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Glucose Uptake and Catabolite Repression in Dominant *HTR1* Mutants of *Saccharomyces cerevisiae*

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Growth and carbon metabolism in triosephosphate isomerase (*Δtpi1*) mutants of *Saccharomyces cerevisiae* are severely inhibited by glucose. By using this feature, we selected for secondary site revertants on glucose. We defined five complementation groups, some of which have previously been identified as glucose repression mutants. The predominant mutant type, *HTR1* (hexose transport regulation), is dominant and causes various glucose-specific metabolic and regulatory defects in *TPI1* wild-type cells. *HTR1* mutants are deficient in high-affinity glucose uptake and have reduced low-affinity transport. Transcription of various known glucose transporter genes (*HXT1*, *HXT3*, and *HXT4*) was defective in *HTR1* mutants, leading us to suggest that *HTR* mutations affect a negative factor of *HXT* gene expression. By contrast, transcript levels for *SNF3*, which encodes a component of high-affinity glucose uptake, were unaffected. We presume that *HTR1* mutations affect a negative factor of *HXT* gene expression. Multicopy expression of *HXT* genes or parts of their regulatory sequences suppresses the metabolic defects of *HTR1* mutants but not their derepressed phenotype at high glucose concentrations. This suggests that the glucose repression defect is not a direct result of the metabolically relevant defect in glucose transport. Alternatively, some unidentified regulatory components of the glucose transport system may be involved in the generation or transmission of signals for glucose repression.

Glucose repression is an important global regulatory system in the yeast *Saccharomyces cerevisiae*. Expression of a large number of functions required for the utilization of alternate carbohydrates and nonfermentable carbon sources is repressed by glucose (for reviews, see references 16 and 23). Since glucose control is ultimately executed at the level of transcription, most of the genetic work on this regulatory mechanism has been focused on the analysis of transcriptional activation or repression of glucose-sensitive genes. Dissection of various gene systems, like *SUC*, *GAL*, *MAL*, *ADH2*, or *CYC1/CYC7*, has revealed a multitude of nuclear factors which regulate the transcription of these genes in response to glucose (for a review, see reference 48). By contrast, relatively little is known about how metabolic signals in glucose repression are generated and in which way they are relayed to the various components of the transcriptional machinery. For a subset of glucose-repressible genes, evidence that the RAS-adenyl cyclase signalling pathway is involved has been presented (for a review, see reference 46), although it remains speculative by which signal this pathway is triggered. A number of other cytoplasmic factors have been identified upon isolation of mutants defective in derepression or with derepressed expression of glucose-sensitive genes. The activity of a protein kinase encoded by the gene *SNF1* (= *CAT1* = *CCR1*), together with an associated factor (*CAT3* = *SNF4*), appears to be crucial for derepression of all glucose-sensitive genes (for a review, see reference 48). The signals which regulate the activity of the kinase and its target proteins are unknown. Two other cytoplasmic factors have been identified upon repression mutants: the product of the *GRR1/CAT80* gene (4, 18, 21, 43), which apparently affects glucose metabolism and several other cellular functions and cell morphology, and gene *HXK2*, which encodes the major hexokinase isoenzyme PII in *S. cerevisiae* (22). It has been suggested that hexokinase PII has a regulatory role in sugar metabolism in addition to its metabolic role (17). However, in a detailed mutant analysis of the *HXK2* gene, Ma et al. (35) could not verify distinct domains for catalytic versus regulatory functions of the hexokinase PII protein, and Walsh et al. (50) showed that the defect in hexokinase PII catalytic function has little effect on the glycolytic flux. Thus, it remains to be elucidated in which way sugar phosphorylation triggers catabolite repression or how hexokinase PII acts as an initial sensor for repressing glucose levels.

These findings do not rule out the possibility that glucose repression is in some way connected to the glycolytic flux or to sugar uptake. The latter determinant of glucose utilization in *S. cerevisiae* poses specific genetic problems because glucose transporter genes apparently are repetitive (see, e.g., references 31 and 33). To define the regulatory functions required for glucose utilization and repression genetically, we isolated suppressors of *Δtpi1* mutations (lacking triosephosphate isomerase) in which the strong inhibitory effects of glucose are partly suppressed. Many of these mutants were indeed deficient in glucose uptake. We report here on the predominant mutant type, *HTR1*, obtained with this approach and discuss implications of these findings on the glucose effect in *S. cerevisiae*.

MATERIALS AND METHODS

Strains, media, and growth conditions. The wild-type strains used in this study were MC971A (*MATa ura3-52 his3-11,15 MAL GAL SUC MEL*) and a set of congenic strains constructed by one-step gene replacements (see below). A closely related strain, MC992B (*MATα ura3-52 his3-11,15 leu2-3,112 MAL GAL SUC MEL*), served as a host strain for YEp13-based plasmids. Standard yeast genetic techniques for mating, sporulation, and tetrad analysis were used as previously described (45). Yeast cells were

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grown at 30°C on YEP medium (2% Difco Bacto Peptone, 1% Difco yeast extract) that contained 2% glucose (YEPD), maltose, galactose, or raffinose. Nutritional requirements were scored on medium consisting of 2% glucose or maltose and 0.67% Difco yeast nitrogen base (YNB) supplemented with the appropriate amino acids, adenine and uracil. Antimycin A (final concentration, 10 μM) was used as a respiration inhibitor. Resistance to 2-deoxyglucose (2DG) was scored on YEPD plates containing 0.25% 2DG (Sigma, St. Louis, Mo.). Plasmid transformations into yeast cells were carried out by the freeze method (15). *Escherichia coli* DH5α/F′ was transformed by the rubidium chloride method (29).

Plasmids and constructions of disruption mutants. Plasmid pTPI1 contains the deletion construct Δ*tpi1*::*HIS3* in vector pUC19 and was kindly provided by J. Dohmen (Massachusetts Institute of Technology, Boston). This plasmid was used for disruption of the *TPI1* gene by transformation of diploid wild-type cells by a *Sna*BI-*Eco*RI fragment to histidine prototrophy. Haploid Δ*tpi1*::*HIS3* strains were obtained by sporulation and tetrad analysis. Plasmid pSO31 contains the *SNF3* gene on a 3.4-kb *Sal*I-*Eco*RI fragment (42), and pSO3 contains the 4.2-kb *Hin*dIII fragment of gene *HXT1*. pSO59 contains the 0.4-kb *Hin*dIII-*Xho*I fragment, and pAHT1 contains the 0.8-kb *Hin*dIII fragment encompassing the 5′ noncoding regions of genes *HXT3* and *HXT4*, respectively (31). The *HXT* clones were isolated as multicopy suppressors of the *HTR1* mutation by using a YEp13-based library (39; see Result). All fragments were cloned into the multicloning site of shuttle vector YEp352 (30). Plasmid pSO21 contains the *SNF3* *Sal*I-*Eco*RI fragment cloned in vector pT7/T3 18U (Pharmacia, Uppsala, Sweden) and was used for disruption of the *SNF3* gene. The 1.4-kb *Bam*HI-*Bgl*II fragment within *SNF3* was replaced by a 1.8-kb *Bam*HI fragment containing the *HIS3* sequence. The *Eco*RI-*Sal*I fragment of this construct was used to disrupt the chromosomal locus by transformation of MC971A to histidine prototrophy. For constructions of *SNF3*::*lacZ* fusions, a 3.4-kb *Sal*I-*Eco*RI fragment from plasmid pSO31 containing the *SNF3* gene was transferred into *Sal*I-*Eco*RI-cleaved *lacZ* fusion vectors YEp357R and YIp357R (38). The multicopy fusion construct was identical to that reported by Marshall-Carlson et al. (36) and Celenza et al. (11). Single-copy integration of the YIp357R vector was verified by Southern analysis of transformants.

DNA and RNA isolation, electrophoresis, and hybridization. Plasmid DNA from *E. coli* was prepared as described by Birnboim and Doly (7). Total yeast DNA or RNA was isolated from *S. cerevisiae* as previously described (32, 45). For Northern (RNA) blot analysis, poly(A)$^+$ RNA was separated on 1% agarose gels containing formaldehyde. DNA probes were labelled by random priming (20).

Fermentation studies and determination of glycolytic metabolites. Sugar consumption rates were determined in steady-state batch cultures, i.e., at low cell densities (1×10^7 to 5×10^7 to cells per ml). Cells were pregrown on maltose and transferred to YEP or YNB media with different concentrations of glucose. Samples were removed at intervals of 30 min. Enzymatic methods for glucose and ethanol measurement were applied as described previously (5). Determination of glycolytic metabolites was done in accordance with reference 13. Fructose 2,6-bisphosphate was assayed as described in reference 49.

Preparation of crude extracts and enzyme assays. Yeast cells were broken with glass beads in accordance with reference 12. Invertase activity was measured as described by Goldstein and Lampen (25), by using whole cells collected from mid-exponential-phase cultures. Hexokinase, maltase, and cytochrome oxidase activities were assayed in crude extracts from mid-exponential-phase cells as described previously (47, 52). For determination of β-galactosidase activity as described by Guarente (28), crude extracts were fractionated by centrifugation for 10 min at 10,000 × *g*. The supernatant and pellet fractions were used for this assay. Protein was determined by the microbiuret method (51) with bovine serum albumin as the standard. The Bio-Rad protein assay kit was used for determination of protein concentrations in the 10,000 × *g* pellet fractions.

Glucose uptake assays. For glucose transport assays, cells were pregrown on 2% maltose medium, harvested, washed, split into two equal portions, and suspended in medium containing either 2 or 0.05% glucose. After incubation at 30°C for 2 h, the cultures were washed twice by centrifugation in distilled water at 3,000 × *g*. The cells were suspended in distilled water (40 mg/ml), and the assay was started by addition of 100-μl cell suspensions to 100-μl samples of labelled sugar (D-[U-^{14}C]glucose; Amersham, Amersham, United Kingdom) at room temperature. Samples (100 μl) were removed after 5 s, and transport was stopped by dilution in 10 ml of ice-cold water. The cells were immediately collected on glass fiber filters (2.4-cm diameter; Whatman, Kent, United Kingdom), washed once with 10 ml of ice-cold water, and transferred to scintillation vials (Quicksafe A; Zinsser Analytic, Frankfurt, Germany). A 10-μl portion of the reaction mixture was transferred to a separate vial and used as a reference for determination of total radioactivity. Substrate concentrations were 0.2 to 100 mM.

RESULTS

Isolation and genetic characterization of Δ*tpi1* suppressor mutants. Yeast mutants deficient in any of the glycolytic reactions are strongly inhibited for growth on fermentable sugars (e.g., see references 13 and 14). This phenotype may result from a number of physiological effects caused by the given block, e.g., accumulation of sugar and sugar phosphates, and low ATP levels. This phenotype has been used for selection of suppressors, i.e., of a Δ*pgi1* strain (1). These revertants have partly overcome the inhibitory effect of extracellular sugar. We chose a Δ*tpi1*::*HIS3* mutant (deficient in triosephosphate isomerase; 2, 13) for this purpose. By contrast to other reactions of the glycolytic pathway, a deficiency in triosephosphate isomerase would not completely block glycolytic sugar utilization and therefore might allow selection of revertants which had reduced levels of glucose transport or metabolism. A detailed analysis of the physiology of Δ*tpi1* cells on fermentable sugars revealed that glucose at concentrations higher than 0.1% was inhibitory (doubling times, >15 h), while growth on 2% raffinose was comparable to growth on nonfermentable carbon sources (ethanol-glycerol; raffinose is hydrolyzed extracellularly by invertase, thereby providing low extracellular levels of monosaccharides). Further, we noticed that glucose metabolism in Δ*tpi1* cells is reduced to approximately 5 to 10% of the wild-type rate. Only traces of ethanol, but unusually high levels of acetate, were found as extracellular products. Acetate accounted for roughly 10% of the glucose carbon and may contribute substantially to growth inhibition by glucose.

From a total of 5×10^7 cells, we selected on YEP–2% glucose 31 spontaneous isolates which were to some degree resistant to the inhibitory effects of glucose. We thought that

TABLE 1. Phenotypes of Δ*tpi1* suppressor mutants[a]

Complementation group	No. of isolates	Growth[b] on:			
		Glucose	Raffinose	Maltose	2DG + glucose
Wild type		+	+	+	−
HTR1	12	±	−	+	+
grr1/cat80	2	±	−	+	+
pgk1	2	−	−	−	±
grr2	1	±	−	+	−
hex2/reg1	1	±	±	−	+

[a] Growth was monitored on YEP medium containing 2% sugar in the presence of antimycin A (10 μM). 2DG (0.25%) was used in combination with 2% glucose (without antimycin A).
[b] Phenotypes refer to strains wild type for *TPI1*. +, normal growth; ±, reduced growth; −, no growth.

TABLE 2. Glucose consumption rate (V_{Glc}), ethanol yield (Y_{EtOH}), and doubling time (t_d) in wild-type and *HTR1-23* mutant cells[a]

Strain	V_{Glc}[b]	Y_{EtOH}[c]	t_d (h)
Wild type	1.55	0.47	1.6
HTR1-23	1.04	0.46	3.1

[a] Each culture was pregrown to the mid-log phase on rich medium containing 2% maltose and then transferred to YEP medium containing 2% glucose. Initial cell densities were 0.5×10^7 to 1.0×10^7/ml. The results shown are averages of duplicate determinations.
[b] Calculation of V_{Glc} (in grams per hour per gram of dry weight) was based on the decrease in glucose concentration from 2% to approximately 1%.
[c] Expressed as grams of ethanol per gram of glucose.

this might result from impaired entry of glucose, reduced sugar phosphorylation, or other regulatory effects which relieve the toxic effects of glucose. We further postulated that such mutations may negatively interfere with glucose metabolism in a *TPI1* wild-type cell. Thus, further characterization was carried out in a congenic *TPI1* wild-type background. With 18 isolates, a more or less strong effect on growth on fermentable sugars was observed. These mutants were further classified by resistance to 0.25% 2DG on YEPD plates. 2DG resistance suggests reduced glucose uptake or phosphorylation (6). By standard genetic methods, these 18 isolates could be attributed to mutations in five different genes (Table 1). Surprisingly, we obtained mutants previously described in screens for glucose repression mutants: two mutants were allelic to *grr1/cat80* (4, 18); one mutant was allelic to *hex2/reg1* (18, 37). Two isolates carried leaky *pgk1* (phosphoglycerate kinase) mutations. This was concluded from the lack of phosphoglycerate kinase activity in crude extracts and from mapping data for one mutation which was localized on chromosome III, tightly linked to *LEU2* and *MAT*. The predominant mutant type (12 of 18 isolates), designated *HTR1* for hexose transport regulation, was dominant. Allelism of these isolates was verified by crossing one isolate (*HTR1-23*) to all others and subsequent analysis of at least seven tetrads from each cross. Allelism was indicated by exclusively parental segregation for the slow-growth phenotype on YEP-raffinose without antimycin. Furthermore, extensive tetrad analysis of wild-type × mutant diploids revealed exclusively 2:2 segregation, demonstrating that all *HTR1* mutations affect a single gene. As will be shown below, *HTR1* mutants are deficient in glucose uptake. A quite similar effect was observed in recessive *grr1* mutations (43).

Sugar utilization and glucose repression in *HTR1* mutants. Growth of *HTR1* mutants on glucose and, in particular, on raffinose was strongly reduced. There was some allele-specific variation in this phenotype; the following experiments were carried out on one representative mutant (*HTR1-23*). Table 2 shows some important features of this isolate: the glucose utilization rate was reduced by 30%, while ethanol yield as a measure of fermentative metabolism, was not significantly different from that of the wild type; the generation time (t_d) was nearly doubled. By contrast, on 2% maltose there was no significant difference in these features compared with wild-type cells. We next determined whether this reduced glucose turnover is somehow reflected by the levels of glycolytic metabolites (Fig. 1). Most remarkably, *HTR1-23* cells on glucose did not accumulate fructose 1,6-bisphosphate, while the levels of other sugar phosphates or pyruvate were only slightly affected. In vivo activities of phosphofructokinase (PFK) in wild-type versus *HTR1-23* mutant cells were, however, not significantly different. Likewise, glucose-induced accumulation of the PFK effector fructose 2,6-bisphosphate (3) was not very different in *HTR1-23* cells (6 pmol/mg [dry weight]) from that of wild-type cells (9 pmol/mg [dry weight]). These findings do not rule out the possibility that PFK activity in *HTR1-23* is down-regulated in vivo to some extent by other, unknown effectors. Also, the kinetics of cyclic AMP levels were monitored. We found no interference of the *HTR1* mutation with the cyclic AMP signal (data [not shown] kindly provided by J. M. Thevelein).

HTR1 mutants uniformly showed fully derepressed invertase levels on 2% glucose (Table 3). Likewise, maltose-induced α-glucosidase (maltase) activity was not repressible by glucose. As a glucose-sensitive mitochondrial activity, we determined cytochrome oxidase activity. The degree of repression of this enzyme in wild-type cells is usually much smaller than for, e.g., invertase. Nevertheless, we observed a more-than-threefold increase in cytochrome oxidase activity in *HTR1-23* mutant cells. Thus, the mutation also effects glucose repression of mitochondrial functions. By contrast, glucose repression of alcohol dehydrogenase II was not affected in *HTR1* mutants (results not shown). In summary,

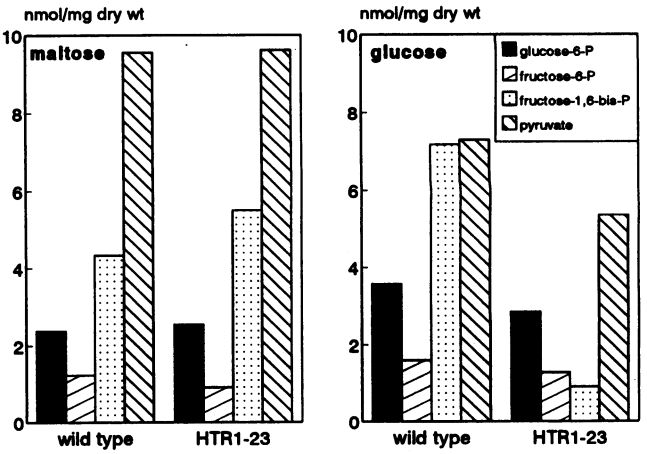

FIG. 1. Effects of glucose and maltose on intracellular concentrations of some glycolytic metabolites in wild-type and *HTR1-23* mutant cells. Extraction of metabolites was carried out after a 2-h incubation in 2% glucose- or 2% maltose-containing YEP medium at a density of 2×10^7 to 4×10^7 cells per ml.

TABLE 3. Specific activities of glucose-repressible enzymes in wild-type and *HTR1* mutant cells[a]

Strain	Invertase sp act (nmol/min/mg of dry wt)		Maltase sp act (μmol/min/mg of protein)		Cytochrome oxidase sp act (nmol/min/mg of protein)	
	R	D	R	D	R	D
Wild type	<0.5	118	0.03	1.5	12	67
HTR1-23	135	103	1.3	1.6	40	66
HTR1-23/+[b]	124	94	1.4	1.6	29	59

[a] Repressed (R) enzyme levels were assayed after growth on rich medium containing 4% glucose. For induction of maltase, 1% maltose was added. For determination of derepressed (D) enzyme levels, cells were pregrown on 2% maltose, washed, and suspended in 0.05% glucose or 2% maltose and incubated for another 3 h. Mean values of duplicate determinations are shown. Standard errors were <20%.

[b] Diploid cells heterozygous for *HTR1*.

HTR1 mutants belong to a class of repression mutants that affect the expression of a subset of glucose-sensitive functions. The dominance of *HTR1* mutants was not only shown by growth defects on glucose and raffinose but was also reflected in the activities of glucose-repressible enzymes (Table 3, last line).

***HTR1* mutants are deficient in glucose uptake.** Resistance to 2DG on glucose and the glucose-specific reduction in metabolic activity suggested that *HTR1* mutants are in some way affected in glucose uptake or phosphorylation. Glucose uptake in *S. cerevisiae* is dependent on two systems that differ in affinity for the sugar (8). The high-affinity system has been analyzed genetically in some detail (11, 33, 34). This type of transport system is required for growth at low extracellular sugar concentrations and is dependent on the expression of genes *SNF3* and *HXT1-HXT4*, which encode glucose transporters (9, 31). *HTR1-23* cells pregrown on 2% glucose showed a decrease in the affinity for glucose, as shown by the slope of the Eadie-Hofstee plot (Fig. 2A). At the highest glucose concentration tested (100 mM), we reproducibly found a 30% reduction in transport velocity relative to wild-type cells. When the transport kinetics of glucose-derepressed cells (pregrown on 0.05% glucose) were examined, the effect of the *HTR1-23* mutation was dramatic (Fig. 2B). Obviously, the high-affinity moiety of glucose uptake is not expressed in *HTR1-23* mutant cells. Thus, high- and low-affinity glucose transport is defective in *HTR1* mutants.

We wished to know whether this high-affinity transport system in our strain is dependent on *SNF3* function. In fact, Δ*snf3*::*HIS3* mutants in this strain background did not show high-affinity uptake and resembled *HTR1-23* cells at a low glucose concentration (Fig. 2B). Δ*snf3*::*HIS3* cells grown at a high glucose concentration, however, showed transport kinetics indistinguishable from those of wild-type cells. Together with the finding that Δ*snf3* mutant cells do not grow on raffinose, these data on the high-affinity uptake system in this genetic background agree with previous reports on *SNF3* function (9, 11). It appeared that *HTR1* mutant cells lack the high-affinity component of glucose uptake and that in cells grown at a high glucose concentration, the apparent affinity for glucose is lowered. This deficiency in transport also became manifest when glucose utilization rates (V_{Glc}) were determined with respect to the extracellular sugar concentration. The data depicted in Fig. 3 show that the reduction in V_{Glc} in mutant versus wild-type cells was much

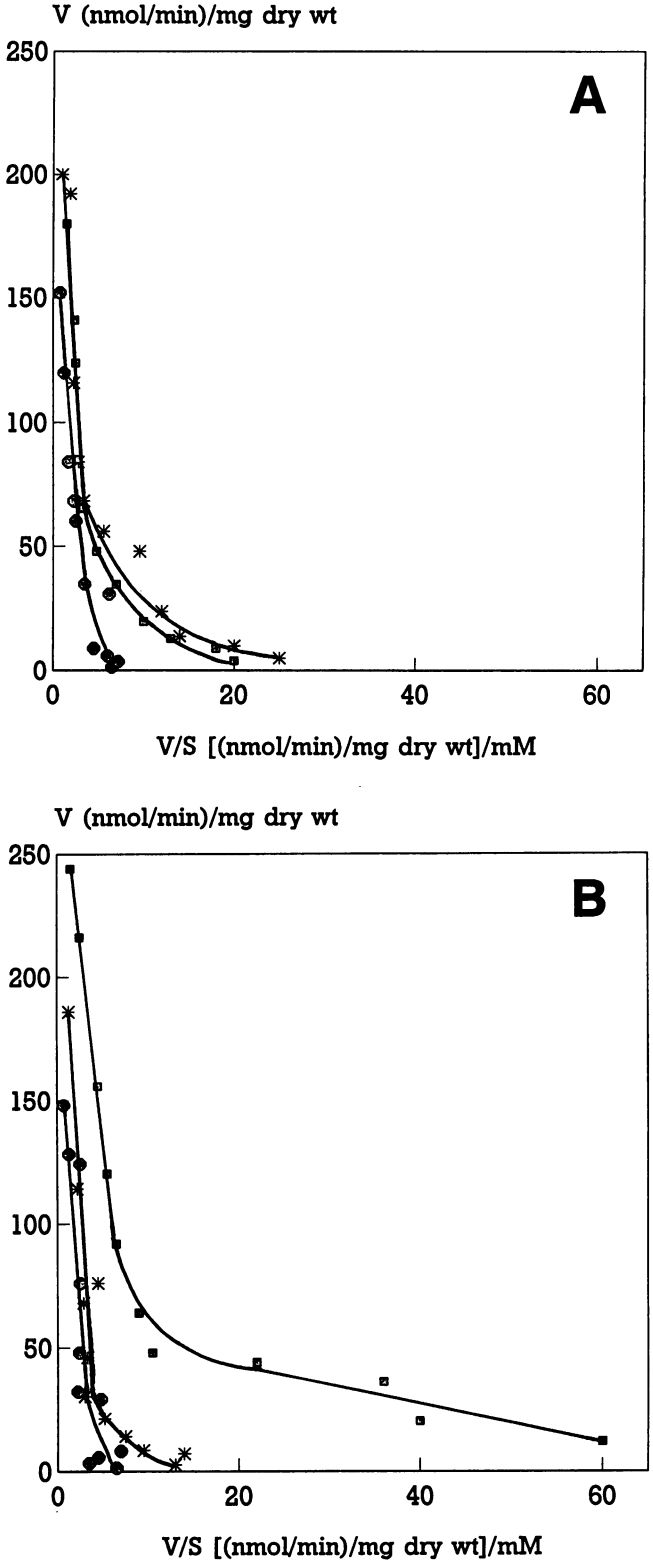

FIG. 2. Eadie-Hofstee plots of glucose uptake in wild-type (■), *HTR1-23* (●), and *snf3* (*) mutant strains grown at a high (A) or low (B) glucose concentration. Cells were pregrown to the early logarithmic phase in YEP–2% maltose medium, harvested, washed, and suspended in medium containing either a high (2%) or a low (0.05%) glucose concentration. After incubation for another 2-h period, uptake kinetics were monitored as described in Materials and Methods.

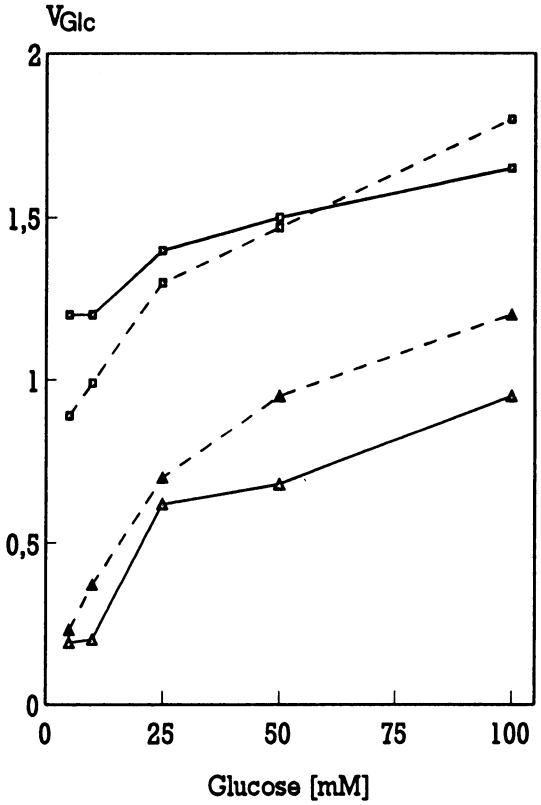

FIG. 3. Glucose utilization as a function of extracellular sugar concentration in wild-type (□) and *HTR1-23* mutant (△) cells. The glucose consumption rate V_{Glc} (solid line) is given as grams per hour per gram of dry weight. Growth conditions were as those described in Table 2, footnote *a*. Calculations of V_{Glc} were based on duplicate samples taken at 30-min intervals. Glucose transport data (dashed line) were taken from Fig. 2.

stronger at a low glucose concentration (e.g., at 5 mM it was 8-fold) than at a high glucose concentration (at 100 mM it was 1.6-fold). Remarkably, the kinetics of V_{Glc} in both mutant and wild-type cells were largely identical to the glucose uptake kinetics. This implies that the reduced V_{Glc} of *HTR1* mutant cells is a direct result of the *HTR1*-mediated uptake defect.

Glucose transporter expression in *HTR1* mutants. The apparent deficiency in glucose uptake could indicate that *HTR1* mutations affect the expression of genes that encode glucose transporters. This was examined by Northern blot analysis of the *SNF3* transcript. As evident from Fig. 4, *SNF3* mRNA was present at normal levels in derepressed *HTR1-23* mutant cells. We next monitored *SNF3* expression at the protein level. We took advantage of the fact that SNF3::lacZ fusion proteins are correctly inserted in the plasma membrane (36). Such fusions appear to function as glucose transporters since they can complement the *snf3* defect. By using a *SNF3*::*lacZ* construct on a single-copy vector, we found only a slight decrease in β-galactosidase activity in *HTR1-23* relative to that in wild-type cells. However, this could hardly explain the complete absence of high-affinity transport in mutant cells (results not shown).

Recent work on another class of yeast glucose transporter genes revealed the existence of at least four genes, *HXT1* to *HXT4* (31, 33, 34). We tested the expression of several *HXT* genes by Northern analysis. As shown in Fig. 5, *HXT1* and

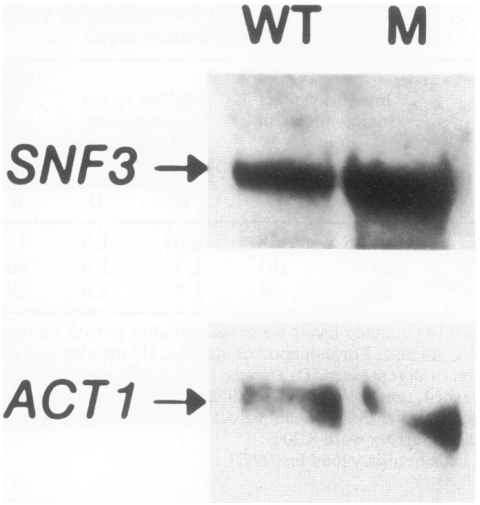

FIG. 4. Northern blot analysis of *SNF3* transcription. Wild-type (WT) and *HTR1-23* mutant (M) cells were grown to the mid-log phase under derepressing conditions. Poly(A)$^+$ RNA (15 μg per lane) was separated on a 1% agarose-formaldehyde gel and hybridized to ^{32}P-labelled probes. The blot was also probed with the actin gene (*ACT1*) as an internal control for loading.

HXT3 transcription was almost completely abolished in derepressed *HTR1* mutant cells. *HXT4* transcripts were also not detectable. However, this gene is expressed only at very low levels in wild-type cells (data not shown). Thus, the defect in glucose uptake can be attributed to impaired transcription of *HXT* genes. From these results we conclude

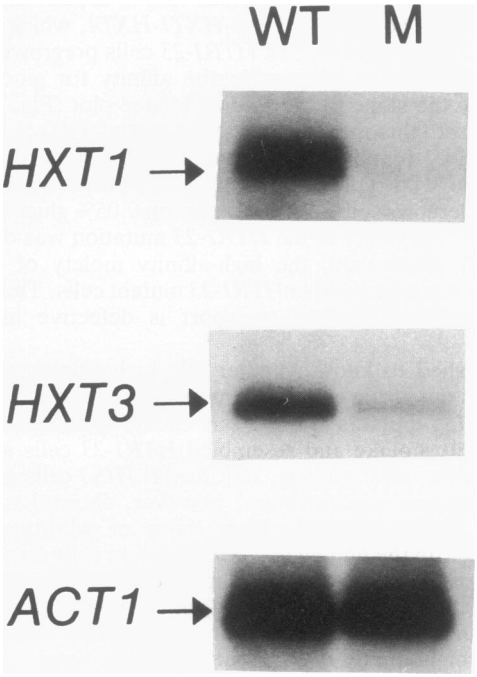

FIG. 5. Northern analysis of *HXT1* and *HXT3* transcripts in wild-type (WT) and *HTR1-23* mutant (M) cells grown on a low glucose concentration. *HXT1* probe, 2.8-kb *Eco*RI-*Hin*dIII fragment; *HXT3* probe, 1.2-kb *Hin*dIII-*Hpa*I fragment (31). The actin gene (*ACT1*) was used as an internal control for loading. A 5-μg sample of poly(A$^+$) RNA was applied per lane.

TABLE 4. Invertase activities, glucose consumption rates (V_{Glc}), and growth phenotypes on raffinose of *HTR1-23* transformants

Strain/plasmid	Invertase activity (nmol/min/mg of dry wt)	V_{Glc}[a]	Growth on raffinose + antimycin A[b]
Wild type	<0.5	1.1	+++
HTR1/YEp352[c]	112	0.7	−
HTR1/YEp352-*SNF3*	85	1.0	++
HTR1/YEp352-*HXT1*	68	ND[d]	++
HTR1/YEp352-*AHT1*	82	1.0	+++
HTR1/pSO59	94	1.1	+++

[a] V_{Glc} was determined in YNB–2% glucose cultures pregrown on maltose. Samples were removed at 30-min intervals.
[b] +++, normal growth; ++, reduced growth; −, no growth.
[c] Control: *HTR1-23* mutant transformed with multicopy vector YEp352.
[d] ND, not determined.

that the inability of *HTR1* mutants to express *HXT* genes is not caused by preventing activation but more likely results from enhanced repression of these genes.

Multicopy suppression of the *HTR1* phenotype. We tried to isolate the *HTR1* wild-type gene by standard yeast cloning techniques. This attempt failed when YCp50-based gene libraries were used, probably because of the dominant nature of *HTR1* mutations. When a multicopy YEp13-based library was used, many transformants which were wild type by growth criteria were obtained. By genetic criteria, however, none of the plasmids recovered from transformants carried the *HTR1* gene. Interestingly, one class of plasmids contained the *HXT1* gene, which was recently identified as a glucose transporter gene (34). This finding led us to examine whether the *SNF3* gene on a multicopy plasmid may phenotypically suppress the *HTR1-23* mutation. This was indeed the case; both YEp352-*SNF3* and YEp352-*HXT1* transformants of *HTR1-23* cells grew normally on glucose- or raffinose-containing media and were sensitive to 2DG (Table 4). Glucose uptake was monitored in these *SNF3* and *HXT1* transformants and in appropriate controls. As seen in Fig. 6, glucose uptake was largely restored in *SNF3* and *HXT1* transformants in comparison with that of a wild-type strain. The glucose consumption rate (V_{Glc}) in *SNF3*-transformed *HTR1-23* cells (Table 4, second column) was very similar to that of wild-type cells. By contrast, the defect in glucose repression of invertase was unaffected in these transformants (Table 4, first column). Also, there was no effect on glucose repression of invertase in congenic wild-type cells transformed with these multicopy suppressors (data not shown).

This suppressive effect of *SNF3* on a multicopy plasmid was abolished when the first half of the coding region was removed, suggesting that suppression was caused by SNF3 protein functions. Nevertheless, we obtained evidence that multicopy suppression can also be caused by a *cis* effect of sequences which interfere with expression of the *HTR1* defect. We observed such an effect for two plasmids, pSO59 and pAHT1, which contain part of the 5′ noncoding regions of genes *HXT3* (0.4-kb *Hin*dIII-*Xho*I fragment) and *HXT4* (0.8-kb *Hin*dIII fragment), respectively. Both plasmids restored growth and glucose uptake in *HTR1*, *grr1*, and *snf3* mutants. Northern blot analysis of *HTR1-23*/YEp352-*AHT1* transformants did not reveal whether the suppressive effect of the *AHT1* sequence on multicopy plasmids can be attributed to enhanced expression of a particular *HXT* gene. Preliminary data indicate that only *HXT3* expression was significantly increased, although not to wild-type levels (data not shown). The glucose repression defect was not affected in YEp352-*AHT1* and pSO59 transformants (Table 4). These findings suggest that the deficiency in glucose uptake and the resulting decrease in glycolytic flux is not responsible per se, for the *HTR1*-mediated defect in glucose repression.

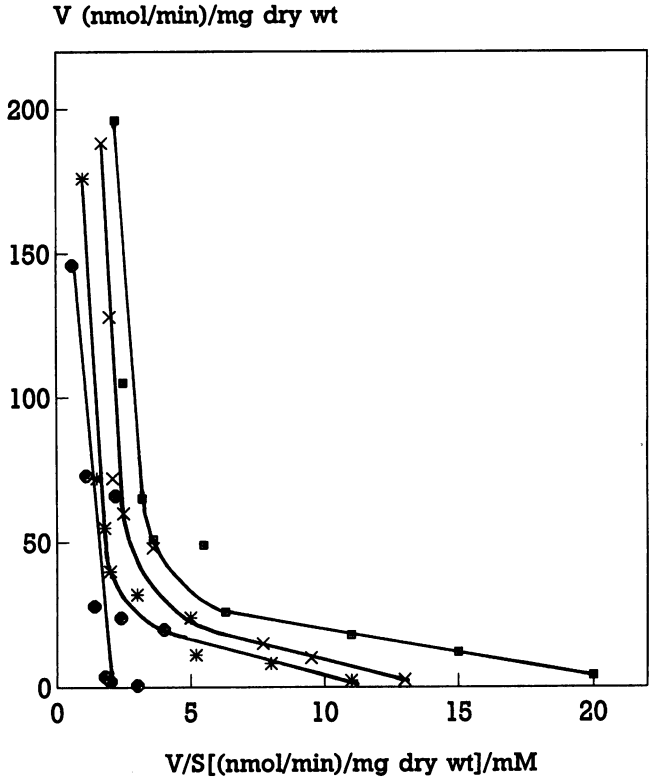

FIG. 6. Eadie-Hofstee plot of glucose uptake in the glucose-derepressed wild-type (■) strain and the *HTR1-23* mutant strain containing glucose transporter gene SNF3 (*) or *HXT1* (×) on a multicopy plasmid. Cells were pregrown in YNB–2% maltose and shifted to 0.05% glucose–YNB before uptake was assayed. ●, *HTR1-23* mutant transformed with vector YEp352.

DISCUSSION

By selecting for revertants of Δ*tpi1* cells which had overcome the strong inhibitory effect of glucose, we isolated five classes of mutants. This selection strategy was used to obtain mutants with reduced glucose uptake or other glucose-specific regulatory defects which would lead to partial relief from glucose inhibition of Δ*tpi1* cells. The preponderant, dominant *HTR1* mutation generates a number of glucose-specific metabolic and regulatory defects. Kinetic analysis of glucose transport in *HTR1* mutants revealed a significant reduction in low-affinity uptake (about 30%) and a complete lack of high-affinity transport (Fig. 2B). Analysis of glucose consumption rates with respect to the extracellular glucose concentration (Fig. 3) indicated a strong correlation between utilization and transport of glucose. At a high glucose concentration (100 mM), we found a decrease of about 30 to 40% in the glucose consumption rate, which is consistent with the observed reduction in low-affinity transport. In *HTR1* mutants, loss of high-affinity transport results in a drastic reduction of the glycolytic flux (about 90%) at low glucose concentrations (5 to 10 mM) at which the transport of glucose is mainly mediated by this system (K_m,

1 to 2 mM; reference 8). It is very likely that the reduced level of transport is responsible for the growth defects of these mutants at high glucose or raffinose concentrations. As shown for *snf3* mutants, growth on raffinose requires high-affinity uptake (9). The *SNF3* gene has been assumed to encode the major component of high-affinity transport or to be required for expression of this transport system (9, 11). These data, taken together, indicate that in *HTR1* mutant cells, sugar uptake is limiting for metabolism.

Interestingly, analysis of glycolytic metabolites showed a significant reduction in the fructose 1,6-bisphosphate level in *HTR1* cells; this might be a consequence of low PFK activity. However, this could not be verified by in vitro PFK activity in *HTR1* mutants, nor were the levels in the PFK effector fructose 2,6-bisphosphate significantly altered. The growth defect of *HTR1* cells on glucose was more pronounced in the absence of respiration (addition of antimycin A). The function of oxidative phosphorylation has a stronger effect on energy metabolism in *HTR1* mutants than in wild-type cells. Surprisingly fermentative metabolism at high glucose concentrations, as reflected in ethanol yield from glucose, was not significantly lowered. One explanation for this phenomenon is that oxidative phosphorylation in glucose-grown wild-type cells may be irrelevant for energy metabolism. Although yeast cells metabolize a small amount of glucose (2 to 5%) by respiration, it does not contribute to ATP production (24). This could be explained if one assumed uncoupling of respiration and oxidative phosphorylation in wild-type cells. In *HTR1* mutants, this uncoupling of ATP synthesis from respiration might be abolished as a corollary of impaired ATP synthesis from glycolysis. Consequently, antimycin inhibition of respiration would result in an ATP deficit and a reduced growth rate.

In *HTR1* cells, transcript levels of *HXT* genes (*HXT1*, *HXT3*, and *HXT4*) were strongly diminished (Fig. 5). This defect was probably responsible for the observed reduction in glucose uptake. It has recently been shown that deletion of all known *HXT* genes (*HXT1* to *HXT4*) leads to severe growth defects at a high glucose concentration or on raffinose (31). The latter finding suggests that high-affinity transport is also mediated by HXT transporters. Combination of *SNF3* with any single *HXT* gene seems to be sufficient for growth on raffinose, indicating some interaction between these transporters (31). As shown by Northern analysis (Fig. 4), *SNF3* transcription was not affected in derepressed *HTR1-23* mutant cells. Also, the slight decrease in the level of the SNF3::lacZ fusion protein could hardly account for the complete absence of high-affinity transport.

To clone the *HTR1* gene, we obtained multicopy suppressors of the *HTR1-23* mutation. One type of suppressor plasmid contained the *HXT1* gene, which codes for a glucose transporter (34). *HXT1* expression is about 10-fold inducible by glucose and appears to be expressed at a comparably high level (43). The *HXT1* gene expressed on multicopy plasmids complements the growth defects of *HTR1* mutants by elevating glucose transport. The same suppressive effect could be observed with the *SNF3* gene on a multicopy plasmid. Obviously, overproduction of individual transporter proteins compensates to some extent for the negative effect of the *HTR1-23* gene product on the expression of HXT transporters.

Another type of multicopy suppressor, plasmids pAHT1 and pSO59, contained only part of the 5′ noncoding sequence of genes *HXT3* and *HXT4*, respectively (31). Both plasmids were able to complement the growth defects of *HTR1*, *grr1*, and *snf3* mutants. With all three mutant types, defects in high-affinity transport and growth on glucose or raffinose were largely restored by these sequences. The finding that regulatory sequences of *HXT3* and *HXT4* genes can function as multicopy suppressors of *HTR1*, *grr1*, and *snf3* mutants by elevating the transport capacity for glucose in these mutants suggests that expression of *HXT* genes is normally regulated by a repression mechanism. These regulatory sequences can probably bind and titrate out a repressor(s) of glucose transporter genes, thus leading to enhanced transcription of those genes, although wild-type expression of *HXT1* or *HXT3* was not observed (12a). Such titration effects were also observed with *SUC4* promoter sequences (27). These findings suggest a negative role of the *HTR1* gene product in *HXT* gene expression: *HTR1* could code for a repressor of glucose transporter genes; in *HTR1* mutants this repressor is in some way overexpressed or binds with increased affinity to its target sequences. This would explain the dominant nature of *HTR1* mutations.

HTR1 mutant cells are released from glucose repression for a subset of normally glucose-repressible functions. In this regard, they resemble nonallelic repression mutants like *hex2*, *hxk2*, and *grr1/cat80* (4, 18). Allelism to various derepression mutants like *snf1/cat1*, *cat3/snf4*, *mig1*, and *cif1* has been ruled out (19, 26, 40, 41). In the course of these studies, we found that *HTR1-23 snf1* double mutants were not viable (results not shown). This dependence of *SNF1* function has recently been demonstrated by Flick and Johnston (21) for *grr1* mutants which resemble *HTR1* mutants in phenotype (43). Tentatively, this suggests that the *SNF1* gene (which encodes a protein kinase; 10) has an essential function during glucose growth and not only in the derepression pathway.

The derepressed phenotype of *HTR1* mutants could be an indirect corollary of the reduced glycolytic flux or of low levels of glycolytic intermediates (especially fructose-1,6-bisphosphate) or could reflect an unknown role of sugar transport in triggering glucose repression. It is not very likely that fructose-1,6-bisphosphate levels play a major role in this context, since *pgi1* mutations (deficient in phosphoglucoisomerase) do not affect invertase repression (44). Likewise, *pfk1* or *pfk2* single mutants have very low levels of fructose-1,6-bisphosphate at a rather normal glycolytic flux but are not affected in invertase repression (29a). We noted also that invertase is derepressed in maltose-grown cells which contain rather high fructose-1,6-bisphosphate levels (cf. Table 3 and Fig. 1). The role of the glycolytic flux on invertase expression has been investigated by Walsh et al. (50), who used defined mutations that affect glucose phosphorylation. From their results one may conclude that glucose repression can hardly be attributed to flux changes, although effects caused by subtle changes cannot be excluded.

An important signal for glucose repression in *S. cerevisiae* is mediated by hexokinase PII and, to a lesser extent, hexokinase PI (44). Since hexose phosphorylation in vivo is reduced in *HTR1* mutants, one might assume that the glucose repression defect is caused by reduced in vivo hexokinase activity. However, *HTR1-23* multicopy transformants show normal phosphorylation activity, as determined from the flux rates (cf. Table 4), but are still defective in repression. This suggests that phosphorylation activity in vivo is not the only determinant for glucose repression. Generation of a signal may be dependent on specific interactions between hexokinase PII and specific HXT transporters which are not sufficiently expressed in *HTR1* mutants or in *HTR1* transformants carrying multicopy suppressors.

Thus, a systematic analysis of the repetitive hexose transporter genes in *S. cerevisiae* (31) would be significant in elucidating the role of glucose uptake in generating signals for glucose repression and other regulatory effects exerted by glucose on growth and metabolism.

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