 std1-23* h⁻	This study
3/8	*std1-8* h⁻	This study
3/8-30T1A	*leu1-32 std1-8* h⁺	This study
3/8-16T2B	*leu1-32 ura4-D18 std1-8* h⁻	This study
YGS5	*leu1-32 ght1* h⁺	M. Hoefer
YGS4	*leu1-32 ght1* h⁺	M. Hoefer

Transformation and plasmid recovery. Yeast transformation was carried out by the alkali cation method described by Alfa et al. (2) with minor modifications. Ten milliliters of yeast cells grown overnight was reinoculated into fresh prewarmed YES medium containing permissive carbon supplements and allowed to grow logarithmically until the cell density reached 10⁷ cells/ml. The cells were harvested, washed with sterile water followed by 0.1 M lithium acetate, and resuspended in 0.1 M lithium acetate to a final concentration of 10⁹ cells/ml. Two micrograms of plasmid DNA along with 10 μl of 10-mg/ml salmon sperm DNA was mixed with 200 μl of cells. Following incubation at room temperature for 60 min, 300 μl of 50% polyethylene glycol 3350 was added, and this mixture was incubated again for 60 min. Heat shock was given at 46°C for 15 min. Cells were removed from polyethylene glycol by centrifugation and incubated in 1 ml of recovery medium containing YES medium. Transformed cells free of recovery medium were plated on selective plates and incubated at 30°C to get the transformants. Yeast plasmid isolation was carried out as described by Alfa et al. (2).

E. coli transformation was carried out by electroporation with a Bio-Rad gene pulser, and the transformants were selected with 100 μg of ampicillin per ml. Cells carrying plasmids were grown with antibiotic selection, and the plasmids were recovered by the alkaline lysis method (4).

[¹⁴C]glucose uptake studies. Uptake of labeled sugar was carried out as described by Bisson and Fraenkel (5). Cells were grown in YES medium containing permissive carbon sources (1% glycerol, 0.4% ethanol, 10 mM δ-gluconolactone) until the cell density reached 10⁷ cells/ml. Cells were collected by centrifugation, washed once with sterile water and twice with phosphate buffer (pH 7.4), and resuspended in phosphate buffer to a final concentration of 2 to 5 mg/ml (wet weight). Uptake was initiated by addition of radiolabeled glucose. Aliquots of 100 μl were taken at different time intervals, and the uptake was terminated by addition of 1 ml of cold water. Cells were collected and washed on a filter paper before radioactivity was measured with an LKB 1219 RACKBETA liquid scintillation counter. D-[U-¹⁴C]glucose was obtained from Bhabha Atomic Research Center, Mumbai, India.

Glucose estimation. Free glucose was estimated by the glucose oxidase-peroxidase method by examining the formation of reduced *o*-dianisidine at A₄₂₀ with an LKB ULTROSPEC II spectrophotometer. The reagents were purchased from Sigma Chemical Co.

RESULTS

Isolation of 2-DOG-resistant mutants. Wild-type strain Sp972 h⁻ of *S. pombe* was mutagenized as described by Sherman et al. (26) with ethylmethanesulfonate and plated on YES plates containing 1% glycerol, 0.4% ethanol, 10 mM δ-gluconolactone, and 1 mM 2-deoxyglucose (2-DOG) as a carbon source (21). From the total of 300 2-DOG-resistant colonies obtained, only two failed to grow on glucose and fructose. We selected these two mutants for further characterization. Growth of these mutants on a permissible carbon source (1% glycerol, 0.4% ethanol, 10 mM δ-gluconolactone) in the presence of glucose clearly demonstrated that the growth defect of these mutants is not due to the inhibition exerted by glucose, as shown in Fig. 1 for 3/23. The fact that hexokinase activity in these mutants was similar to that in the wild type indicated that loss of growth on glucose or 2-DOG resistance is not due to a defect in the glucose phosphorylation step (Table 2).

Uptake of labeled sugar. In order to confirm the transport deficiency of the mutants, we performed radioactive sugar uptake experiments along with the wild-type strain, Sp972 h⁻,
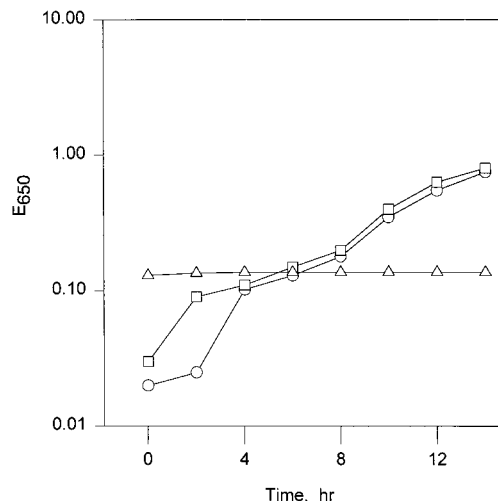

FIG. 1. Growth of mutant 3/8 on a permissive carbon source in the presence of glucose. Cells grown on YES medium containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone cells were freshly inoculated into YES medium containing 20 mM glucose (△); 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone (□); and 1% glycerol, 0.4% ethanol, 10 mM δ-gluconolactone, and 20 mM glucose (○). Growth was monitored by measuring the E₆₅₀ as a function of time. Similar results were obtained with 3/23.

with 20 mM glucose. [¹⁴C]glucose uptake experiments were carried out as described in Materials and Methods. Both of the mutants are indeed defective in glucose uptake, as demonstrated in Fig. 2.

Growth properties of the mutants. We checked the growth properties of our *std1* mutants on various carbon sources and compared them with those of the glucose transporter mutants. Both of the mutants failed to grow on the monosaccharides glucose, fructose, mannose, and xylulose and the disaccharides sucrose and maltose (Table 3). Galactose does not serve as a sole carbon source for *S. pombe*. Among these two mutants, 3/23 failed to grow only at low concentrations of glucose, but 3/8 failed to grow even at high concentrations (Fig. 3).

S. cerevisiae has maltose permease, which transports maltose inside that is finally hydrolyzed by maltase intracellularly (7). Since *std1* mutants failed to grow on maltose, we examined whether in *S. pombe* maltose is extracellularly broken down to glucose. The mutants as well as the wild-type strains were grown in presence of 25 mM maltose, and samples were taken at different time intervals. Glucose was estimated in the medium as described in Materials and Methods. In the wild type, the glucose concentration in the medium increases till 12 h of growth but later decreases. In both of the mutants, glucose accumulates at the same rate as the wild type; however, the

TABLE 2. Hexokinase activity in the mutants

Strain	Sp act (mU/mg)ᵃ		
	Hexokinase		Glucose-6-P dehydrogenase
	Glucose	Fructose	
Sp972	88	153	43
3/23	172	190	65
3/8	200	224	96

ᵃ Cultures were grown in YES medium containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone to the log phase. Enzymes were assayed in cell extracts, and enzyme activity has been expressed in milliunits per milligram of protein.
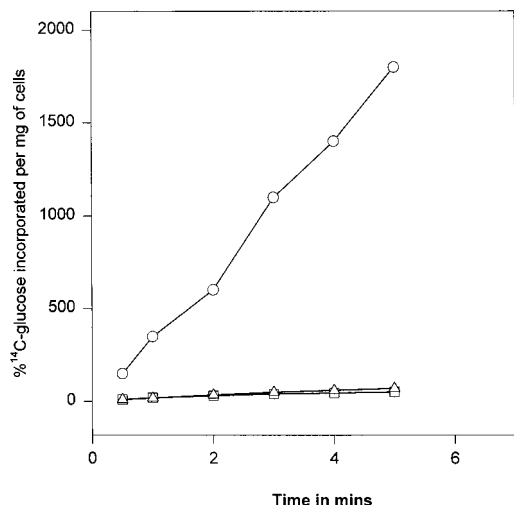
FIG. 2. Uptake of [^{14}C]glucose in *std1* mutants and the wild type. Labeled glucose uptake experiments were performed as described in Materials and Methods. Uptake of labeled glucose within 5 min by the wild type (○), 3/8-30T1A (□), and 3/23-1T1A (△) is shown. The amount of radioactivity incorporated was monitored with a scintillation counter.

glucose concentration does not decrease in the mutants (Fig. 4). This clearly indicated that maltose is extracellularly hydrolyzed in fission yeast.

Genetics of *std1* mutants. Both *std1* mutants 3/8 and 3/23 were crossed to wild-type PN (*leu1-32 ura4-D18* h$^+$) to introduce an auxotrophic marker for complementation studies. Both of the mutants were obtained with auxotrophic markers (Table 1). They were backcrossed to wild-type PN h$^+$ and 20T1B h$^-$ strains to confirm the single-gene mutation. In both

TABLE 3. Growth of mutants on mono- and disaccharides

Sugar	Growth of strain[a]			
	Sp972	3/23-1T1A	3/8-30T1A	YGS4
Fructose				
20 mM	+	−	−	−
50 mM	+	±	−	−
Mannose				
20 mM	+	−	−	−
50 mM	+	±	−	−
Maltose				
10 mM	+	−	−	−
25 mM	+	±	−	−
Sucrose				
10 mM	+	−	−	−
25 mM	+	±	−	−
Xylulose (25 mM)	+	−	−	−
Galactose (50 mM)	−	−	−	−
Glycerol (1%) + ethanol (0.4%) + δ-gluconolactone (10 mM)	+	+	+	+
δ-Gluconolactone (50 mM)	+	+	+	+

[a] Growth on YES medium containing various carbon sources was scored in 4 days at 30°C as follows: +, growth; ±, leaky growth; −, no growth.
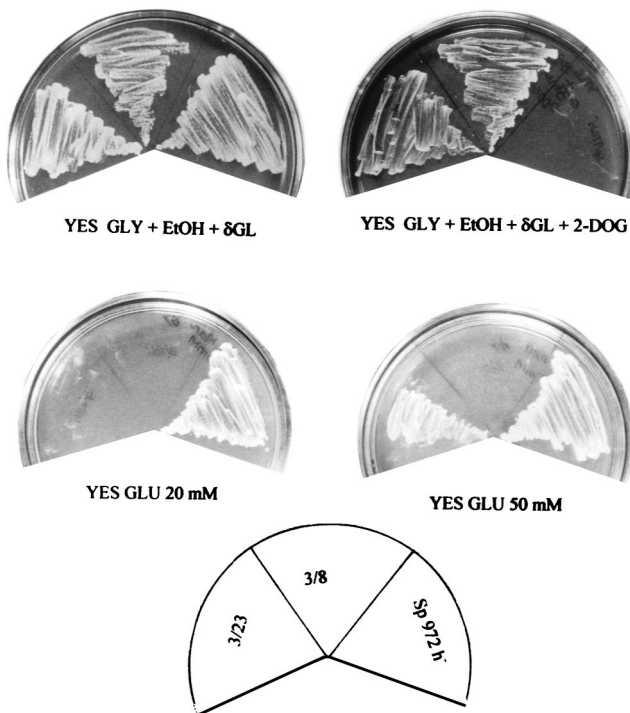

FIG. 3. Growth characteristics of the mutants. Wild-type and the mutant strains of *S. pombe* were streaked on YES agar plates containing the indicated carbon supplements, and growth was monitored after 3 days of incubation at 30°C. Strains are enumerated in Table 1. GLY, 1% glycerol; EtOH, 0.4% ethanol; δGL, 10 mM δ-gluconolactone; GLU, glucose.

3/8-to-wild-type and 3/23-to-wild-type crosses (10 complete tetrads in the first and 5 in the second), the glucose uptake defect cosegregated with the property of resistance to 2-DOG. In the case of the 3/23-to-wild-type cross, the phenotype of the lag period on glucose cosegregated with the glucose uptake defect
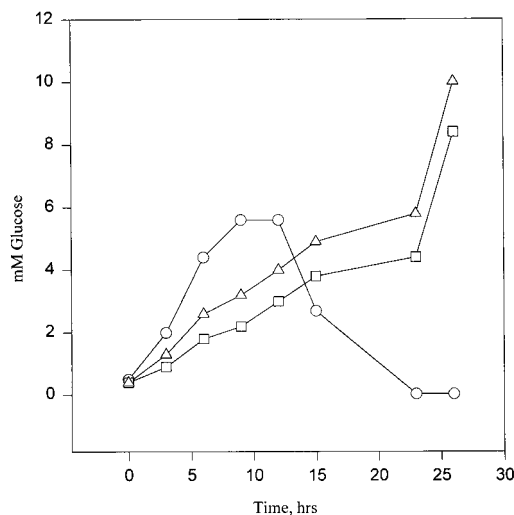

FIG. 4. Estimation of glucose in the supernatant produced from hydrolysis of maltose. Wild-type, 3/23-1T1A, and 3/8-30T1A cells were grown in YES medium containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone, washed, and inoculated in YES medium containing 25 mM maltose. Samples were taken at various time intervals, and the cell medium was used to detect the glucose remaining in the medium for the wild type (○), 3/23-1T1A (□), and 3/8-30T1A (△).
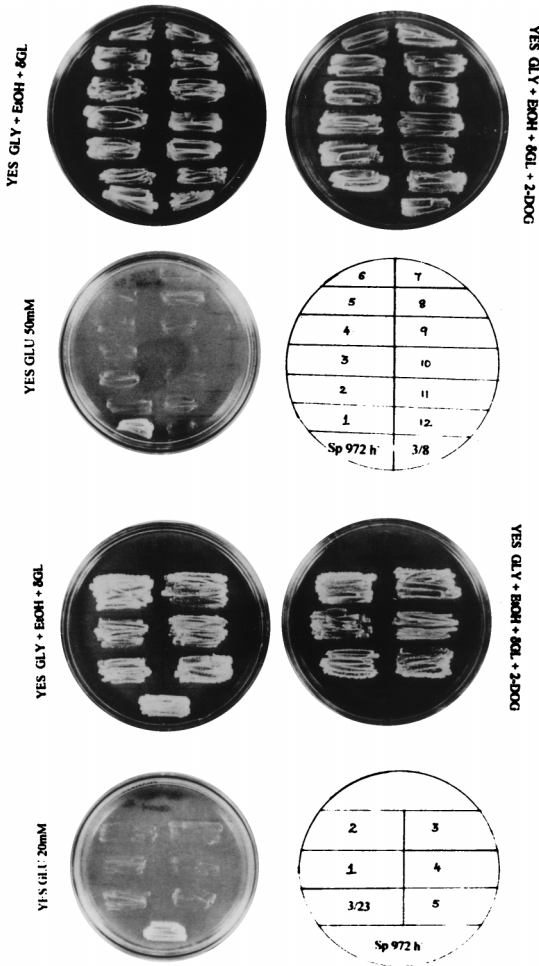
FIG. 5. The 2-DOG resistance property cosegregates with the *std1* mutation. The wild type, mutants, and their segregants were grown on YES agar plates containing the respective carbon supplements, and growth was monitored in 3 days. (A) represents 3/8 and its segregants. (B) represents 3/23 and its segregants. GLY, 1% glycerol; EtOH, 0.4% ethanol; δGL, 10 mM δ-gluconolactone; GLU, glucose.

cose, such as 50 and 100 mM, but with a lag of 12 to 20 h. The lag decreases at higher concentrations of glucose (Fig. 6). The strain carrying mutation *std1-23* yielded a biomass similar to that of the wild type by utilizing less glucose (Fig. 6). Surprisingly, the other mutant with an allelic mutation, 3/8, failed to grow on both 10 and 20 mM glucose, and hence showed no glucose utilization.

Lag disappears when cells are transferred from the log phase. We tried to assess the significance of the lag period at high sugar concentrations by transferring cells from different stages of its growth. The lag seen at a high concentration of glucose disappeared when the culture was transferred from the log phase at high concentrations of glucose but not from the stationary phase (Fig. 7). However, this mutant did not show any growth at low glucose concentrations (20 mM) when transferred from different stages of its growth at high glucose concentrations. Stage-specific transfer did not bring about any improvement in the mutant's phenotype when the cells were transferred from gluconeogenic sources.

Defect in induction and repression of shunt pathway and enzymes. Earlier studies of *S. cerevisiae* glucose transport mutants have shown that some mutations that create a defect in transport also affect catabolite repression (6, 9). We analyzed the induction and repression of the enzymes of the shunt pathway and the glycolytic enzymes by using the 3/23-1T1A mutant. δ-Gluconolactone is known to induce the expression of shunt pathway enzymes in *S. cerevisiae* (27). Induction of shunt pathway enzymes was examined on δ-gluconolactone as compared to glucose in the 3/23-1T1A mutant and the wild-type strain. In *std1* mutants, most of the shunt pathway enzymes are derepressed and show constitutive expression, while some fail to be induced by δ-gluconolactone (Table 4). Among the gly-

at low concentrations of glucose. Further, random spore analysis revealed that out of 200 spores of each cross examined, 70 from the mutant 3/8 and 60 from the mutant 3/23 were glucose uptake deficient and resistant to 2-DOG (Fig. 5). This confirmed the linkage of sugar transport defect to the resistance to the sugar analog; this was not so in the case of YGS mutants (22). For allele testing 3/23-1T1A h$^+$ was crossed to 3/8-16T1B h$^-$. All of the segregants from five complete tetrads failed to grow on glucose; however, the auxotrophic markers as well as the lag at high concentrations of glucose segregated 2:2. To further confirm these findings, random spore analysis was performed, and 200 segregants were checked for growth on 20 and 50 mM glucose as well as for the auxotrophic marker. No glucose-positive segregant was obtained, which clearly confirmed that the mutations are allelic.

3/23 mutant is altered in affinity and shows a growth lag on glucose at high concentrations. Since 3/23 showed growth at high concentrations of glucose, we checked the growth and glucose utilization kinetics of this mutant on different concentrations of glucose. We used the 3/23-1T1A segregant for all of the experiments. It grows well at high concentrations of glu-
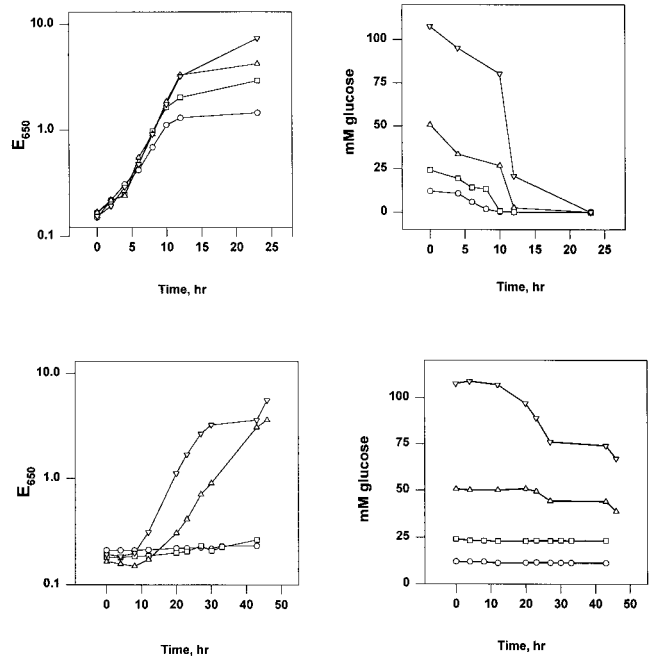

FIG. 6. Growth and glucose utilization of the wild type and the 3/23-1T1A mutant of *S. pombe*. The top panels represent the wild type and the bottom panels represent the 3/23-1T1A mutant. Cells were grown in YES medium containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone, washed, and inoculated in YES medium containing 10 (○), 20 (□), 50 (△), or 100 (▽) mM glucose. Samples were taken at various time intervals to measure the growth (left) and the cell media were used to measure glucose remaining in the medium (right).
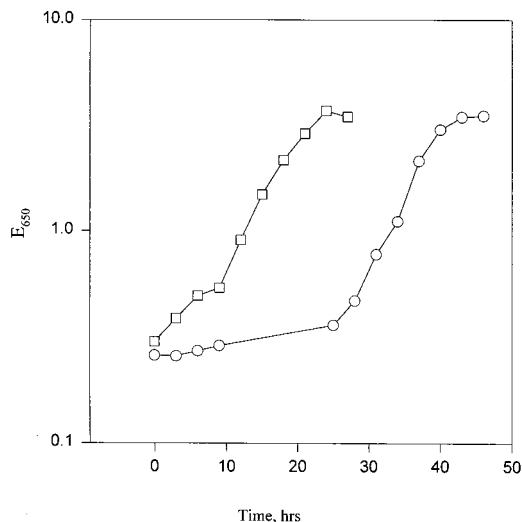
FIG. 7. Growth characteristics of 3/23-1T1A on 50 mM glucose during stage-specific transfer. Mutant 3/23-1T1A was grown in YES medium containing 50 mM glucose. A portion of the cells was harvested during the log phase (E_{650} = 1.0) and transferred into YES medium containing 50 mM glucose ($\square$). The remaining cells were harvested during their stationary phase (E_{650} = 8.0) and inoculated in a similar manner ($\bigcirc$). Growth was measured by taking samples at different time intervals. The viability of the cells was checked by streaking them on a YES plate containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone, and the revertants were examined by streaking them on 20 mM glucose plates.

colytic enzymes induction of only pyruvate decarboxylase (PDC) is affected on glucose in these mutants.

3/23 and 3/8 complement YGS mutants. Recently isolation of somewhat similar mutants from *S. pombe* that were defective in glucose transport was reported (22). They obtained two mutants with allelic mutations, YGS4 and YGS5, which have a mutated glucose transporter gene and are phenotypically similar to 3/8. To rule out the possibility of another allele of the glucose transporter, we crossed *std1* mutants (3/8-16T1A and 3/23-1T1A) with YGS4 and YGS5 (a generous gift from M. Hoefer). Because of the mating defect in the *std1* mutants, we could examine no more than five complete tetrads for the 3/8-16T1A-to-YGS4 and -YGS5 crosses. The pattern of segregation for absence to presence of growth on glucose in four cases was 3:1, and in one case it was 2:2. Similar results were obtained with 3/23-1T1A-to-YGS4 and -YGS5 crosses.

DISCUSSION

We obtained two mutants defective in glucose transport (*std1* gene mutation) by using 2-DOG resistance as a selection pressure. Both of the mutants failed to grow on many of the mono- and disaccharides that we have tested. Genetic analysis with these two mutants confirmed them to have mutations in a single gene. Complementation study as well as tetrad analysis of these two mutants proved that the mutations are allelic. The inability of the mutants to grow on glucose is not due to inhibition of growth by glucose (Fig. 1), and the 2-DOG resistance property is not due to the lack of glucose kinase activity but to lack of transport. The transport deficiency of these mutants was confirmed by [14C]glucose uptake studies (Fig. 2).

Of the two *std1* mutants, 3/23 is able to grow on high concentrations of glucose, whereas 3/8 cannot. At high glucose concentrations, 3/23 showed a lag period of 10 to 12 h, in contrast to the wild-type strain. The lag decreases with increasing concentrations of glucose. When the culture inoculum

came from the cells growing on high concentrations of glucose (50 to 100 mM), the lag disappeared when it was from the exponential growth phase, but not when it came the stationary phase of growth. Stage-specific transfer did not improve the phenotype of the mutant on 20 mM glucose. This indicated the necessity for induction of some factor(s) by glucose for its growth at a higher concentration. This induction could be at the level of transport itself or at a step downstream to it. The lack of [14C]glucose uptake during the lag period in the 3/23 mutant at the higher glucose concentration clearly indicated that the transport itself requires induction (Fig. 8). Since this phenotype was observed only in the mutant strain, we ascribe it to the locus *std1*.

It has been shown in *S. cerevisiae* that *SNF3*, which is involved in the detection of glucose and affects high-affinity transport, also plays a role in catabolite repression (9). To investigate this possibility, we measured the levels of shunt pathway and glycolytic enzymes in the *std1-23* mutant and compared them to those in the wild type. Our results indicate that the mutant is also defective in the induction and repression of most of the shunt pathway enzymes (Table 4) and in the induction of PDC among the glycolytic enzymes (data not shown). We believe that *std1* is perhaps defective in some step in the regulation of synthesis of the sugar transporter.

In *S. cerevisiae* and *Candida utilis*, as well as in most prokaryotes, there exists a separate transporter for maltose, which subsequently gets hydrolyzed intracellularly (7, 19, 31). However, in case of the protozoan *Trichomonas vaginalis*, maltose is hydrolyzed extracellularly to glucose (29). Interestingly, *S. pombe* also seems to have an extracellular maltase. The *std1* mutation revealed the extracellular hydrolysis of maltose to glucose in *S. pombe*. Since mutants carrying this mutation also fail to grow on fructose and mannose apart from glucose, it is possible that fission yeast has a common transporter for the monosaccharides and no separate transporter for disaccharides. Hence, a mutation affecting glucose transport impairs the cell's ability to grow on all of the sugars. Surprisingly, *S. pombe* is known to have a separate transporter for gluconate (15).

YGS4 and YGS5 are the two sugar transport mutants ob-

TABLE 4. Constitutive expression of shunt pathway enzymes in the wild type and mutant

Enzyme[a]	Sp act (mU/mg)[b]			
	Sp972 h−		3/23-1T1A	
	Glucose	Glycerol + ethanol + δ-gluconolactone	Glucose	Glycerol + ethanol + δ-gluconolactone
ZWF	220	152	132	209
TAL	6	13	20	13
TKT	6	15	15	17
PGL	3	12	4	5
GNK	27	56	43	46
GND	552	1,549	1,196	1,255
PPI	4	22	7	8
PPE	16	50	53	45

[a] ZWF, glucose-6-P dehydrogenase; TAL, transaldolase; TKT, transketolase; PGL, phosphogluconolactonase; GNK, gluconate kinase; GND, 6-phosphogluconate dehydrogenase; PPI, pentose phosphate isomerase; PPE, pentose phosphate epimerase.
[b] Cultures were grown on complete synthetic medium containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone to the log phase. The cells were harvested, washed and inoculated into complete synthetic medium containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone. Enzymes were assayed in the cell extracts, and enzyme activity has been expressed in milliunits per milligram of protein.
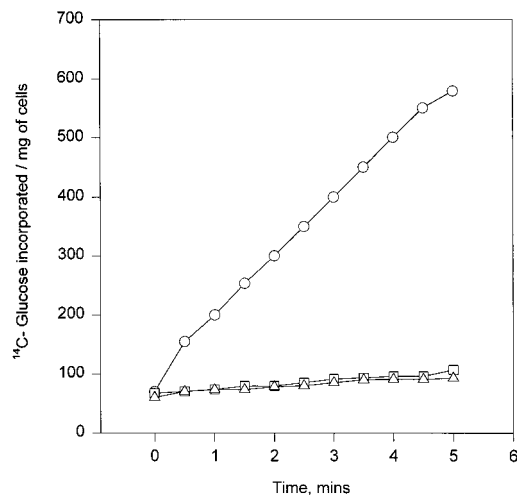
FIG. 8. Uptake of [^{14}C]glucose in the 3/23-1T1A and 3/8-30T1A mutants during the lag phase compared to that of the wild type. The wild-type strain Sp972 h$^-$ and the 3/8-30T1A and *3*/23-1T1A mutants were grown on YES medium containing 1% glycerol, 0.4% ethanol, and 10 mM δ-gluconolactone, washed, and reinoculated into YES medium containing 50 mM glucose. One hour after the transfer, the cells were harvested and the [^{14}C]glucose uptake was measured in the wild type (○), 3/8-30T1A (△), and 3/23-1T1A (□) as described in Materials and Methods. The amount of radioactivity incorporated was monitored with a scintillation counter.

tained by Milbradt and Hoefer (22) in a similar screen. The *GHT1* clone complementing YGS5 has been identified and characterized (18). The *GHT1* gene encodes a 2.6-kb transcript and codes for a transporter protein with 12 transmembrane domains. Isolation and characterization of *std1* mutants which are different from the mutants in this structural gene for the symporter uncovered the presence of some additional step that controls sugar transport in *S. pombe* at the genetic level. Further analysis of this mutant and identification of the mutated gene would reveal the step blocked in the transport pathway and would also illuminate the area of catabolite repression in fission yeast.

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