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The Mutation *DGT1-1* Decreases Glucose Transport and Alleviates Carbon Catabolite Repression in *Saccharomyces cerevisiae*

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Glucose in ethanol-glycerol mixtures inhibits growth of *Saccharomyces cerevisiae* mutants lacking phosphoglycerate mutase. A suppressor mutation that relieved glucose inhibition was isolated. This mutation, *DGT1-1* (*d*ecreasing *g*lucose *t*ransport), was dominant and produced pleiotropic effects even in an otherwise wild-type background. Growth of the *DGT1-1* mutant in glucose was dependent on respiration, and no ethanol was detected in the medium within 7 h of glucose addition. When grown on glucose, the mutant had a reduced glucose uptake and both the low- and high-affinity transport systems were affected. In galactose-grown cells, only the high-affinity glucose transport system was detected. This system had similar kinetic characteristics in the wild type and in the mutant. Catabolite repression of several enzymes was absent in the mutant during growth in glucose but not during growth in galactose. In contrast with the wild type, the mutant grown in glucose had high transcription of the glucose transporter gene *SNF3* and no transcription of *HXT1* and *HXT3*. Expression of multicopy plasmids carrying the *HXT1*, *HXT2*, or *HXT3* gene allowed partial recovery of both fermentative capacity and catabolite repression in the mutant. The results suggest that *DGT1* codes for a regulator of the expression of glucose transport genes. They also suggest that glucose flux might determine the levels of molecules implicated as signals in catabolite repression.

Addition of glucose or other easily fermentable sugars to cultures of *Saccharomyces cerevisiae* produces repression of the transcription of many genes (16, 22, 33), inactivation of several enzymes, and activation of others like phosphofructokinase, pyruvate kinase, and plasma membrane ATPase (for a review, see reference 15). In spite of the importance of this general catabolic control exerted by glucose, it is not known how the yeast cell senses the presence of glucose and transduces this information to trigger all the mentioned phenomena. The important question of the existence of a glucose receptor remains unanswered, in spite of claims that the product of the *TPS1* gene (coding for trehalose-6-phosphate synthase [2, 37]) is the "general glucose sensor" (35). In order to identify elements implicated in the sensing and transduction of the signal for glucose presence, we have started the isolation of mutants with altered responses to glucose. Our strategy was to search for suppressors of the toxic effect of glucose in a mutant defective in phosphoglycerate mutase. This enzyme, coded for by the *GPM1* gene (23), catalyzes the interconversion between 3- and 2-phosphoglycerates (14) and is therefore an essential enzyme for glucose metabolism. Glucose does not allow growth of mutants affected in different steps of the glycolytic pathway and inhibits their growth on alternative carbon sources (7, 8). We reasoned that among suppressors of the inhibitory effect of glucose on *gpm1* mutants, we could uncover mutants affected in the sensing and transducing machinery of glucose, in transport or phosphorylation of this sugar, or in catabolite repression. We used a yeast strain with a chromosomal deletion of the *GPM1* gene as starting material. We report in this article the characterization of a dominant suppressor mutation, *DGT1-1*, that presents an important decrease of glucose transport and alleviates catabolite repression.

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MATERIALS AND METHODS

Yeast strains and plasmids. *S. cerevisiae* W303-1A *MAT*a *ade2-1 his3-11,15 leu2,3-112 trp1-1 ura3-52* was used in this work. Strain CJM198, in which the *GPM1* gene was deleted and replaced by *LEU2*, was constructed as follows: the 3.1-kb *Sph*I fragment from plasmid YRPGM2 (30) containing the *GPM1* gene was introduced into a pUC18 in which the *Sal*I site was eliminated. In this construction, a 1.5-kb *Bgl*II-*Sal*I fragment containing the entire coding sequence of *GPM1* was replaced by a 2.4-kb *Bgl*II-*Sal*I fragment from YEp13 bearing the *LEU2* gene. A 4.1-kb *Sph*I fragment from this construction containing the disrupted-deleted *GPM1* gene was used to replace the *GPM1* gene in W303-1A by the one-step gene replacement procedure (31).

Plasmids pYEp351-HXT1 and pYEp351-HXT3 are multicopy plasmids carrying the *HXT1* and *HXT3* yeast genes, respectively, and were kindly provided by F. Portillo (Madrid, Spain). Plasmids pAK-1a and pGT7 (kindly provided by L. Bisson, Davis, California) are multicopy plasmids containing the *HXT2* and *HXT4* genes, respectively, subcloned into YEp352.

Plasmid pYEp351-SNF3 carrying the *SNF3* gene into the YEp351 multicopy plasmid was constructed as follows: plasmid pJF177 carrying the yeast *SNF3* gene (kindly given by J. M. François, Toulouse, France) was digested with *Eco*RI, blunt ended, and digested with *Sal*I. The 3.2-kb fragment carrying *SNF3* was ligated into pYEP351 digested with *Sma*I and *Sal*I.

Escherichia coli TG1 and DH5α were used for the propagation of plasmids. Transformants were selected by growth on Luria-Bertani ampicillin broth.

Growth media. Yeast cells were grown on 1% yeast extract–2% peptone with one of the following carbon sources: 2% glucose, 3% glycerol–2% ethanol, or 3% glycerol–2% ethanol–2% glucose. In some cases, other carbon sources were

used as indicated in each particular case. Yeast strains bearing plasmids were grown in Difco yeast nitrogen base supplemented with the adequate auxotrophic requirements. Antimycin A, when used, was added at a final concentration of 2 μg/ml. Ethidium bromide was used at a final concentration of 10 μg/ml. Growth was monitored by measuring the A_{660} of the cultures in a Bausch & Lomb Spectronic 20.

Enzyme analysis. Cell extracts were obtained by shaking 100 mg (wet weight) of cells in 0.5 ml of ice-cold 20 mM imidazole (pH 7) with 1 g of glass beads (diameter, 0.5 mm) in a vortex for four periods of 1 min each, with 1-min intervals in ice after each period. The extract was centrifuged for 3 min at 700 × *g*, and the supernatant was used for the enzymatic assays. Phosphoglycerate mutase was assayed as described in reference 3, fructose-1,6-bisphosphatase was assayed as described in reference 17, glutamate dehydrogenase was assayed as described in reference 10, malate dehydrogenase was assayed as described in reference 39, isocitrate lyase was assayed as described in reference 9, and cytochrome *c* oxidase was assayed as described in reference 19. Protein was determined as described in reference 26, with bovine serum albumin as the standard.

Transport of glucose. For glucose transport assays, yeast cells were harvested at a cell density of 3 mg/ml (wet weight) after growth in glucose or 1 mg/ml (wet weight) after growth in galactose. The transport of glucose was measured at 20°C with uniformly labelled ^{14}C-glucose in a final volume of 55 μl with 5 mg of yeast cells (wet weight) in 0.1 M potassium phosphate buffer (pH 6). The reaction was triggered by the addition of the labelled sugar (0.5 to 4 mCi/mmol). Ten seconds after the glucose was added, 10 ml of ice-cold 0.5 M glucose in 0.1 M potassium phosphate buffer (pH 6) was added as described in reference 38 and the sample was filtered under vacuum conditions and washed in the filter with the same volume of the previous solution. Control samples were treated similarly, but the reaction was started by adding the yeast cells to the labelled glucose and placing this mixture in the 10 ml of ice-cold glucose. The final glucose concentrations ranged from 0.5 to 100 mM. The filters were counted in scintillation vials in OptiPhase HiSafe liquid (Fisons Chemicals, Loughborough, United Kingdom). Kinetic parameters were determined by Eadie-Hofstee plots, and all data were analyzed by computer-assisted nonlinear regression analysis with IGOR software. Data were fitted to a model with one kinetic component, $v = V_{max} \cdot S/K_m + S)$, or with two kinetic components, $v = V_{max1} \cdot S/(K_{m1} + S) + V_{max2} \cdot S/(K_{m2} + S)$, where v is the rate of glucose uptake and S is the substrate concentration.

Fermentation and respiration measurements. Fermentation and respiration of glucose were measured in a conventional Warburg respirometer in 0.15 M sodium phosphate buffer and 0.1 M KCl (pH 6.5) in cell suspensions of 20 mg of yeast cells (wet weight) per ml.

Determination of fermentation products in the medium by NMR. Yeast cells grown in rich medium with glucose were collected during the exponential phase of growth, resuspended at 20 mg (wet weight)/ml in yeast nitrogen base with 1% 1-^{13}C-glucose, and incubated at 30°C. Samples were taken at different time intervals and centrifuged, and the supernatant was used for nuclear magnetic resonance (NMR) analysis. High-resolution ^{13}C NMR analysis was performed at 8.4 T on a Bruker AM-360 NMR spectrometer. Broad-band proton-decoupled ^{13}C spectra (22°C, pH 4.7) were obtained at 90.55 MHz with a WALTZ-16 decoupling sequence gated only during the acquisition (0.3 W of average forward power). ^{13}C NMR conditions were as follows: 55° pulses, 200-ppm sweep width, 64-kiloword data table (1.57-sec acquisition time), and 6.0-sec total recycle time. Usually, 8,000 scans representing 13 h of accumulation time were collected. Free-induction decays were zero filled to 128 kilowords prior to Fourier transformation. Chemical shifts were calibrated with the signal of a 10% dioxane solution (67.4 ppm) placed in a concentric capillary.

Genetic methods. Crosses, sporulation, and dissection of tetrads were done by conventional methods. Diploids homozygous for the *gpm1::LEU2* mutation failed to sporulate in several media tested. Sporulation was restored in the presence of a plasmid carrying the *GPM1* gene. Yeast cell transformation was performed with lithium acetate according to the method of Ito et al. (20).

DNA and RNA isolation and Northern (RNA) blot. Plasmid DNA was obtained from *E. coli* as described previously (32). For Northern blot analysis, total yeast RNA was extracted as described in reference 25 and poly(A)$^+$ was obtained basically as described in reference 21. The probes, labelled as described in reference 13, were a 0.5-kb *Eco*RI-*Eco*RI fragment from plasmid YEp351-SNF3 corresponding to the 160 C-terminal amino acids of the protein for *SNF3*, a 0.13-kb *Eco*RI-*Acc*I fragment from the gene corresponding to the 60 N-terminal amino acids of the protein for *HXT1*, and a 1.75-kb *Bam*HI-*Hin*dIII fragment from plasmid pYactI for actin (28).

Mutagenesis and isolation of mutants. Cells were grown on glycerol-ethanol to a concentration of 3 to 4 mg/ml (wet weight), washed twice with water, suspended in water at a concentration of about 10^7 cells per ml, and irradiated with a germicidal UV lamp to reach a mortality of about 90%. After overnight incubation in glycerol-ethanol medium, the cells were spread on plates with glycerol-ethanol-glucose medium. Colonies appearing after 4 to 5 days were picked and streaked on plates with glycerol-ethanol and glycerol-ethanol-glucose. Those growing on the last medium were selected for further study.

RESULTS

Isolation and genetic characterization of suppressors of the *gpm1* mutation. The *gpm1* mutation causes inability to grow in media containing glucose. A yeast strain with a deletion of the *gpm1* gene was initially selected (see Materials and Methods), and suppressors of the glucose inhibitory effect were isolated as described in Materials and Methods. Twenty-five independent colonies were studied. Each of them was crossed with a wild-type strain; the resulting diploid was sporulated, and 12 complete tetrads of each cross were scored for growth in glycerol-ethanol-glucose plates. Ten mutants showing a phenotype segregation indicative of a monogenic mutation were retained. A spore of each of the previous crosses carrying the new mutation and the *gpm1::LEU2* disruption was crossed with a strain bearing the disrupted *GPM1* gene, and the resulting diploids were tested for the ability to grow on glucose. All diploids grew on glucose, showing that the isolated suppressor mutations were dominant. Analysis of the progeny of pairwise crosses between the independent isolates showed that they belonged to one complementation group. One mutant was chosen for further study, and after characterization of its properties (see below), the gene responsible for the phenotype was termed *DGT1* (decreasing glucose transport) and the allele studied was termed *DGT1-1*.

Generation times of strains with the *DGT1-1* mutation. The double mutant *gpm1 DGT1-1* grew on glycerol-ethanol plates with additions of glucose of up to 10%. Table 1 shows the generation times of strains carrying the *DGT1-1* mutation alone or in combination with the *gpm1* mutation. The double mutant did not grow in glucose, but the *DGT1-1* single mutant did, with a doubling time twice that of a wild type. The

TABLE 1. Effect of the *DGT1-1* mutation on the generation time of yeast strains grown in different media[a]

Carbon source	Generation time (min) for genotype:			
	GPM1	*gpm1*	*gpm1 DGT1-1*	*GPM1 DGT1-1*
Glycerol-ethanol	190	245	230	200
Glycerol-ethanol-glucose	110	NG[b]	360	180
Glucose	90	NG	NG	180

[a] The different strains were grown in rich media with the indicated carbon sources as described in Materials and Methods.
[b] NG, no growth.

mutation increased the doubling time in glycerol-ethanol mixtures compared with that of the wild type when glucose was present.

Utilization of glucose and accumulation of products in the medium by the *DGT1-1* mutant. The following studies were carried out with the *DGT1-1* mutation in an otherwise wild-type background. The mutant did not grow on glucose plates with antimycin A or ethidium bromide but grew on galactose plates with these substances. Fermentation of glucose was undetectable in the mutant grown in glucose, while its respiration was almost three times higher than that of the wild type (Table 2). When it was grown on galactose, the mutant fermented and respired glucose or galactose like the wild type (Table 2).

Wild-type and *DGT1-1* mutant cells grown in glucose were transferred to a fresh medium containing 1-^{13}C-glucose, and samples were taken at different time intervals. As shown in Fig. 1, the wild type had consumed all the glucose after 5 h and fermented it basically to ethanol; however, with the *DGT1-1* mutant, glucose had not disappeared after 7 h, and no ethanol was detectable. A slight quantity of acetate, about 2 mM, was detected at this time in the medium. It appears therefore that the *DGT1-1* mutant respires most of the consumed glucose. These results confirm that the mutant has a decreased glycolytic flux compared with that of the wild type when grown in glucose and that the *DGT1-1* mutant respires most of the consumed glucose. This is also in agreement with the effect of antimycin A on growth mentioned above.

Effect of *DGT1-1* on other mutations. Phosphoglycerate kinase, *pgk1* (7), and trehalose-6-phosphate synthase, *tps1* (2, 37), mutants do not grow on glucose. Glucose inhibits growth in permissive carbon sources. The *DGT1-1 pgk1* double mutant grew normally in glycerol-ethanol-glucose plates. Also, the *DGT1-1 tps1* double mutant grew on glucose plates like the

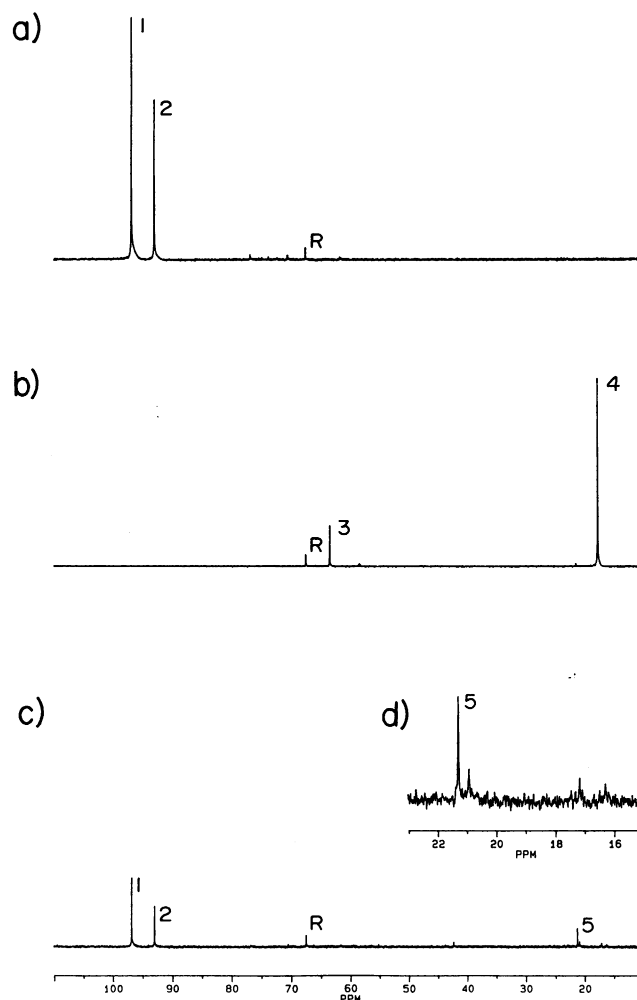

FIG. 1. Analysis of products excreted by wild-type and *DGT1-1* mutant yeasts. Wild-type or *DGT1-1* mutant cells were suspended in media with 1% 1-^{13}C-glucose and treated as described in Materials and Methods. Samples were taken at the times indicated below and centrifuged, and ^{13}C NMR spectra (90.55 MHz) of the supernatants were analyzed as described in Materials and Methods. (a) Time zero. (b) Wild type, 5 h after glucose addition. (c) *DGT1-1* mutant, 7 h after glucose addition. (d) Region where peak 5 appeared (enlargement). Peaks: 1 and 2, glucose C-1 β and α, respectively; R, dioxane (reference); 3, glycerol C-1 and C-3; 4, ethanol C-2; 5, acetate C-2.

TABLE 2. Fermentation and respiration of sugars in yeast strains carrying the *DGT1-1* mutation[a]

Strain	Carbon source	Fermentation (μmol of sugar/g of yeast cells [wet wt]/h) of:		Respiration (μmol of sugar/g of yeast cells [wet wt]/h) of glucose
		Glucose	Galactose	
W303-1A	Glucose	700	ND[b]	30
(wild type)	Galactose	175	170	85
CJM198	Glucose	<5	ND	85
(*DGT1-1*)	Galactose	140	150	80

[a] Yeast cells were grown in rich media with 2% glucose or 2% galactose as described in Materials and Methods, harvested during the exponential phase of growth, and washed with water. Fermentation and respiration were measured as described in Materials and Methods.
[b] ND, not determined.

wild type. This result indicates that *DGT1-1* suppresses the toxic effects of glucose on a variety of mutants.

Catabolite repression in the *DGT1-1* mutant. If glucose is utilized mainly by respiration by the *DGT1-1* mutant, the genes coding for the respiratory enzymes should have escaped catabolite repression. A series of different enzymes encoded by catabolite-repressed genes presented in the mutant elevated levels of activity during growth in glucose, indicating a loss of catabolite repression, although the degree of derepression was not the same in all cases (Table 3). However, after the *DGT1-1* mutant was grown in galactose, the repression was similar to that of the wild type (Table 3).

Glucose transport in the *DGT1-1* mutant. The results obtained so far indicated that the *DGT1-1* mutant could be affected in its glucose uptake. After the kinetics of glucose

TABLE 3. Specific activities of some repressible enzymes in wild-type and *DGT-1* mutant yeast strains grown in rich media with different carbon sources[a]

Enzyme	Sp act (milliunits/mg of protein) in:					
	W303-1A (wild type) grown in:			CJM198 (*DGT-1* mutant) grown in:		
	Glycerol + ethanol	Glucose	Galactose	Glycerol + ethanol	Glucose	Galactose
Fructose-1,6-bisphosphatase	40	<1	<1	40	10	<1
Isocitrate lyase	29	<1	<1	22	8	<1
Malate dehydrogenase	980	180	390	960	960	465
Glutamate dehydrogenase	110	10	17	113	106	17
Cytochrome *c* oxidase[b]	38	6	19	30	46	20

[a] Yeast strains were grown in rich media with the indicated carbon sources as described in Materials and Methods. They were harvested during the exponential phase of growth and washed twice with water. Enzymes were assayed as described in Materials and Methods.
[b] For cytochrome *c* oxidase, specific activities are expressed as the rate constant k (in seconds^{-1}) per milligram of protein $\times 10^2$.

transport of the *DGT1-1* mutant was analyzed in cells grown in glucose and harvested during the exponential phase of growth, a striking difference was observed with respect to the wild-type cells (Fig. 2). The V_{max} of the high-affinity system decreased in the mutant from 5.3 to 1.9 μmol/min/g, while that of the low-affinity system decreased from 13.5 to 3.6 μmol/min/g. There was no marked variation in the K_m values measured for the high-affinity component (wild type, 1.7 mM; mutant, 1 mM), and the changes in the low-affinity system (wild type, 30 mM; mutant, 10 mM) are likely not significant. After the wild type and the mutant were grown in galactose, only the high-affinity system was observed in both cases, and the kinetic parameters were similar for the two strains (Fig. 3).

Effect in *DGT1-1* mutants of overexpression of genes coding for glucose transporters. Since the transport measurements showed a defect in this step, we tried to complement the *DGT1-1* mutation with genes known to be implicated in glucose transport (for a review, see reference 4). Transformation of *DGT1-1* cells with multicopy plasmids carrying gene *SNF3* or genes *HXT1* through *HXT4* allowed growth on glucose-antimycin A plates, although growth was impaired with respect to the wild type. In the case of the mutant transformed with *SNF3*, growth was particularly weak. Overexpression of *SNF3* slightly increased fermentation of glucose in the mutant without affecting respiration and catabolite repression (Table 4). However, overexpression of *HXT2* increased fermentation of glucose to about 75% of that of the wild-type rate, decreased respiration, and almost completely restored catabolite repression of the enzymes tested (Table 4). Overexpression of *HXT1* or *HXT3* also increased fermentation and partially restored catabolite repression in most of the enzymes tested (Table 4), although these genes were less effective than *HXT2*. These results suggest that the extent of restoration of glucose repression parallels that of glucose fermentation.

Effect of the *DGT1-1* mutation on the expression of genes related to glucose transport. The observed decrease in glucose uptake in the *DGT1-1* mutant might be mediated through changes in the expression of other genes involved in glucose transport (24). We examined the expression of *SNF3* and *HXT1* in the wild type and in the *DGT1-1* mutant grown in glucose and galactose. The *DGT1-1* mutant expressed *SNF3* to high levels after being grown in glucose, in contrast to the wild type. There was a high level of expression of *SNF3* in both the wild type and the mutant after they were grown in galactose (Fig. 4). On the other hand, *HXT1* and *HXT3* expression could not be detected in the mutant grown in glucose, while there was a clear expression in the wild type. Neither the wild type nor the mutant showed expression of *HXT1* during growth in galactose (Fig. 5).

DISCUSSION

We have identified in *S. cerevisiae* a dominant mutation that caused pleiotropic effects in glucose metabolism. The primary effect of the *DGT1-1* mutation appears to be a severe reduc-

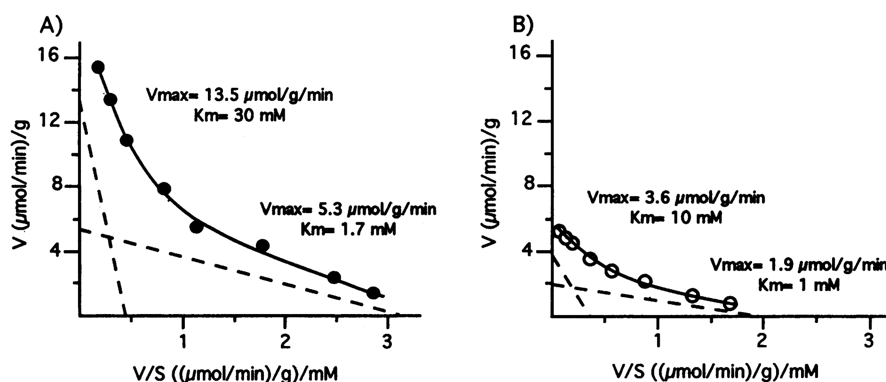

FIG. 2. Kinetic analysis of glucose uptake by wild-type and *DGT1-1* cells grown in glucose. Glucose uptake by the wild type (A) or *DGT1-1* mutant (B) was measured as described in Materials and Methods. The experimental points were fitted to a model consisting of two systems with different parameters as described in Materials and Methods. The dashed lines represent the kinetics of the two uptake systems provided by the model. The kinetic parameters are indicated.

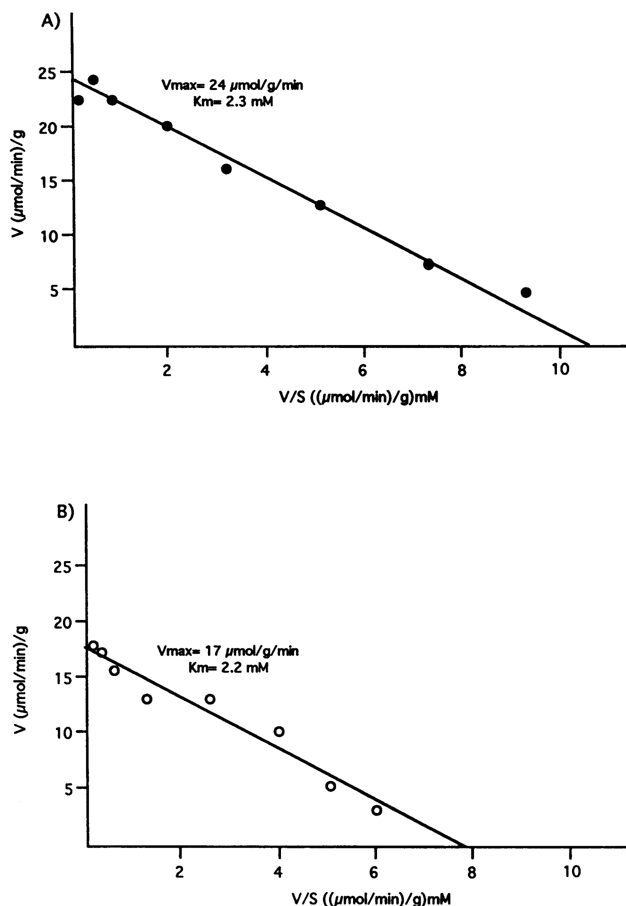

FIG. 3. Kinetic analysis of glucose uptake by wild-type and *DGT1-1* mutant cells grown on galactose. Glucose uptake in galactose-grown wild-type cells (A) and *DGT1-1* mutant cells (B) was measured as described in Materials and Methods. The experimental points were adjusted by a nonlinear regression analysis program as mentioned in Materials and Methods. Kinetic parameters are indicated.

tion of glucose uptake. The classical work of Bisson and Fraenkel (5) showed the existence of two kinetically distinct uptake systems. The *DGT1-1* mutation affected both systems, although the one with low affinity was more influenced. During growth of yeast cells in galactose, only the high-affinity system was detected and no significant difference between the mutant

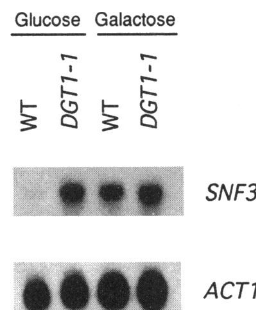

FIG. 4. Northern blot analysis of *SNF3* transcription. Wild-type (WT) and *DGT1-1* mutant cells were grown in glucose or in galactose as described in Materials and Methods. Poly(A)$^+$ RNA (5 μg per lane) separated by electrophoresis as described in Materials and Methods was hybridized to ^{32}P-labelled probes. As an internal control for loading, the blot was also probed with the actin gene (*ACT1*).

and wild type could be seen, suggesting that the effects of the *DGT1-1* mutation are directly related with growth in glucose. The V_{max} of transport in the wild type was the same in galactose as the sum of the V_{max} values of the high- and low-affinity systems during wild-type growth in glucose. Walsh et al. (38) have reported that during cell growth in glucose, the total V_{max} of the glucose uptake system remains unchanged and that only the distribution between the high- and low-affinity forms changes. Our results indicate that this constancy of V_{max} extends also to cells grown in galactose.

A family of genes that affect glucose transport has been identified, and it comprises *SNF3* and *HXT1* through *HXT4*. The proteins encoded by these genes have the structure of sugar transporters, although their roles are not definitely established (for a review, see reference 4). Snf3p could play a role in the proposed interconversion (38) of the low- and high-affinity systems. The mutation *DGT1-1* perturbed the expression of *SNF3*, *HXT1*, and *HXT3* so that during cell growth in glucose, *SNF3* was expressed to high levels while expression of *HXT1* and *HXT3* was not detected. This suggests that the mutation *DGT1-1* blocks the expression of *HXT1* and *HXT3*, with the possibility that the effect is mediated by increased levels of *SNF3*. However, a disruption of *SNF3* did not influence the *DGT1-1* phenotype (results not shown). The observed facts, including the dominance of the *DGT1-1* mutation, may be explained by a model in which *DGT1* represses expression of *HXT1* and *HXT3*, with this repression being counteracted by glucose. The mutation *DGT1-1* would abolish

TABLE 4. Specific activities of some glucose-repressible enzymes and fermentation and respiration of glucose in *DGT1-1* mutants overexpressing the *SNF3*, *HXT1*, *HXT2*, or *HXT3* gene[a]

Strain	Sp act (milliunits/mg of protein) of[b]:				Glucose fermentation (μmol/g/h)	Glucose respiration (μmol/g/h)
	Isocitrate lyase	Malate dehydrogenase	Glutamate dehydrogenase	Cytochrome *c* oxidase[c]		
W303-1A/YEp351	<2	172 ± 7	6.6 ± 0.3	8.5 ± 0.6	700	40
CJM198/YEP351	22 ± 4	835 ± 21	46 ± 3	31 ± 3	<5	95
CJM198/YEp351-SNF3	25 ± 5	765 ± 30	21 ± 2	31 ± 2	60	100
CJM198/YEp351-HXT1	9 ± 2	546 ± 21	14 ± 1	27 ± 2	337	57
CJM198/pAK1a (HXT2)	<2	228 ± 21	7.1 ± 0.7	12 ± 2	425	50
CJM198/YEp351-HXT3	15 ± 3	482 ± 25	13 ± 1	21 ± 3	210	70

[a] Yeast strains were grown in minimal medium with 2% glucose as described in Materials and Methods. They were harvested during the exponential phase of growth and washed twice with water. Glucose fermentation and respiration were measured as described in Materials and Methods.
[b] Values are means ± standard deviations of three separate experiments.
[c] For cytochrome *c* oxidase, specific activities are expressed as the rate constant k (in seconds^{-1}) per milligram of protein × 10^2.

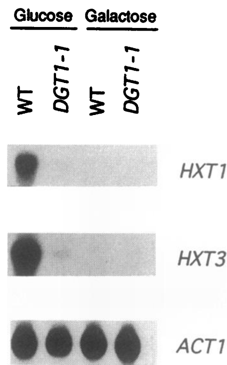

FIG. 5. Northern blot analysis of *HXT1* transcription. Wild-type (WT) and *DGT1-1* mutant cells were grown in glucose or in galactose as described in Materials and Methods. Poly(A)$^+$ RNA (5 μg per lane) was separated by electrophoresis as described in Materials and Methods and hybridized to ^{32}P-labelled probes. The blot was also probed with the actin gene (*ACT1*) as a control for loading.

the action of glucose and therefore expression of *HXT1* and *HXT3* in this sugar. Since *SNF3* is subject to catabolite repression (4), its observed expression in the *DGT1-1* mutant may be explained by the loss of catabolite repression. It is noteworthy that *DGT1-1* affected the expression of *HXT1* and *HXT3*, genes that according to the results of Ko et al. (24) are differentially expressed during growth in glucose.

The *DGT1-1* mutation allowed growth in glucose of *tps1* (formerly called *cif1* or *fdp1*) mutants and also suppressed the toxic effect of glucose on *pgk1* mutants. These results may be explained as follows: *tps1* mutants have an uncontrolled glucose flux, likely due to their lack of inhibition of hexokinase by trehalose-6-phosphate (6), and are rapidly depleted of ATP upon addition of glucose (18, 27, 36). A decrease in glucose transport would alleviate this problem and allow cell growth in glucose. In the case of *pgk1* mutants or the original *gpm1* mutants, the *DGT1-1* mutation in the presence of glucose allows derepression of several genes whose products are necessary for cell growth in alternative carbon sources. What is the mechanism that would allow derepression during growth in glucose? The fact that overexpression of *HXT* genes increased fermentation and partially restored repression by glucose suggests a correlation between transport and catabolite repression. However, we cannot presently exclude the possibility that *DGT1-1* influences transport and catabolite repression by different mechanisms and that overexpression of *HXT* genes partially corrects both. It is tempting to speculate that either transport proteins are directly involved in the mechanism of catabolite repression or a decreased glucose flux could cause a change in the levels of some molecule(s) that triggers catabolite repression. In this way, glucose transport could function as a glucose sensor. Note that flux as such cannot be a regulator, since flux is a kinetic, dynamic value and regulation systems can respond only to changes in the concentration of some molecule. It is interesting that catabolite repression was only alleviated during cell growth in glucose, reinforcing our idea that *DGT1-1* is implicated in catabolite repression only when the signal is given by glucose. Two other mutations, *HTR1-23* and *cat80/grr1*, that affect glucose transport and catabolite repression (11, 12, 29, 34) have been identified. We believe that the mutation *DGT1-1* is different from these mutations for the following reasons. The *HTR1-23* mutant fermented most of the consumed glucose to ethanol, while the *DGT1-1* mutant did not produce ethanol. In addition, *DGT1-1* affects both the high- and low-affinity systems, while from the data of Özcan et al. (29), it can be calculated that *HTR1-23* affected basically the high-affinity system. Also, expression of multicopy plasmids carrying the *SNF3* and *HXT1* genes had different effects. In the *HTR1-23* mutant, both suppressed the defective growth phenotype but did not have an effect on the lack of catabolite repression, while in the *DGT1-1* mutant, overexpression of *HXT1* partially restored both glucose fermentation and catabolite repression and *SNF3* was without a significant effect. Moreover, *HTR1-23* did not influence transcription of *SNF3* (29), while *DGT1-1* did.

The *cat80/grr1* mutation is a recessive one that affects the high-affinity glucose transport system. Moreover, it produces an aberrant morphology (1) that was not observed in *DGT1-1* mutants. However, we cannot yet completely discard the possibilities that *DGT1-1* is an allele of one of these genes and that the observed differences in phenotype are due to differences in the mutation or the genetic background.

The results presented here, together with those of other authors (12, 29, 34), stress the importance of the transport step for a coordinated functioning of the yeast cell.

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