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The *HXT1* Gene Product of *Saccharomyces cerevisiae* Is a New Member of the Family of Hexose Transporters

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Two novel genes affecting hexose transport in the yeast *Saccharomyces cerevisiae* have been identified. The gene *HXT1* (hexose transport), isolated from plasmid pSC7, was sequenced and found to encode a hydrophobic protein which is highly homologous to the large family of sugar transporter proteins from eucaryotes and procaryotes. Multicopy expression of the *HXT1* gene restored high-affinity glucose transport to the *snf3* mutant, which is deficient in a significant proportion of high-affinity glucose transport. *HXT1* was unable to complement the *snf3* growth defect in low copy number. The HXT1 protein was found to contain 12 putative membrane-spanning domains with a central hydrophilic domain and hydrophilic N- and C-terminal domains. The HXT1 protein is 69% identical to GAL2 and 66% identical to HXT2, and all three proteins were found to have a putative leucine zipper motif at a consensus location in membrane-spanning domain 2. Disruption of the *HXT1* gene resulted in loss of a portion of high-affinity glucose and mannose transport, and wild-type levels of transport required both the *HXT1* and *SNF3* genes. Unexpectedly, expression of β-galactosidase activity by using a fusion of the *lacZ* gene to the *HXT1* promoter in a multicopy plasmid was maximal during lag and early exponential phases of growth, decreasing approximately 100-fold upon further entry into exponential growth. Deletion analysis of pSC7 revealed the presence of another gene (called *ORF2*) capable of suppressing the *snf3* null mutant phenotype by restoring high-affinity glucose transport and increased low-affinity transport.

At least two types of hexose uptake systems have been described in the yeast *Saccharomyces cerevisiae*. Bisson and Fraenkel (3) defined a high-K_m (15 to 20 mM for glucose and 50 to 100 mM for fructose), low-affinity transport system that appears to be constitutive. A low-K_m (1 to 2 mM for glucose and 5 to 7 mM for fructose), high-affinity transport system that is glucose repressible and dependent upon the activities of at least one of the hexose-phosphorylating enzymes, hexokinase P1, hexokinase P2, or glucokinase, for activity has also been characterized (3, 4).

The *snf3* (sucrose nonfermenting) mutation was found to result in loss of high-affinity glucose uptake in the S288C genetic background and to confer a growth defect on raffinose and low glucose concentrations (0.05%), particularly under conditions in which respiration is inhibited (7). Other genes involved in hexose transport were isolated by cloning multicopy suppressors of the *snf3* mutation which restored growth on raffinose (5). Complementation groups included plasmids which either restored high-affinity transport (pSC7 and pSC2) or elevated low-affinity transport (pSC3 and pSC8). Subsequent cloning and sequencing of SNF3 (8, 33) revealed the protein to be homologous to other known glucose transport proteins from mammals (GLUT) (30), the procaryotic pentose permeases for xylose (XylE) and arabinose (AraE) (26), and the yeast galactose transporter (GAL2) (40) and disaccharide transporters for maltose (MAL61) (11) and lactose (LAC12) (9).

One multicopy suppressor of the *snf3* mutation, *HXT2*, also encodes a putative glucose transporter (23). We describe here the isolation and analysis of another homolog of this family, *HXT1*. The low level and pattern of expression of the *HXT1* gene during growth are inconsistent with this protein playing a major role in sugar catabolism, and this raises the question of the true physiological function of the *HXT1* gene product.

MATERIALS AND METHODS

Materials. The Sequenase version 2.0 sequencing kit was from U.S. Biochemical Corp. (Cleveland, Ohio). The T7 and T3 sequencing primers were from Stratagene Cloning Systems (La Jolla, Calif.). Nytran filters were from Schleicher & Schuell, Inc. (Keene, N.H.). Restriction endonucleases and other enzymes were from either New England BioLabs, Inc. (Beverly, Mass.), Pharmacia-LKB Biotechnology (Piscataway, N.J.), Promega Corp., (Madison, Wis.), or Boehringer Mannheim Biochemicals (Indianapolis, Ind.). The isotopes [α-^{32}P]dCTP (3,000 Ci/mmol) and [α-^{35}S]dATP (>1,000 Ci/mmol) were from Amersham Corp. (Arlington Heights, Ill.). The synthetic oligonucleotides used for sequencing were synthesized by Operon Inc. (Alameda, Calif.). Isotopes for transport assays, D-[U-^{14}C]glucose, D-[U-^{14}C]fructose, D-[6-^{3}H(N)]galactose, and D-[2-^{3}H]mannose, were from Dupont, NEN Research Products (Boston, Mass.).

Strains, genetic methods, and media. *Escherichia coli* DH5α was used throughout, with plasmid transformations performed by the calcium chloride method (27). Either Luria broth or TB broth was used for the culturing of *E. coli* and was supplemented with ampicillin for the selection of transformants as described previously (27).

All MCY, LBY, and YPH strains of *S. cerevisiae* are of S288C background and therefore isogenic (Table 1). Alleles of *snf3* have previously been described (5, 7). Yeast transformation was done by the lithium acetate method (22). Yeast cells were grown in either YP medium containing 1% yeast extract (Difco Laboratories) and 2% Bacto-Peptone (Difco) or minimal medium (YNB) containing 0.67% yeast nitrogen base (Difco), the appropriate nitrogen source, and

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TABLE 1. Yeast strains

Strain	Genotype	Source or reference
DFY1-1C	*MATα lys1 ura3-52 SUC MAL MEL GAL*	F. Sherman
MCY1407	*MATa snf3-Δ4::HIS3 Δhis3 ura3-52 lys2-801 gal2*	M. Carlson
LBY405	*MATa/MATα snf3-Δ4::HIS3/snf3-Δ4::HIS3 ura3-52/ura3-52 lys2-801/lys2-801 ade2-100/ade2-100 trp1-Δ63/trp1-Δ63 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1*	23
LBY403	*MATa snf3-Δ4::HIS3 ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	23
LBY202	*MATa hxt1-Δ1::URA3 ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	This work
LBY203	*MATa hxt1-Δ1::URA3 snf3-Δ4::HIS3 ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	This work
X2180-1A	*MATa SUC2 mal mel gal2 CUP1*	R. K. Mortimer
YPH501	*MATa/MATα ura3-52/ura3-5 lys2-801/lys2-801 ade2-100/ade2-100 trp1-Δ63/trp1-Δ63 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1*	37
YPH500	*MATα ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	37
YPH499	*MATa ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	37
YP80 (YNN295)	*MATα ura3-52 lys2 ade1 ade2 trp1-Δ1 his7*	Yeast Genetics Stock Center

the specified carbon source. Yeast mating, sporulation, and tetrad analysis were performed by standard procedures (36).

Plasmids. Plasmids used in this study were either *S. cerevisiae-E. coli* shuttle vectors YEp351, YEp352 (20), pUN75, and pUN85 (14) or the *E. coli* plasmids pUC18 and -19 and M13mp18 and -19 (29, 43). Isolation of the YEp24-based clone pSC7 has been described (5). Plasmids pDL306 and pDL307 (opposite orientation of pDL306) were constructed by excision of the 3.2-kb *Hin*dIII-*Dra*I fragment of pSC7 (Fig. 1) and ligation of the Klenow-repaired fragment into the *Sma*I site of YEp352. The 3.2-kb *Hin*dIII-*Dra*I fragment was also cloned into pUN85 (to give pDL406), a centromere-based plasmid (not shown). Plasmid pDL304 was constructed by excision of the 4.2-kb *Hin*dIII fragment of pSC7 and ligation into the *Hin*dIII site of YEp352; the same *Hin*dIII fragment was ligated into the *Hin*dIII site of pUN75 in two orientations to generate pDL400 and -401. The 5.7-kb *Eco*RI fragment of pSC7 was ligated into the *Eco*RI site of YEp352 to give pDL302 (not shown). Since the *Eco*RI site is at the starting ATG of the *HXT1* gene, this

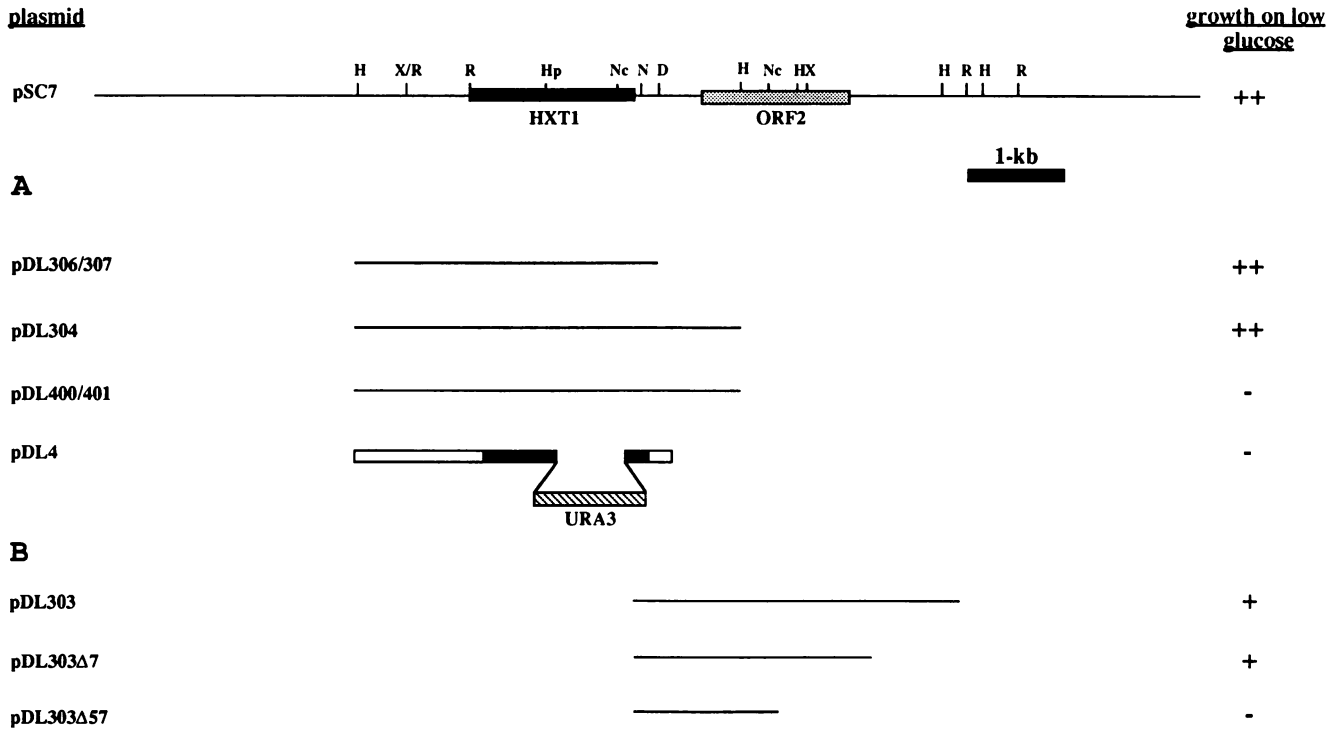


FIG. 1. Complementation of MCY1407 by subclones of pSC7. Plasmids were tested for the ability to complement MCY1407 on 0.05% glucose with 1 μg of antimycin A per ml. (A) Subclones defining *HXT1*; (B) subclones defining *ORF2*. Horizontal lines indicate DNA fragments. Arrows indicate direction of open reading frames. All plasmids are multicopy except pDL400 and pDL401, which are centromeric (see text). The black box indicates the open reading frame of *HXT1*; the hatched box indicates the assigned functional region for *ORF2*; the striped box indicates the *URA3 Hin*dIII fragment; the open box indicates the *HXT1* noncoding DNA. Abbreviations: D, *Dra*I; H, *Hin*dIII; Hp, *Hpa*I; N, *Nar*I; Nc, *Nco*I; R, *Eco*RI; X, *Xba*I. Colony sizes: ++, 2 mm; +, 1 mm; −, no growth.

construct contains no upstream regulatory region for expression of *HXT1*, while the *ORF2* region is intact. Plasmid pDL303 is the minimal *snf3*-complementing exonuclease III (ExoIII) deletant of pDL302.

Deletion and complementation analysis of pSC7 and DNA sequencing. Complementation of the *snf3* growth defect by subclones was tested on low-glucose (0.05%) medium with antimycin A (1 μg/ml). Plasmid pDL302 was used to construct nested ExoIII deletions (19) to map the minimal region necessary to complement the *snf3* null strain and also to sequence in part the sense strand of *HXT1*. A minimal complementing deletant from pDL302 (pDL302Δ6a), which had remaining the *Nar*I-to-3′ *Eco*RI site, designated pDL303, was used to obtain deletants from the 3′ direction of *ORF2* (pDL303Δ7 and pDL303Δ57 in Fig. 1). The 750-bp *Hin*dIII fragment in pDL303 as well as the 1.5-kb *Hin*dIII fragment 3′ of the *Xba*I site were sequenced from either end in the M13mp18 vector, using single-stranded sequencing (29). Both ExoIII deletants and pDL406 were used to sequence *HXT1*, using primers and double-stranded templates (10), by the dideoxy method (35, 41). Nucleic acid and protein sequence data were analyzed by using the University of Wisconsin Genetics Computer Group (GCG) software, version 6.1 (13).

DNA isolation, mapping, and hybridization techniques. Plasmid DNA was prepared and purified on cesium chloride gradients as described previously (27). Small-scale DNA from *E. coli* was prepared by the rapid boiling method (41). Genomic DNA from *S. cerevisiae* was isolated by the glass bead method (21). The molecular biological techniques used have been described (27). Southern blot hybridization analysis (39) was performed as recommended by Schleicher & Schuell. Yeast chromosomes from the tester strain YP80 were separated by pulsed-field gel electrophoresis on the Hexi-CHEF 6,000 system (C.B.S. Scientific Co., Del Mar, Calif.) as suggested by the manufacturer with minor modifications and probed with the *Eco*RI-*Xba*I *HXT1* fragment from pDL302 under high-stringency conditions.

Disruption of *HXT1*. The *Hin*dIII-*Dra*I fragment of pSC7 (which contained the entire *HXT1* coding region, including 1.1 kb 5′ and 0.3 kb 3′) was ligated into the *Sma*I site of pUC19, followed by excision of the 0.734-kb *Hpa*I-*Nco*I fragment, Klenow repair, and insertion of the *URA3* gene (Klenow-repaired 1.166-kb *Hin*dIII fragment of YEp24) to give pDL4 (Fig. 1A). The *Eco*RI-*Sal*I fragment of pDL4 was used to disrupt the chromosomal locus by transformation of YPH501 and LBY405 strains to uracil prototrophy (34). Genomic DNA was prepared from diploid transformants and from all four spore progeny of four randomly selected tetrads and digested with diagnostic enzymes, and null strains were confirmed by high-stringency Southern analysis (39) using the 2.8-kb *Hin*dIII-*Nco*I *HXT1* fragment from pDL306 and the 1.166-kb *Hin*dIII *URA3* fragment from YEp24 as probes (15).

Growth and hexose transport assays. The zero *trans* influx of radioactively labeled D-[U-^{14}C]glucose, D-[U-^{14}C]fructose, D-[6-^{3}H(N)]galactose, and D-[2-^{3}H]mannose by yeast cells was determined as previously described (3) and monitored with a Packard Tri-Carb 2000CA scintillation counter with Optifluor scintillation cocktail. Glucose concentrations of duplicate culture supernatants were measured in triplicate with a Yellow Springs Instruments model 27 glucose analyzer. Cells containing plasmids were grown on yeast nitrogen base (0.67%) supplemented with the appropriate amino acids to maintain plasmid selection with 2% hexose. Medium shifts were performed by dilution of overnight cultures (A_{580} = 9 to 10) to 10^{-3} in 100 ml of fresh medium; cells were grown to an A_{580} of 1, harvested by filtration, and washed in fresh medium lacking sugar, and the filtrant was split into two equal portions and resuspended in medium containing either 0.05 or 2% hexose. For strains without plasmids, cultures were grown as described above on YP medium with 2% hexose to either late log phase (A_{580} = 6, <0.1% glucose) or early log phase (A_{580} = 1.5 to 2) for the medium shift to either 0.05 or 2% hexose.

Construction of a *HXT1* promoter-*lacZ* fusion vector for expression of β-galactosidase. The *HXT1* regulatory region and first codon of the *HXT1* open reading frame were fused in frame into the yeast multicopy *lacZ* fusion shuttle vector YEp357R (32). The *Hin*dIII-*Dra*I *HXT1* fragment was ligated into the *Hin*dIII site of the YEp357R polylinker, partially digested with *Eco*RI, and religated to generate the in-frame fusion (pDL502) of the *HXT1* regulatory sequences (1.1 kb 5′ of the putative start codon of HXT1) with the *lacZ* gene. The pDL502 construct was verified by sequencing. The β-galactosidase activity of crude yeast cell extracts was determined by measurement of *o*-nitrophenyl-β-D-galactoside (ONPG) hydrolysis as described previously (18, 31); initial rates were determined by using a Perkin-Elmer 3840/7500 spectrophotometer, and activity was expressed in Miller Units.

Nucleotide sequence accession number. The *HXT1* sequence appears in the EMBL/Genbank/DDBJ nucleotide sequence data base under accession number M38709.

RESULTS

Cloning and deletion analysis of pSC7, a multicopy suppressor of the *snf3* mutant. Plasmids capable of complementing the *snf3* defect by restoring growth on raffinose were previously isolated from a multicopy plasmid genomic library (5). Subclones of pSC7 were tested for their ability to complement in multicopy the *snf3* null strain MCY1407 by restoration of growth on low glucose (0.05%) in the absence of respiration (antimycin A) (Fig. 1). Two independent regions of pSC7 were found to complement the *snf3* growth defect. Plasmid pDL306 fully complemented (Fig. 1A) but did not complement in the low-copy-number centromeric plasmids pDL400 and pDL401. Complementation was found to be direction independent (pDL306 and pDL307 are in opposite orientations), which suggested that the *HXT1* gene regulatory region was intact.

Plasmid pDL303 (Fig. 1B), which shared no overlapping regions with pDL306, also complemented the *snf3* null strain in multicopy but at a lower level, as judged from relative colony size. Complementation analysis of ExoIII deletions in pDL303 further defined the minimal region (pDL303Δ7) necessary for full *ORF2* function. Preliminary sequence analysis 3′ of *HXT1* has revealed several as yet unjoined open reading frames, all in the same direction within the 750-bp *Hin*dIII fragment. This gene was designated simply *ORF2*, pending further characterization.

Effect of complementing plasmids on the hexose uptake kinetics of the *snf3* null. The *snf3* null strain MCY1407 was transformed with multicopy plasmids containing the *HXT1* gene (pDL306), the second complementing gene *ORF2* (pDL303Δ7), and both genes on the same fragment (pSC7). Transformants were grown to mid-log phase in minimal medium with 2% glucose lacking uracil to maintain selective pressure for the plasmids and were shifted to fresh medium containing either 2 or 0.05% glucose for 3 h (two doublings). Under repressing conditions (2% glucose), all strains showed approximately the same glucose uptake kinetics, displaying

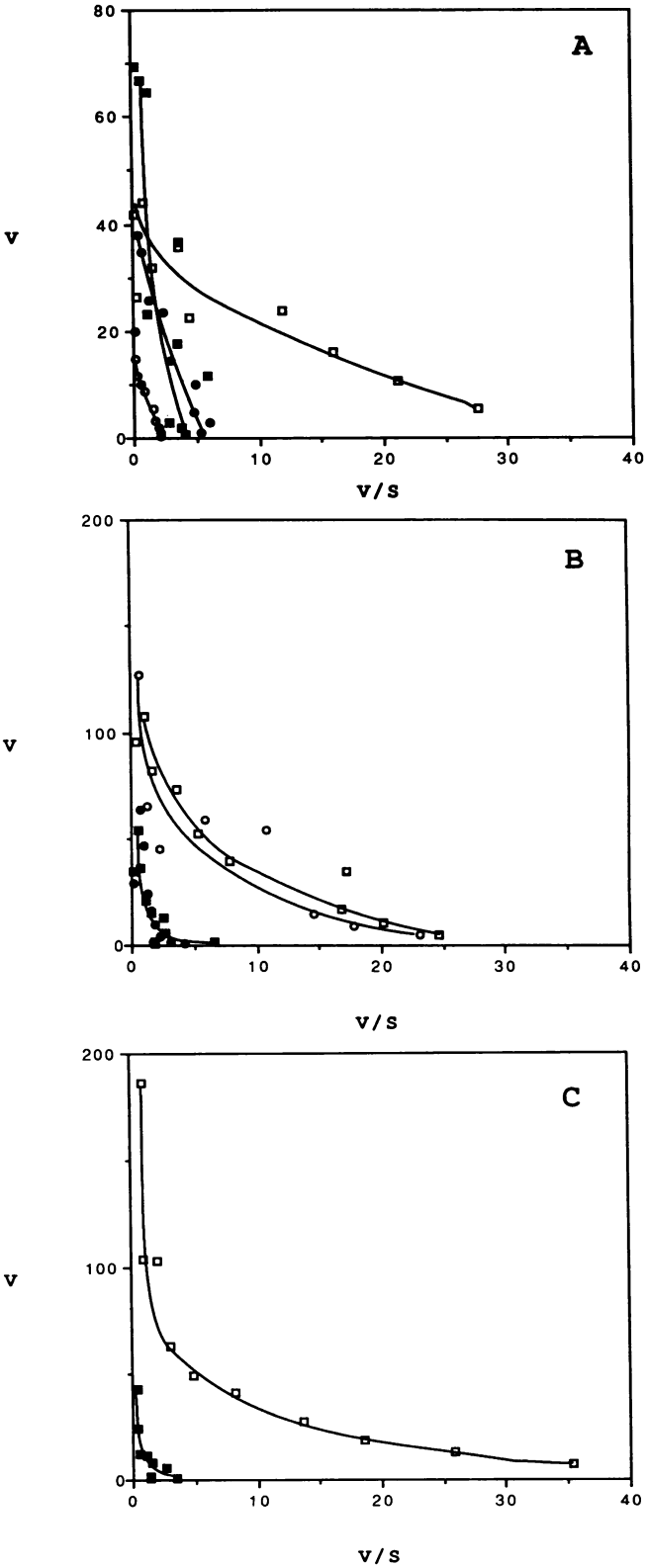


FIG. 2. Eadie-Hofstee plots of glucose uptake in the *snf3*::*HIS3* null strain containing complementing plasmids. Cells were grown in 2% glucose to early exponential phase (A_{580} = 1) and shifted to either 2% (solid symbols) or 0.05% (open symbols) glucose-containing YNB, supplementing with amino acids without uracil, for 3 h. Symbols: (A) □, YPH500, 0.05%; ■, YPH500, 2%; ○, MCY1407, 0.05%; ●, MCY1407, 2%; (B) MCY1407 with following plasmids: □, pSC7, 0.05%; ■, pSC7, 2%; ○, pDL306, 0.05%; ●, pDL306, 2%; (C) □, pDL303Δ7, 0.05%; ■, pDL303Δ7, 2%. Scales: v, nanomoles of glucose per minute per milligram (dry weight); s, millimolar. Note that axis scales are different.

low-affinity uptake and minimal-high affinity transport. The *snf3* mutant did not display high-affinity transport under derepressing conditions (0.05% glucose) (Fig. 2A). Plasmid pSC7 or pDL306 (*HXT1*) restored high-affinity transport to the *snf3* null strain under these conditions (Fig. 2B). Plasmid pDL303Δ7, carrying the minimum complementing region of *ORF2*, also showed a derepression in high-affinity transport under low-glucose conditions, and low-affinity transport also seemed to be slightly elevated (Fig. 2C). Thus, both *HXT1* and *ORF2* are capable of restoring high-affinity glucose uptake, but only *ORF2* seems to affect low-affinity uptake as well.

DNA sequence analysis of the *HXT1* gene. The nucleotide and deduced amino acid sequences of the *HXT1* gene were determined (Fig. 3). The sequence revealed a 1,707-nucleotide open reading frame predicted to encode 569 amino acids and a 64,044-Da protein. Southern analysis of YPH500 genomic DNA indicates that there are one *HXT1* gene and one *ORF2* gene per haploid genome (data not shown). The *HXT1* gene was localized to chromosome VIII as described in Materials and Methods.

On the basis of Kyte-Doolittle hydropathy analysis (24), the HXT1 protein was predicted to be highly hydrophobic and to contain two groups of six hydrophobic domains separated by a 68-amino-acid hydrophilic loop with a 59-amino-acid hydrophilic N-terminal and a 58-amino-acid C-terminal domain. The boxed regions in Fig. 3 indicate contiguous hydrophobic domains, each 21 amino acids in length; similar secondary structures were found for the erythrocyte glucose transporter and proposed to be membrane-spanning domains (30). Each putative membrane-spanning domain is separated by a hydrophilic domain, the largest of which occurs at a consensus position between membrane-spanning domains 6 and 7. Like other members of the hexose transporter family, the HXT1 protein lacks an N-terminal signal sequence (17, 26). Several conserved amino acid motifs which are common to the hexose transporter family are also present in HXT1 (26, 40).

The HXT1 protein is unique in both the positioning and number of N-linked glycosylation sites within the protein. Four putative glycosylation sites are located in the N-terminal region: Asn-2, -14, -36, and -42. A fifth, Asn-227, follows domain 5. The HXT1 protein sequence is also unusual because it does not contain a PEST consensus region which occurs in the GAL2 and SNF3 proteins (40), in MAL61 (11), and in HXT2 (23).

HXT1 contains a potential leucine zipper-like motif starting five amino acids N terminal to membrane-spanning domain 2 and extending through that domain (Fig. 3). The leucine zipper heptad repeat motif has been proposed as a tertiary structure involved in dimer formation (6, 25). Interestingly, the leucine zipper motif is highly conserved in position in HXT1, HXT2, and GAL2 (Fig. 4) but is not present in the more distantly related SNF3 protein in the analogous position. The human glucose transporters have degenerate leucine zipper motifs in a similar position (28, 42).

A high degree of sequence similarity was seen in comparisons among HXT1, HXT2, and GAL2 (Table 2). The SNF3

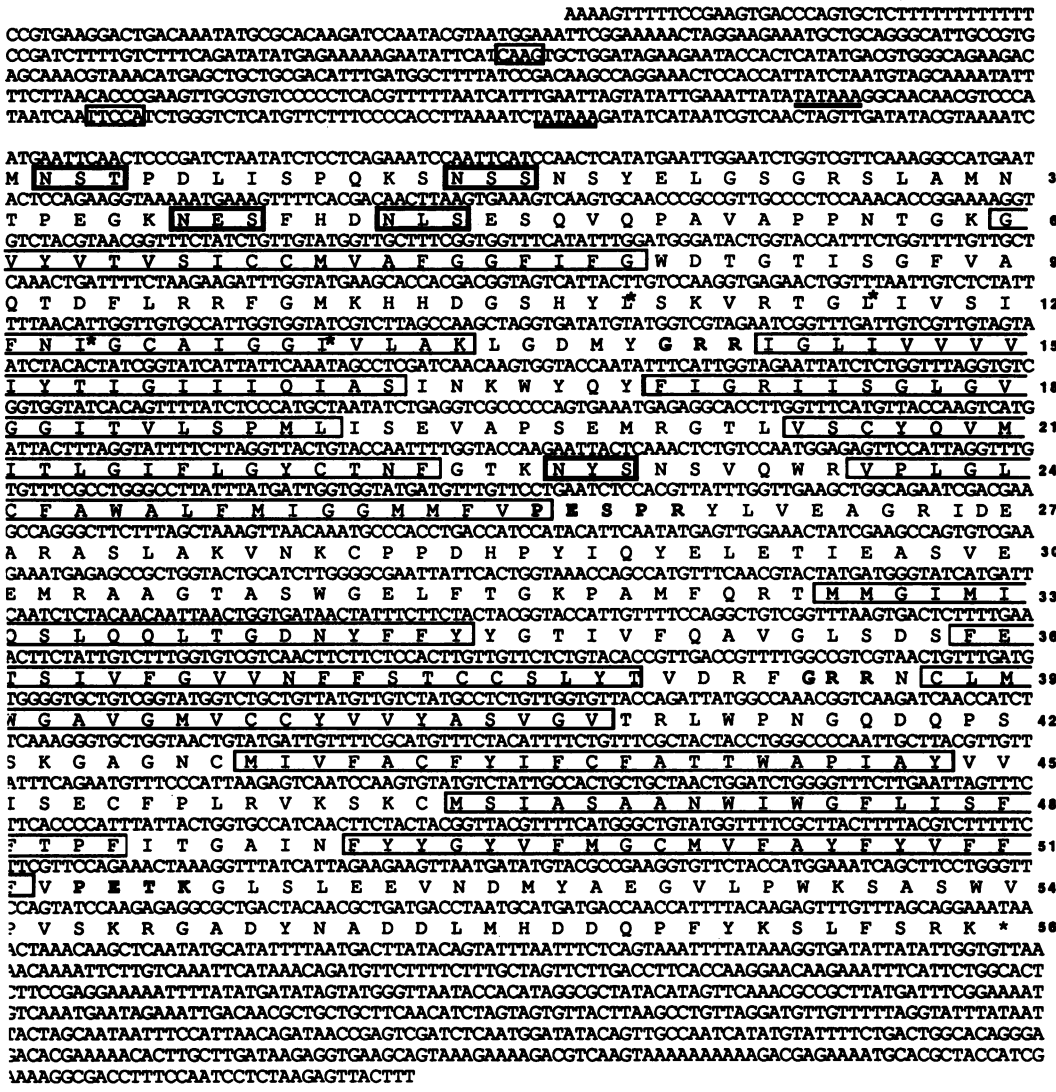


FIG. 3. Nucleotide and deduced amino acid sequences of the *HXT1* gene. Nucleotides are numbered on the left, and amino acids are numbered on the right. Potential regulatory elements in the 5′ region are underlined at −39 and −106 and boxed at −79 and −314. Highly hydrophobic domains of 21 amino acids in length are boxed, and potential N-linked glycosylation consensus sites are enclosed in bold boxes. Conserved amino acid motifs are shown in bold type. Stars indicate residues proposed to form a putative leucine zipper motif.

protein is much less homologous, only 30% identical, an identity similar to that for the mammalian glucose transporters. This high degree of relatedness between the three yeast hexose transport genes *HXT1*, *HXT2*, and *GAL2* was further demonstrated physically by low-stringency Southern analysis of *HXT1*, *HXT2*, *GAL2*, and *SNF3* (data not shown). The *SNF3* fragment failed to hybridize to the *HXT1* probe under these low-stringency conditions, while *HXT1*, *HXT2*, and *GAL2* did hybridize. Multicopy expression of the *HXT1* gene in *gal2* mutant strains (MCY1407 and YPH500) did not, however, confer good growth on galactose (data not shown), so although *GAL2* and *HXT1* are highly homologous at the amino acid level, they do not share substrate specificities.

Disruption of the *HXT1* gene. To determine the necessity for and functionality of the *HXT1* gene, the genomic locus was disrupted by gene replacement (Fig. 1). To confirm a single insertion event of *hxt1::URA3* at the *HXT1* locus, representative tetrads were screened by Southern analysis as detailed in Materials and Methods (data not shown). Tetrad analysis of the disruptants was also performed. A total of 22 tetrads were scored, and each displayed 2:2 segregation for Ura^+:Ura^-. All spores were viable on complex medium (YPD), indicating that *HXT1* is not an essential gene. The *hxt1* null (LBY202) and *hxt1 snf3* double-null (LBY203) haploid strains had the same colony sizes as and displayed growth rates similar to those of the wild-type strain, YPH500, on 2% glucose medium.

Hexose transport kinetics of the *hxt1* mutant strains. The effect of loss of *HXT1* on glucose, fructose, and mannose transport kinetics was analyzed in the presence and absence of the *SNF3* gene (YPH500 and LBY403, respectively). Glucose transport kinetics for the wild type (YPH500) and the *hxt1* null strain (LBY202) were compared (Fig. 5A) in complex media under derepressing glucose concentrations (<0.1%). The loss of the *HXT1* gene resulted in a decrease in high-affinity uptake under these conditions, although some

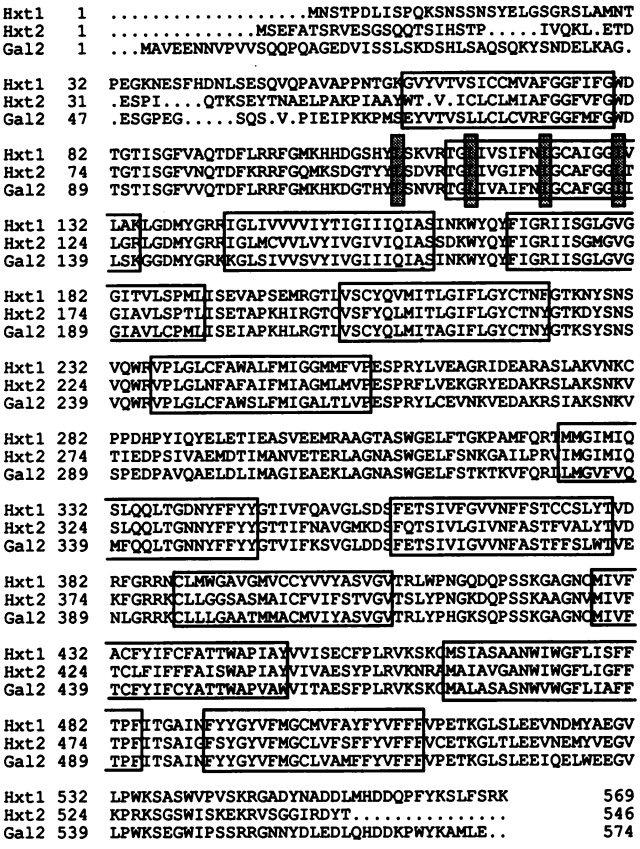


FIG. 4. Amino acid similarity among the HXT1, HXT2, and GAL2 family of sugar transporters. Amino acid sequences of HXT1, HXT2 (23), and GAL2 (40) are aligned; gaps introduced by the GAP program are indicated by periods. Putative membrane-spanning domains are boxed, and the conserved putative leucine zipper motifs for all three proteins are indicated as shaded boxes. Numbering for each protein is indicated at the left.

high-affinity uptake was still visible, which may be due to the presence of other transporters (HXT2 and SNF3). The effect of loss of both *HXT1* and *SNF3* was analyzed (Fig. 5B). The dramatic drop in high-affinity transport due to the loss of *SNF3* (LBY403) is only slightly more decreased in the *hxt1*::*URA3 snf3*::*HIS3* strain.

It has been shown previously that the glucose high-affinity transport system in *S. cerevisiae* is also specific for fructose (4). The proportion of fructose transport due to *HXT1* was therefore assessed by testing fructose transport in cells grown in glucose (Fig. 6). Unlike glucose uptake, fructose uptake was not affected by the loss of *HXT1*. It is believed that mannose is also transported via the glucose uptake system (12), although this has not been addressed genetically. Accordingly, mannose transport was also analyzed (Fig. 7). Cells were first grown in glucose to derepressing conditions and tested for mannose transport. As shown in Fig. 7A, there was no high-affinity mannose uptake in these cells but there was significant low-affinity transport. When cells were instead grown on mannose and harvested at a low mannose concentration prior to preparation for mannose transport assays, high-affinity mannose transport was seen. This finding suggests that some component(s) of the mannose transport system requires induction by growth on mannose or, alternatively, that components are repressed by the very low concentrations of glucose in the previous assay. The wild-type and *hxt1* null strains were grown on mannose to late log phase and tested for mannose transport (Fig. 7B). There was a significant decrease (approximately 50%) in the amount of mannose transport due to the loss of *HXT1*. The *snf3* null strain also showed a significant decrease in mannose transport (approximately 75%) compared with the wild type (Fig. 7C). Interestingly, the *hxt1 snf3* double-null strain (LBY203) showed only a 50% decrease in high-affinity mannose transport, similar to the loss seen in the *hxt1* single-null strain but less severe than the loss seen in the *snf3* single-null strain. Thus, loss of *HXT1* seems to affect both glucose and mannose uptake moderately while not affecting fructose transport. The loss of *SNF3* affected high-affinity uptake of all three sugars.

Expression of the *HXT1-lacZ* fusion in the wild type and in the *snf3* null mutant. To further characterize the role of the *HXT1* gene in sugar catabolism, the *HXT1* promoter region was fused to the reporter gene for β-galactosidase on a multicopy plasmid. Expression of β-galactosidase activity was assessed by using a multicopy vector because the *HXT1* gene complements the *snf3* null mutation only in multicopy. The wild-type (YPH500) and *snf3* null (LBY403) strains were transformed with the *HXT1-lacZ* fusion multicopy plasmid pDL502, and β-galactosidase activity and glucose concentration were monitored throughout the growth curve. Expression of β-galactosidase activity was analyzed in three separate transformants for each strain, and all transformants yielded the identical pattern of expression. In the wild type (Fig. 8B), β-galactosidase expression increased immediately upon transfer of the stationary-phase culture (−1 h in Fig. 8) to fresh 2% glucose medium and continued to increase to a maximum about 5 h into lag/early exponential phase, at which time the glucose concentration was still at approximately 2% (Fig. 8A). As the culture began to enter exponential-phase growth (after approximately 5.5 h), β-galactosidase expression began to decrease dramatically (<5 Miller units); expression continued to decrease throughout exponential phase and eventually reached a basal level (<1 Miller units) in early stationary phase, when glucose levels were less than 0.05%.

Expression of the *HXT1-lacZ* plasmid in the *snf3* null

TABLE 2. Comparison of glucose transporters with HXT1[a]

Transporter[b]	% Nucleotide identity	Amino acid		Reference
		% Identity	% Similarity	
Yeast				
HXT2	65	66	79	23
GAL2	68	69	83	40
SNF3	17	30	55	8
MAL61	15	22	48	11
Mammalian				
GLUT1	10	27	52	30
RATGT	10	28	52	2
Procaryotic				
AraE		27	55	26
XylE		30	56	26

[a] Nucleic acid and amino acid sequence homologies were analyzed by using the COMPARE program from the GCG sequence analysis software package.

[b] HXT2, hexose transporter 2; GAL2, galactose permease; SNF3, sucrose nonfermenting; MAL61, maltose permease; GLUT1, mammalian glucose transporter from HepG2 cells, erythrocytes, and brain; RATGT, rat glucose transporter; AraE, arabinose transporter (from *E. coli*); XylE, xylose transporter (from *E. coli*).

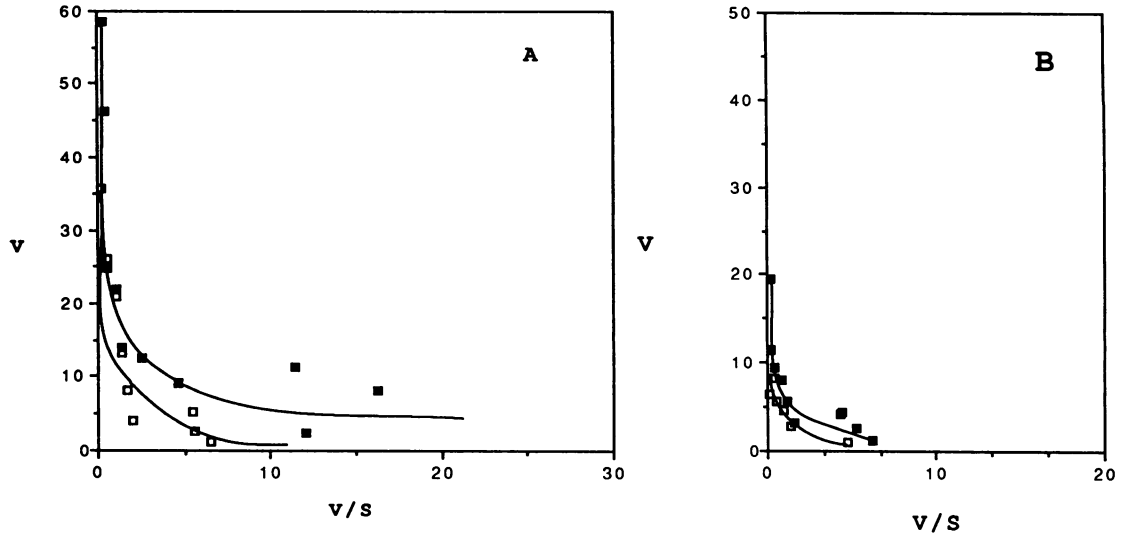


FIG. 5. Eadie-Hofstee plots of glucose uptake in wild-type, *hxt1*::*URA3* null, *snf3*::*HIS3* null, and *hxt1*::*URA3 snf3*::*HIS3* double-null strains. Cells were grown in YPD with 2% glucose to an A_{580} of 5 (late log) and assayed for glucose transport. Symbols: (A) ■, YPH500; □, LBY202; (B) ■, LBY403; □, LBY203. Scales: v, nanomoles of glucose per minute per milligram (dry weight); s, millimolar.

strain (Fig. 8C) paralleled the pattern observed in the wild type, although the maximum activity was slightly, but reproducibly, higher in the *snf3* mutant strain. There was no significant difference in the pattern of glucose utilization between the wild-type and *snf3* null strains throughout the growth curve (Fig. 8A), so the glucose concentrations in the media were comparable. Since the SNF3 protein is thought to be a high-affinity hexose transporter expressed under low-sugar conditions, this pattern of expression of HXT1 was unexpected.

The *HXT1* gene was originally isolated by its ability to complement the *snf3* growth defect on raffinose. Accordingly, the expression of *HXT1* during growth on raffinose was examined. The *HXT1* promoter expression on raffinose was similar to that observed in glucose during lag phase (Fig. 8D).

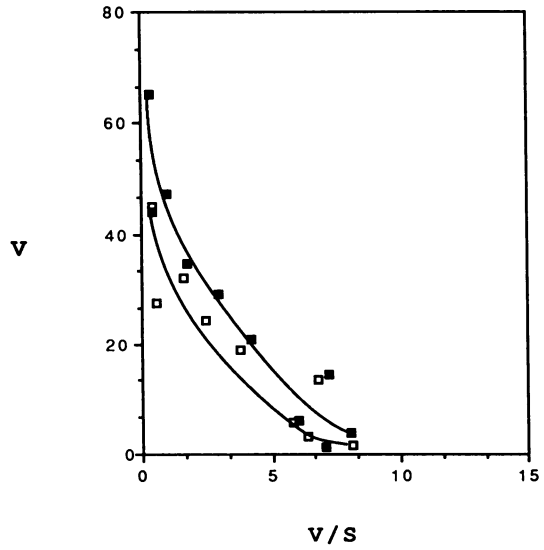


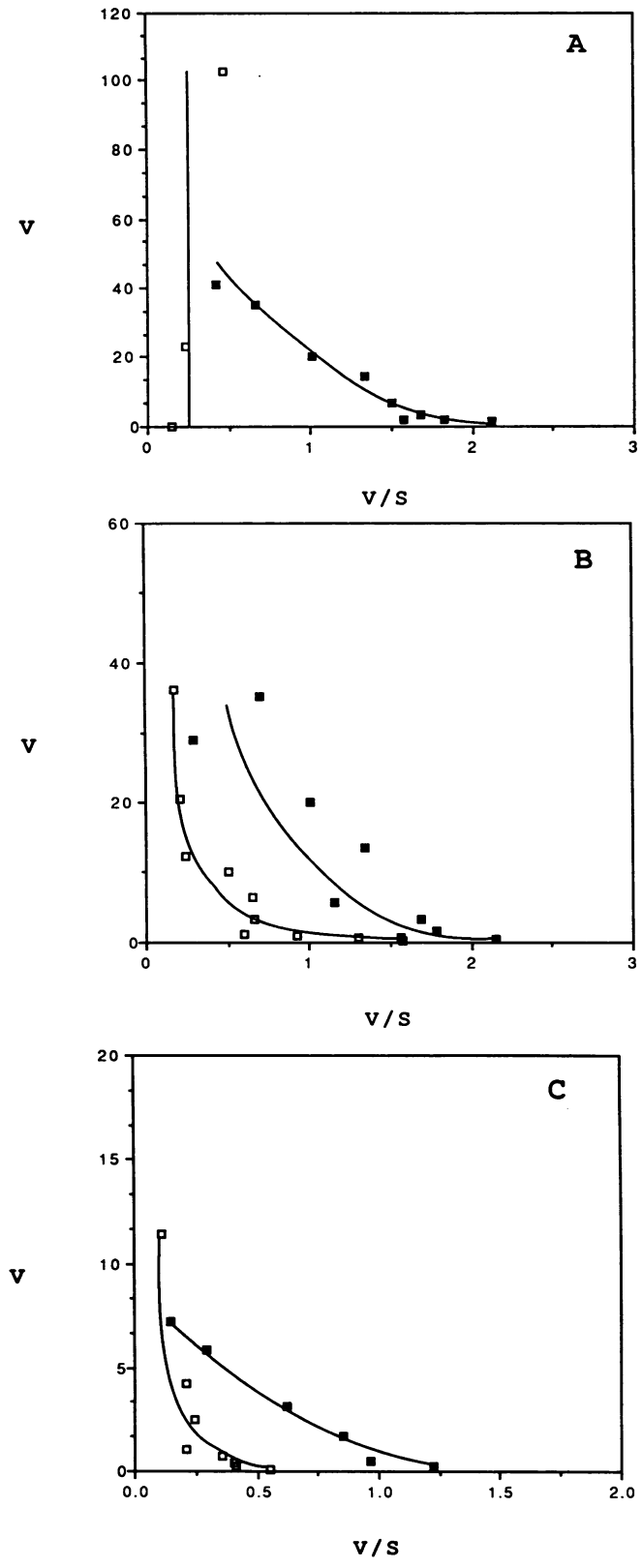
FIG. 6. Eadie-Hofstee plots of fructose uptake in wild-type and *hxt1*::*URA3* null strains. Cells were grown as for Fig. 5 and were tested for fructose transport. Symbols: ■, YPH500; □, LBY202. Scales: v, nanomoles of glucose per minute per milligram (dry weight); s, millimolar.

DISCUSSION

Two separate regions of plasmid pSC7 were identified as capable of complementing the growth defect conferred by the *snf3* null mutation when present on multicopy plasmids. One of these clones, pDL306, was found to contain a gene, *HXT1*, which encodes a 569-amino-acid protein highly homologous to known sugar transporter proteins. The other gene, *ORF2*, has been partially sequenced, and no homology to any previously described transporter was found. Multicopy expression of either *HXT1* or *ORF2* restored high-affinity glucose transport to the MCY1407 strain to the wild-type level.

The sequence analysis of HXT1 revealed that this protein is a member of the superfamily of sugar transporters and that HXT1 is very closely related to two other yeast transporters, HXT2 and GAL2. Previous data suggest that the yeast transporter gene family contains members in addition to *HXT1* and *GAL2*, as revealed by the number of bands observed by low-stringency Southern analysis (23). The degree of conservation in amino acid sequence among HXT1, HXT2, and GAL2 was much greater in their membrane-spanning domains than in the hydrophilic regions. It was also evident that the first half of each protein was more highly conserved than the second half, which may be a clue to the determinants of substrate specificity or affinity.

The loss of *HXT1* resulted in a decrease in high-affinity transport of both glucose and mannose but not fructose, suggesting that this protein might be specific for aldohexoses. This is an interesting parallel to the substrate specificity of glucokinase, thought to be an aldohexose kinase (1). The loss of *HXT1* exerted a greater effect on mannose uptake than on glucose uptake. This could reflect the fact that there are probably residual glucose transporters in the cell, as previously proposed (23). It was also found that SNF3 can apparently mediate mannose transport, and SNF3 is thus a glucose, fructose, and mannose transporter. From sugar



competition studies, mannose, glucose, and fructose were thought to share the same uptake systems (12). Glucose depletion from the growth medium results in derepression of glucose and fructose uptake, but from the work reported here, high-affinity mannose uptake is expressed in mannose-grown cells only following mannose depletion.

The analysis of the *HXT1* promoter-*lacZ* fusions revealed an unexpected pattern of regulation in view of the genetic and sugar transport kinetic analyses. The *HXT1* promoter is stimulated when cells are exposed to high glucose, as observed when stationary cells with glucose levels less than 0.05% are transferred to fresh medium with 2% glucose, although it is not clear whether the signal is glucose itself or the return to growth conditions, since a similar pattern of expression was observed with raffinose as a substrate. The amount of activity expressed by the *HXT1-lacZ* multicopy plasmid (pDL502) was comparable to that of a *SNF3-lacZ* fusion construct expressed in a multicopy plasmid (8); however, expression of the *SNF3-lacZ* fusion was investigated only during exponential growth. Multicopy expression of HXT1 obviously supplies enough HXT1 activity to compensate for the absence of SNF3 in the *snf3* null strain. The temporal pattern of gene expression of other yeast sugar transporters has not been described. In comparison with glycolytic genes which contribute up to 30% of the total soluble cell protein (16), the maximal level of *HXT1* expression obtained is low to moderate and is approximately 100-fold less than the level of expression of β-galactosidase observed with a *HXT2-lacZ* promoter fusion (23a). It should be noted that this level of expression was obtained by using a multicopy vector, and *HXT1* expression from the genome is likely to be significantly less. This low level of expression is inconsistent with a role of the HXT1 protein as a major route of sugar uptake during sugar catabolism. Also, β-galactosidase activity decreased rapidly and dramatically (100-fold drop in levels) upon entry of the cells into exponential growth, suggesting that the HXT1 protein is made largely during lag phase. If it is assumed that HXT1 is in fact a sugar transporter or somehow directly involved in hexose uptake, then these data may be explained by a model of posttranslational regulation whereby the transporter transcript is immediately made upon exposure to substrate and translated and the newly made transporter is then held in an intracellular compartment until needed to scavenge for the last glucose from the media. Such a model is reminiscent of the proposed mechanism of insulin regulation of mammalian glucose transporter (38). However, the timing and low level of expression of HXT1 may indicate an alternative physiological role, as a hexose sensor of some sort necessary early in the growth phase but not essential for continued growth. If HXT1 were required for transport of glucose during lag phase, null mutations would be expected to confer a growth defect of some type or to lengthen lag phase due to substrate limitation, neither of which is observed. These studies raise the question as to whether or not all members of the glucose transporter family are indeed primarily glucose transporters.

FIG. 7. Eadie-Hofstee plots of mannose uptake in wild-type, *hxt1*::*URA3* null, *snf3*::*HIS3* null, and *hxt1*::*URA3 snf3*::*HIS3* double-null strains. Cells were grown in YP plus 2% sugar to an A_{580} of 5. Symbols: (A) □, YPH500 grown in glucose; ■, YPH500 grown in mannose; (B) ■, YPH500 grown in mannose; □, LBY202 grown in mannose; (C) ■, LBY403 grown in mannose; □, LBY203 grown in mannose. Scales: v, nanomoles of glucose per minute per milligram (dry weight); s, millimolar.

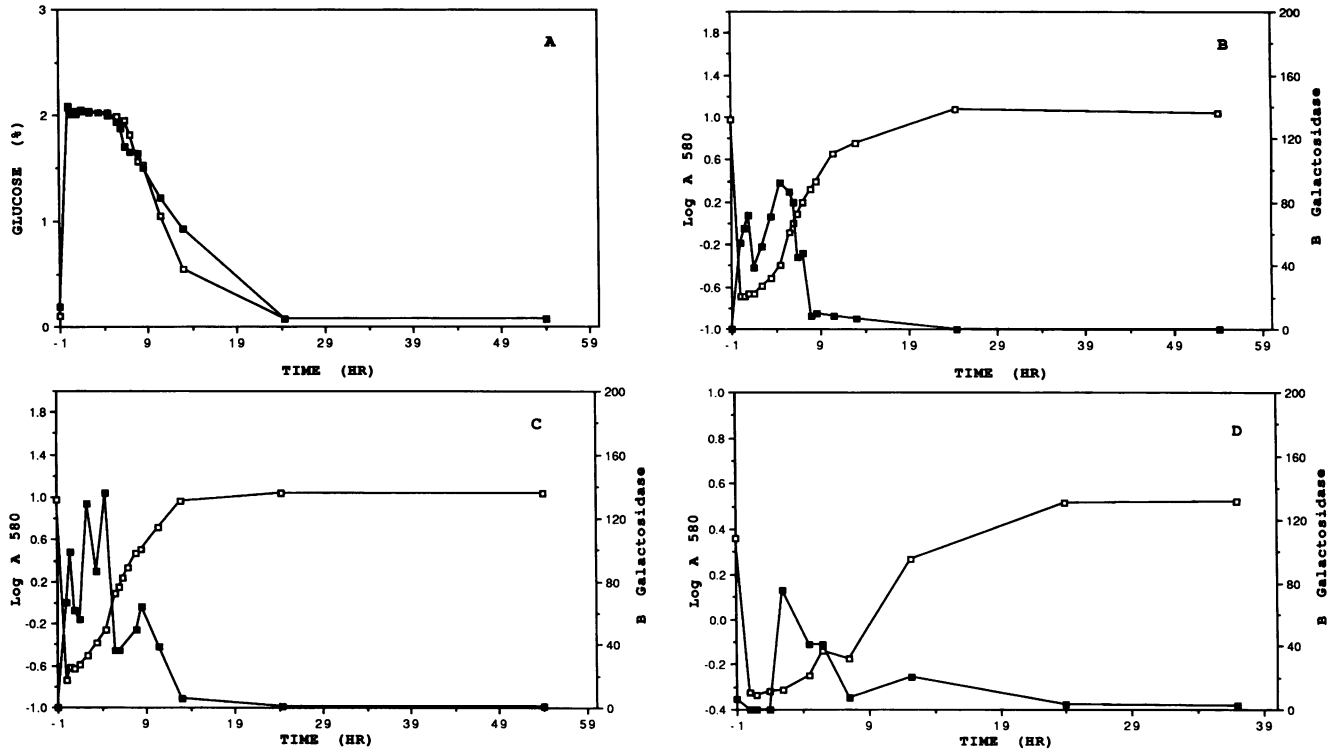


FIG. 8. β-Galactosidase activity of the *HXT1* promoter-*lacZ* fusion in wild-type and *snf3* mutant strains. (A) Glucose concentrations for plots A (open squares) and B (closed squares). Culture samples (0.1 ml) were microfuged to remove cells, and supernatants were analyzed for glucose. (B) Wild-type YPH500 containing pDL502. Three individual transformants were grown 24 h to stationary phase in YNB and 2% glucose with uracil selection, pelleted, and used to inoculate 100 ml of fresh medium at a 1/40 dilution. Samples of 1 A_{580} unit were analyzed in triplicate for β-galactosidase activity throughout the growth phase. β-Galactosidase activity (closed boxes) is expressed in Miller units ($\Delta A_{420} \times 10^3 A_{580}^{-1} \text{ min}^{-1}$); A_{580} is shown by open boxes. Data from one representative strain are shown. (C) *snf3* null mutant LBY403 containing pDL502. Three individual transformants were processed as for panel A; symbols are as for panel A. (D) Wild-type YPH500 containing pDL502 grown in 2% raffinose. A representative transformant from panel A was grown as for panel A except with substitution of 2% raffinose for glucose.

Further gene expression analyses under different physiological and genetic conditions as well as protein localization studies should help to define the role of *HXT1* in hexose transport in *S. cerevisiae*.

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