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The *HXT2* Gene of *Saccharomyces cerevisiae* Is Required for High-Affinity Glucose Transport

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The *HXT2* gene of the yeast *Saccharomyces cerevisiae* was identified on the basis of its ability to complement the defect in glucose transport of a *snf3* mutant when present on the multicopy plasmid pSC2. Analysis of the DNA sequence of *HXT2* revealed an open reading frame of 541 codons, capable of encoding a protein of M_r 59,840. The predicted protein displayed high sequence and structural homology to a large family of procaryotic and eucaryotic sugar transporters. These proteins have 12 highly hydrophobic regions that could form transmembrane domains; the spacing of these putative transmembrane domains is also highly conserved. Several amino acid motifs characteristic of this sugar transporter family are also present in the HXT2 protein. An *hxt2* null mutant strain lacked a significant component of high-affinity glucose transport when under derepressing (low-glucose) conditions. However, the *hxt2* null mutation did not incur a major growth defect on glucose-containing media. Genetic and biochemical analyses suggest that wild-type levels of high-affinity glucose transport require the products of both the *HXT2* and *SNF3* genes; these genes are not linked. Low-stringency Southern blot analysis revealed a number of other sequences that cross-hybridize with *HXT2*, suggesting that *S. cerevisiae* possesses a large family of sugar transporter genes.

The transport of glucose into cells of the yeast *Saccharomyces cerevisiae* has been well characterized kinetically. Two kinetically distinct transport systems have been described, one with low affinity (K_m = 20 mM) and the other with high affinity (K_m = 1 mM) for glucose (6, 55). Glucose is transported into yeast cells via facilitated diffusion (6, 55). The low-affinity system is constitutive, whereas the high-affinity system is repressed at high extracellular glucose concentrations (7). Furthermore, the high-affinity system is dependent on the presence in the cell of a kinase which can phosphorylate the transported sugar (6). Fructose is also a substrate for the transport systems, with K_m values of 6 mM (high affinity) and 40 mM (low affinity). Hexokinase activity is necessary for high-affinity fructose transport (6).

Analysis of mutants isolated on the basis of a defect in growth on sucrose, called *snf*, for sucrose nonfermenting (43), revealed that the *SNF3* gene is involved in hexose transport (8). The *SNF3* gene is required for expression of high-affinity glucose transport and for growth on low-glucose medium when respiration is inhibited (8). The *SNF3* gene product is an integral membrane protein with homology to the glucose facilitated diffusion transporter of human erythrocytes (12) and to a large family of other sugar transporters (2, 15, 53). Expression of a *SNF3-lacZ* gene fusion product is repressed by high glucose concentrations (12). A *snf3* null mutant, lacking high-affinity glucose transport, possesses wild-type levels of hexose-phosphorylating activity (8) and of secreted invertase activity (44). This fact suggests that *SNF3* acts directly at the glucose transport step, since null mutations do not have pleiotropic effects on glucose utilization or the catabolite repression system.

The trisaccharide raffinose is not transported by yeast cells but is cleaved to transportable hexoses by secreted hydrolases. In a Mel$^-$ strain, invertase cleaves raffinose to fructose and melibiose, and the fructose is assimilated. Mel$^-$ *snf3* null mutant strains are unable to grow on rich raffinose medium (44). Multicopy plasmids bearing yeast genomic DNA fragments that restore growth on raffinose to a *snf3* null mutant strain were recovered. Two of these plasmids (pSC2 and pSC7) were shown to restore high-affinity glucose transport to the *snf3* null mutant. They also imparted upon the null mutant the ability to grow on low-glucose medium in the presence of a respiratory inhibitor (8).

We have identified and sequenced the gene responsible for the complementing activity of plasmid pSC2. This gene has been designated *HXT2* (hexose transporter). We show that it is homologous to a large family of sugar transport proteins from procaryotes and eucaryotes, including SNF3, and that it is necessary under certain physiological conditions for high-affinity glucose transport.

MATERIALS AND METHODS

Strains and plasmids. The yeast strains used are shown in Table 1. Strains MCY1407 and YPH500, isogenic derivatives of S288C, were the parents in the genetic cross (51) that produced strain LBY400. Subsequent strains were derived by genetic crosses or by one-step gene disruption (47). Strain DFY1 (originally D585-11C, from Fred Sherman) is unrelated to S288C. The *Escherichia coli* strain used for plasmid amplification was DH5α (Bethesda Research Laboratories, Inc.).

The isolation of episomal plasmid pSC2 containing the original *HXT2* clone has been described (8), as have the yeast-*E. coli* shuttle vectors YEp351 and YEp352 (27). Plasmid pSC2-1C3 was constructed by ligation of a 5.2-kb *Pst*I-*Sph*I fragment of pSC2 into the polylinker of YEp352. The restriction map of the insert is shown in Fig. 1. A 3.1-kb *Pvu*I fragment (including 0.2 kb of vector DNA) was subcloned from pSC2-1C3 into YEp352, to form pAK1a, whose entire sequence is known. Plasmid pAK9a was made by inserting the 2.2-kb *Sma*I-*Hpa*I *LEU2* fragment from YEp351 (27) at the unique *Hpa*I site of pAK1a (codon 122 of *HXT2*).

Growth conditions. *E. coli* was grown at 37°C in LB or TB medium as described previously (39). Yeast strains were

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TABLE 1. Yeast strains

Strain	Genotype[a]	Source or reference
DFY1	*lys1 SUC MAL MEL*	F. Sherman
MCY1407	*MATa snf3-Δ4::HIS3 Δhis3 ura3-52 lys2-801 SUC2 mel*	44
LBY400	*MATa/MATα snf3-Δ4::HIS3/+ ura3-52/ura3-52 lys2-801/lys2-801 +/ade2-100 +/trp1-Δ63, his3-Δ200/his3-Δ200 +/leu2-Δ1*	This work
LBY403	*MATa snf3-Δ4::HIS3 lys2-801 ura 3-52 ade2-101 leu2-Δ1 trp1-Δ63 Δhis3*	This work
LBY404	*MATα snf3-Δ4::HIS3 lys2-801 ura 3-52 ade2-101 leu2-Δ1 trp1-Δ63 Δhis3*	This work
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/Δhis3 leu2-Δ1/leu2-Δ1*	This work
LBY406	*MATa hxt2-1::LEU2 ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	This work
LBY407	*MATa hxt2-2::LEU2 ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	This work
LBY410	*MATa ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	This work
LBY411	*MATα snf3-Δ4::HIS3 lys2-801 ura3-52 ade2-101 leu2-Δ1 trp1-Δ63 his3-Δ200*	This work
LBY413	*MATa hxt2-1::LEU2 ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	This work
LBY416	*MATa hxt2-1::LEU2 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
YPH499	*MATa ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1*	51a
YPH500	*MATα ura3-52 lys2-801 ade2-100 trp1-Δ63 his3-Δ200 leu2-Δ1mel*	51a
YPH501	*MATa/MATα ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1-Δ63/trp1-Δ63 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1*	51a

[a] The *his3-Δ200* allele was found to confer a leaky phenotype.

cultured at 30°C in YP (10 g of yeast extract, 20 g of peptone, 25 mg of adenine, and 25 mg of uracil per liter) or YNB (6.7 g of yeast nitrogen base without amino acids per liter, pH 6.8) medium, to which glucose was added after autoclaving to a final concentration (wt/vol) of 2% (high glucose) or 0.05% (low glucose). Liquid cultures (medium volume 20% of vessel volume) were aerated on a rotatory shaker at 250 strokes per min. Low-glucose plates were overlaid 1 h before use with 200 μl of a solution of antimycin A (Sigma) made by diluting a 1-mg/ml ethanolic stock solution 1:7 (vol/vol) in sterile water; the final concentration of antimycin A in the plate was 1 μg per ml of medium. YNB media were supplemented with amino acids, nitrogenous bases, or both, as required by the auxotrophic markers of the strains being grown, as previously described (51).

DNA sequence analysis. Nested deletions of the yeast genomic DNA insert of plasmid pSC2-1C3 were constructed as previously described (26). Briefly, 10 μg of pSC2-1C3 was digested with the restriction enzymes *Xho*I and *Sph*I (Fig. 1), and another 10 μg was digested with *Sst*I and *Bam*HI (sites in the YEp352 polylinker). These samples were phenol extracted, precipitated with ethanol, resuspended in exonuclease III buffer (26), and incubated with 50 U of exonuclease III (Bethesda Research Laboratories) per μg at 37°C. Samples (0.5 μg) were removed at 30-s intervals, added to excess EDTA, and heated to 65°C for 10 min. Single-stranded DNA was digested with nuclease S1 (Bethesda Research Laboratories). The resulting blunt-ended molecules were self-ligated and transformed into DH5α (39). Plasmid DNA was isolated from ampicillin-resistant transformants as described previously (56) and digested with appropriate restriction enzymes, and the extent of the deletion in each clone was determined. Plasmids that formed a series of nested deletions were tested for their ability to restore growth on low glucose to strain MCY1407. Selected plasmids were used as double-stranded templates (14, 56) for DNA sequence analysis (48) using chemically modified T7 DNA polymerase (United States Biochemical). Both strands were sequenced for the entire region shown in Fig. 2. Each base on each strand was sequenced, on average, from 1.4 deletion derivatives.

Glucose transport assays. For glucose transport assays, 1 ml of stationary-phase cells was added to 100 ml of YP high glucose and cultured for 12 (LBY410, LBY411, and LBY413) or 14 (LBY416) h. Cells were harvested by centrifugation, washed, and resuspended in YP medium. The cells were split into two equal portions, glucose was added to a final concentration of 2 or 0.05% (final volume, 100 ml), and the flasks were returned to culture conditions. After 90 min, the low-glucose culture was harvested and glucose transport was assayed. The assay was completed within 1 h, at which time the high-glucose culture was harvested and glucose transport was assayed.

The method used for determining glucose transport kinetics by yeast cells has been described (6). The assay measured zero trans influx. Cells were harvested by filtration under vacuum and washed four times with 10 ml of 0.1 M potassium phosphate buffer (pH 6.5) at room temperature to remove free glucose. Cells were resuspended in buffer to an A_{580} of ca. 15. Samples (80 μl) of cells were incubated for 5 s with radiolabeled glucose (D-[U-^{14}C]glucose; New England Nuclear) at 30°C. Transport was rapidly quenched by addi-

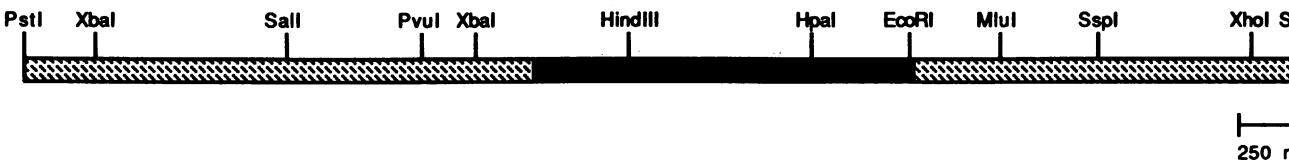

FIG. 1. Restriction map of the region containing the *HXT2* gene. The open reading frame encoding the HXT2 protein is shaded. The designated start methionine is to the right of the *Eco*RI site.

tion of 10 ml of ice-cold water. The cells were collected on glass fiber filters under vacuum and washed twice with 10 ml of ice-cold water. The amount of radioactivity taken up was determined by liquid scintillation counting (Packard TriCarb 2000CA, with OptiFluor scintillation cocktail).

Miscellaneous techniques. Yeast plasmid and genomic DNA was isolated as described by Hoffman and Winston (28). DNA blot (Southern) analysis (52) was performed by using Nytran membranes (Schleicher & Schuell), following general conditions prescribed by the manufacturer. Detailed hybridization and wash conditions are given in the legend to Fig. 6. Restriction digestion, ligation, DNA electrophoresis, and other nucleic acid manipulations were performed as previously described (39). Glucose concentrations were measured with a YSI model 27 glucose analyzer as prescribed by the manufacturer.

Computer analysis. Nucleic acid and protein sequence data were manipulated and analyzed with the University of Wisconsin Genetics Computer Group software version 6.1 (19), running on a VAX 8600 minicomputer at the Computer Center, University of California, Davis. The programs used are cited in the text. Unless otherwise stated, default settings were used for the analyses.

Nucleotide sequence accession number. The sequence reported has been submitted to the GenBank data base (accession number M33270).

RESULTS

Sequence determination and analysis of *HXT2*. The restriction map of the SC2 fragment (8) was refined, and a unique 5.2-kb *Pst*I-*Sph*I fragment (Fig. 1) was subcloned into the multicopy yeast-*E. coli* shuttle vector YEp352 (27). This fragment was able to restore growth to the *snf3* null mutant strain MCY1407 when streaked on YNB low-glucose plates (data not shown). Nested deletions from each end of this fragment were generated by exonuclease digestion (26), and nested deletants were tested for their ability to restore growth on low glucose when transformed into MCY1407.

The deletion-transformation analysis defined a segment of DNA approximately 3 kb in length which was sufficient to complement the growth defect of MCY1407. The nucleotide sequence of 2,890 bp of this segment was determined (Fig. 2). The sequence contains a single open reading frame of 1,623 nucleotides, which is predicted to encode a protein of 541 amino acid residues. The polypeptide has a predicted molecular weight of 59,840 and an isoelectric point of 8.3.

5′ noncoding and regulatory region of *HXT2*. The region 5′ of the putative initiation codon is A+T rich and contains TATA-like motifs at −585, −122, −47, and −14.

Five consensus regulatory sequences were found in the 5′ noncoding region of *HXT2*. A pair of CT-rich imperfect direct repeats occur at −564 and −482; these sequences are identical to one another at 13 of 14 positions. The sequence TTCCA was found at −331, and the sequence CAAG occurs at −310. A near-perfect match to the extended critical promoter of yeast mitochondrial RNA polymerase (40) was found at position −243: CTAACGATA in *HXT2* versus CTAAACGATA among the consensus sequences of the extended critical promoter.

Predicted amino acid sequence of *HXT2*. The environment of the initiation codon is similar to that found in many other efficiently expressed yeast genes, with A at −3 and T at +6 (20). The codon bias index (3) of *HXT2* is 0.47, which means that codon usage is not random, but is not highly biased. This implies that *HXT2* may be expressed at moderate levels.

The GenBank and EMBL nucleotide data banks were searched for sequences similar to *HXT2*. Significant similarity was found to the sequences of a large family of procaryotic and eucaryotic sugar transport genes. The similarity of these genes is even greater when their predicted translation products are compared. In Fig. 3, the amino acid sequence of HXT2 is aligned with the consensus sequences of three subsets of the sugar transporter family. HXT2 is identical to these consensus sequences at a number of positions, particularly in regions which are highly conserved among the members of this family of transporters (see reference 25 for review). HXT2 is most similar to the galactose transporter of *S. cerevisiae*, which is encoded by *GAL2*. These two proteins are identical at 65% of their residues and are 72% identical in regions predicted to be transmembrane domains (see below). The percent identities between HXT2 and a number of other sugar transporters are approximately equal, ranging from 21% for the yeast maltose permease to 31% for SNF3 and the *E. coli* xylose permease. The percent similarities follow the same pattern. Thirteen residues are perfectly conserved in identity and position among all 17 transporters represented in Fig. 3; these residues are circled in Fig. 2.

The secondary structure of HXT2 has been predicted by computer analysis. The results of hydropathy profile analysis (36) are shown in Fig. 4. Two sets of six hydrophobic domains were found, separated by a hydrophilic region of 68 amino acids. The amino and carboxyl termini of the protein are relatively hydrophilic. The hydrophobic domains are approximately 21 amino acids in length. A similar pattern of 12 hydrophobic domains has been found in all of the sugar transporters in this family. It has been hypothesized that these hydrophobic domains traverse the plasma membrane, folding in some manner to form a channel for sugar transport (41).

The predicted alpha helix and beta sheet structure of the protein was determined by the algorithms of Chou and Fasman (16) and Garnier et al. (24). Both methods predicted a high proportion of beta sheet in the hydrophobic domains (data not shown). The Garnier algorithm predicts HXT2 to possess 21.1% alpha helix and 38.6% beta sheet. The protein was therefore analyzed by the Garnier method, with decision constants of 20 to 50% for both alpha helix and beta sheet. Transmembrane domains (TMDs) I to V, VII, and XII were predicted by this analysis to be predominantly or entirely beta sheet, whereas TMDs VI, VIII, IX, and X were predicted to be predominantly alpha helix; TMD XI showed roughly equal propensities for either alpha helix or beta sheet. The long hydrophilic loop between TMDs VI and VII displayed predominantly alpha-helical content. Direct measurements on the secondary structure of HXT2 have not been made.

The primary sequence of the HXT2 protein contains two consensus sites at which N-linked glycosylation may occur (Asn-X-Ser/Thr; 35, 54), at residues 82 and 299 (Fig. 2); these sites are predicted to occur on the extracellular face of the plasma membrane (see below). HXT2 also has two potential cyclic AMP-dependent protein kinase phosphorylation sites (17, 34), at residues 266 (Lys-Arg-Ser) and 539 (Lys-Arg-Val-Ser; Fig. 2); both of these sites are predicted to be exposed on the cytoplasmic face of the membrane (see below).

A periodicity of leucine or isoleucine every seventh residue (leucine zipper motif; 37) has been observed in a number of vertebrate glucose transporters in or near the second putative transmembrane domain (57). A leucine zipper motif involving Leu-101, Leu-108, Ile-115, and Leu-122 occurs in

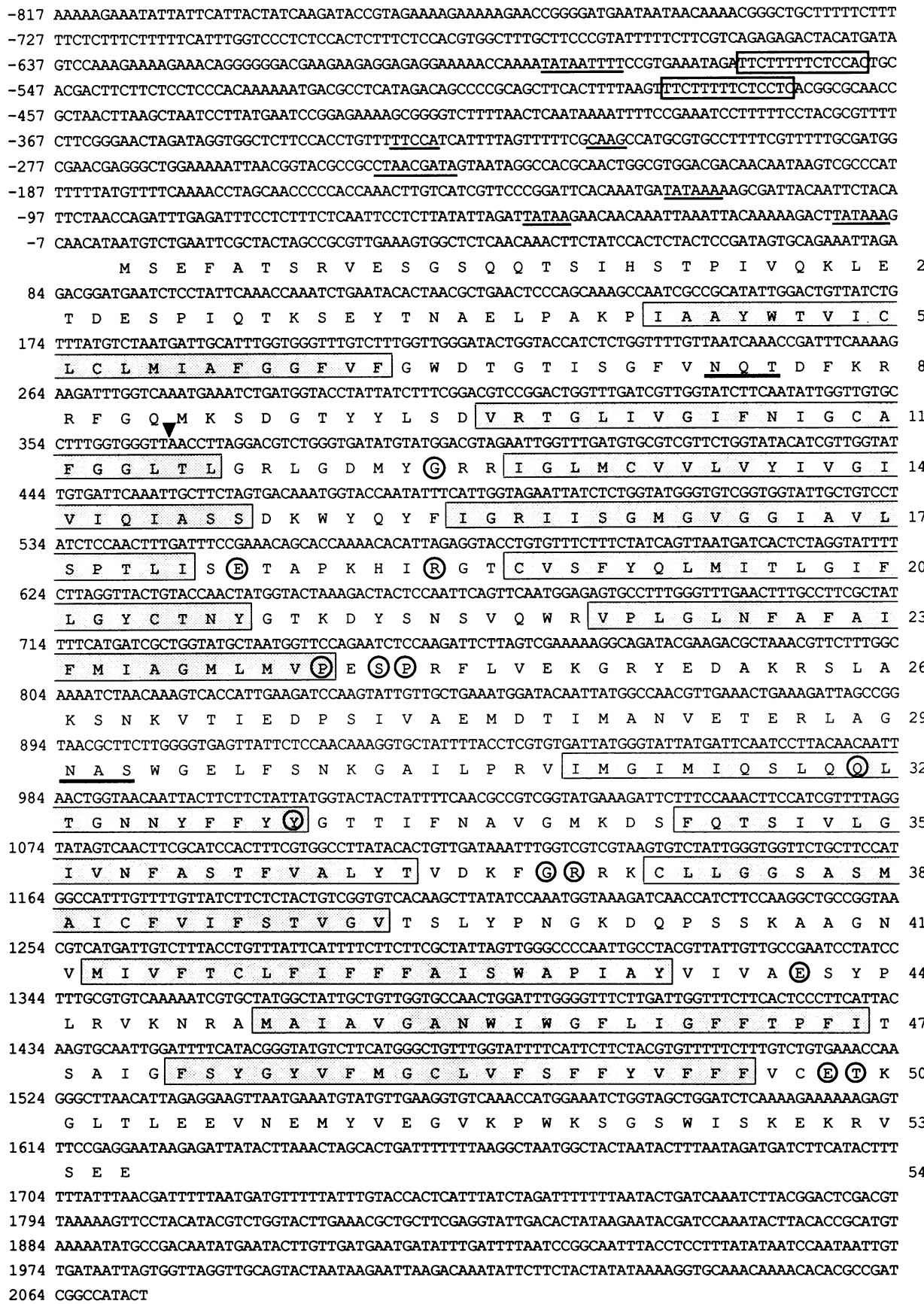
-817 AAAAAGAAATATTATTCATTACTATCAAGATACCGTAGAAAAGAAAAAGAACCGGGGATGAATAATAACAAAACGGGCTGCTTTTTCTTT
-727 TTCTCTTTCTTTTTCATTTGGTCCCTCTCCACTCTTTCTCCACGTGGCTTTGCTTCCCGTATTTTTCTTCGTCAGAGAGACTACATGATA
-637 GTCCAAAGAAAAGAAACAGGGGGGACGAAGAAGAGGAGAGGAAAAACCAAAATATAATTTTCCGTGAAATAGATTCTTTTTCTCCACTGC
-547 ACGACTTCTTCTCCTCCCACAAAAAATGACGCCTCATAGACAGCCCCGCAGCTTCACTTTTAAGTTTCTTTTTCTCCTCACGGCGCAACC
-457 GCTAACTTAAGCTAATCCTTATGAATCCGGAGAAAAGCGGGGTCTTTTAACTCAATAAAATTTTCCGAAATCCTTTTTCCTACGCGTTTT
-367 CTTCGGGAACTAGATAGGTGGCTCTTCCACCTGTTTTTCCATCATTTTAGTTTTTCGCAAGCCATGCGTGCCTTTTCGTTTTTGCGATGG
-277 CGAACGAGGGCTGGAAAAATTAACGGTACGCCGCCTAACGATAGTAATAGGCCACGCAACTGGCGTGGACGACAACAATAAGTCGCCCAT
-187 TTTTTATGTTTTCAAAACCTAGCAACCCCCACCAAACTTGTCATCGTTCCCGGATTCACAAATGATATAAAAAGCGATTACAATTCTACA
-97 TTCTAACCAGATTTGAGATTTCCTCTTTCTCAATTCCTCTTATATTAGATTATAAGAACAACAAATTAAATTACAAAAAGACTTATAAAG
-7 CAACATAATGTCTGAATTCGCTACTAGCCGCGTTGAAAGTGGCTCTCAACAAACTTCTATCCACTCTACTCCGATAGTGCAGAAATTAGA
M S E F A T S R V E S G S Q Q T S I H S T P I V Q K L E 28
84 GACGGATGAATCTCCTATTCAAACCAAATCTGAATACACTAACGCTGAACTCCCAGCAAAGCCAATCGCCGCATATTGGACTGTTATCTG
T D E S P I Q T K S E Y T N A E L P A K P I A A Y W T V I C 58
174 TTTATGTCTAATGATTGCATTTGGTGGGTTTGTCTTTGGTTGGGATACTGGTACCATCTCTGGTTTTGTTAATCAAACCGATTTCAAAAG
L C L M I A F G G F V F G W D T G T I S G F V N Q T D F K R 88
264 AAGATTTGGTCAAATGAAATCTGATGGTACCTATTATCTTTCGGACGTCCGGACTGGTTTGATCGTTGGTATCTTCAATATTGGTTGTGC
R F G Q M K S D G T Y Y L S D V R T G L I V G I F N I G C A 118
354 CTTTGGTGGGTTAACCTTAGGACGTCTGGGTGATATGTATGGACGTAGAATTGGTTTGATGTGCGTCGTTCTGGTATACATCGTTGGTAT
F G G L T L G R L G D M Y G R R I G L M C V V L V Y I V G I 148
444 TGTGATTCAAATTGCTTCTAGTGACAAATGGTACCAATATTTCATTGGTAGAATTATCTCTGGTATGGGTGTCGGTGGTATTGCTGTCCT
V I Q I A S S D K W Y Q Y F I G R I I S G M G V G G I A V L 178
534 ATCTCCAACTTTGATTTCCGAAACAGCACCAAAACACATTAGAGGTACCTGTGTTTCTTTCTATCAGTTAATGATCACTCTAGGTATTTT
S P T L I S E T A P K H I R G T C V S F Y Q L M I T L G I F 208
624 CTTAGGTTACTGTACCAACTATGGTACTAAAGACTACTCCAATTCAGTTCAATGGAGAGTGCCTTTGGGTTTGAACTTTGCCTTCGCTAT
L G Y C T N Y G T K D Y S N S V Q W R V P L G L N F A F A I 238
714 TTTCATGATCGCTGGTATGCTAATGGTTCCAGAATCTCCAAGATTCTTAGTCGAAAAAGGCAGATACGAAGACGCTAAACGTTCTTTGGC
F M I A G M L M V P E S P R F L V E K G R Y E D A K R S L A 268
804 AAAATCTAACAAAGTCACCATTGAAGATCCAAGTATTGTTGCTGAAATGGATACAATTATGGCCAACGTTGAAACTGAAAGATTAGCCGG
K S N K V T I E D P S I V A E M D T I M A N V E T E R L A G 298
894 TAACGCTTCTTGGGGTGAGTTATTCTCCAACAAAGGTGCTATTTTACCTCGTGTGATTATGGGTATTATGATTCAATCCTTACAACAATT
N A S W G E L F S N K G A I L P R V I M G I M I Q S L Q Q L 328
984 AACTGGTAACAATTACTTCTTCTATTATGGTACTACTATTTTCAACGCCGTCGGTATGAAAGATTCTTTCCAAACTTCCATCGTTTTAGG
T G N N Y F F Y Y G T T I F N A V G M K D S F Q T S I V L G 358
1074 TATAGTCAACTTCGCATCCACTTTCGTGGCCTTATACACTGTTGATAAATTTGGTCGTCGTAAGTGTCTATTGGGTGGTTCTGCTTCCAT
I V N F A S T F V A L Y T V D K F G R R K C L L G G S A S M 388
1164 GGCCATTTGTTTTGTTATCTTCTCTACTGTCGGTGTCACAAGCTTATATCCAAATGGTAAAGATCAACCATCTTCCAAGGCTGCCGGTAA
A I C F V I F S T V G V T S L Y P N G K D Q P S S K A A G N 418
1254 CGTCATGATTGTCTTTACCTGTTTATTCATTTTCTTCTTCGCTATTAGTTGGGCCCCAATTGCCTACGTTATTGTTGCCGAATCCTATCC
V M I V F T C L F I F F F A I S W A P I A Y V I V A E S Y P 448
1344 TTTGCGTGTCAAAAATCGTGCTATGGCTATTGCTGTTGGTGCCAACTGGATTTGGGGTTTCTTGATTGGTTTCTTCACTCCCTTCATTAC
L R V K N R A M A I A V G A N W I W G F L I G F F T P F I T 478
1434 AAGTGCAATTGGATTTTCATACGGGTATGTCTTCATGGGCTGTTTGGTATTTTCATTCTTCTACGTGTTTTTCTTTGTCTGTGAAACCAA
S A I G F S Y G Y V F M G C L V F S F F Y V F F F V C E T K 508
1524 GGGCTTAACATTAGAGGAAGTTAATGAAATGTATGTTGAAGGTGTCAAACCATGGAAATCTGGTAGCTGGATCTCAAAAGAAAAAAGAGT
G L T L E E V N E M Y V E G V K P W K S G S W I S K E K R V 538
1614 TTCCGAGGAATAAGAGATTATACTTAAACTAGCACTGATTTTTTTAAGGCTAATGGCTACTAATACTTTAATAGATGATCTTCATACTTT
S E E 541
1704 TTTATTTAACGATTTTTAATGATGTTTTTATTTGTACCACTCATTTATCTAGATTTTTTTAATACTGATCAAATCTTACGGACTCGACGT
1794 TAAAAAGTTCCTACATACGTCTGGTACTTGAAACGCTGCTTCGAGGTATTGACACTATAAGAATACGATCCAAATACTTACACCGCATGT
1884 AAAAATATGCCGACAATATGAATACTTGTTGATGAATGATATTTGATTTTAATCCGGCAATTTACCTCCTTTATATAATCCAATAATTGT
1974 TGATAATTAGTGGTTAGGTTGCAGTACTAATAAGAATTAAGACAAATATTCTTCTACTATATAAAAGGTGCAAACAAAACACACGCCGAT
2064 CGGCCATACT

FIG. 2. Nucleotide and deduced amino acid sequences of the *HXT2* gene. Numbers at the left correspond to the nucleotide position relative to the A of the putative ATG translational start codon; numbers at the right correspond to the amino acid residue position relative to that methionine. In the region 5′ to the putative start codon, two 14-bp direct repeats are enclosed in boxes, and a number of potential regulatory elements are underlined. Highly hydrophobic domains 21 residues in length are enclosed in shaded boxes. Residues conserved in position and identity with 16 other sugar transporters are circled. Potential N-linked glycosylation sites are bold underlined. Potential cAPK sites are double underlined. The site of *Hpa*I cleavage at which *LEU2* was inserted is indicated with an inverted triangle.

the same topographic position in HXT2. We have also observed that a leucine zipper is present in the sequences of GAL2 (53) and SNF3 (12) at this position.

***HXT2* similarities with other cloned genes.** The most similar DNA sequence to *HXT2* found in the search of the GenBank and EMBL data bases was the *rho11* cDNA (45; Fig. 5). Two segments of the *rho11* cDNA, referred to as *rho11-1* and *rho11-2*, have been sequenced (45). After alignment and introduction of nine single-base gaps and one two-base gap, the 177-bp *rho11-2* sequence is perfectly identical to *HXT2*. The *rho11-1* sequence is similar to a DNA segment which is 3′ of the *HXT2* termination codon. These latter sequences are identical at only 59 of 132 positions (44.7%) after introduction of one single-base gap. We note that the *rho11* mRNA is predicted (45) to be transcribed from the strand opposite that encoding the *HXT2* mRNA.

Southern analysis. High-stringency Southern blot analysis revealed only a single band when genomic DNA from four yeast strains was digested with restriction enzymes that have only a single recognition site within the *HXT2* region (Fig. 6A). The intensity of radioactive signal from the genomic *HXT2* sequence is qualitatively equal to that from a single genome equivalent of cloned *HXT2* DNA included as a positive control on the same Southern blot. This finding demonstrates that *HXT2* is present in only a single copy in the yeast genome. However, when the same blot was washed at low stringency, a number of other bands were revealed (Fig. 6B). One of these is probably the *HXT1* gene (D. A. Lewis and L. F. Bisson, unpublished data), and one is probably *GAL2* (53). However, the absence of autoradiographic signal from a single genome equivalent of cloned *SNF3* DNA suggests that the other bands represent other, uncharacterized sugar transport genes in the yeast genome. The same Southern blot was stripped, rehybridized with a

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     1                                                                100
Hxt2 MSEFATSRVE SGSQQTSIHS TPIVQKLETD ESPIQTKSEY TNAELPAKPI AAYWTVICLC LMIAFGGFVF GWDTGTISGF VNQTDFKRRF GQMKSDGTYY
  I  .......... .......... .......... .......... ..mepss.kv tg.rlmlavg g...lgslqf gyntgvInaf ynqt.wnhry ge.ip.tt..
 II  ..d..ls... s.s....int .es.d.l... rq..e..s.s ..g..pg..l ..y.kv...c ..va.ggf.f GyDt.lig.. .sl.aflk.. g.lh......
III  .......... .......... .......... ....q..s.. .......... ........is .vA.lgGlLF G.DtaVI.Ga v......... ..........

     101                                                              200
Hxt2 LSDVRTGLIV GIFNIGCAFG GLTLGRLGDM YGRRIGLMCV VLVYIVGIVI QIASSDKWYQ YFIGRIISGM GVGGIAVLSP TLISETAPKH IRGTCVSFYQ
  I  lttlws.LSV aiFsVGGMig SFsvGlfvnr fGRrnsMLmm llafv..vlm gfskl.ksfe mLIGRfiiGv ycGLttGfVP MYvGE.sPTa lRGAlGTlhq
 II  .s.v.t.l.v si.nlgg.fg .lfaspi.d. yGrkpt.iig lffv.g.iiq .af...k..m livGrv.sgf gvg.isa.vp .y.sE.apk. lRg.l.s.yq
III  ..s.L.g..V ssallG.aiG a...G..s.R .GR..slk.. a.lfvl...s .......... fi..Rv.gGI gVG.AS..aP .YiaE.apah .RGkl.S..Q

     201                                                              300
Hxt2 LMITLGIFLG YCTNYGTKDY SNSVQWRVPL GLNFAFAIFM IAGMLMVPES PRFLVEKGRY EDAKRSLAKS NKVTIEDPSI VAEMDTIMAN VETERLAGNA
  I  LgIVvGILia QvfGL.s.im gnadlWplLL s..f.pAlLQ cilLpfCPES PRfLlInrne Enraksvlkk lrgtadvtrd lqemkeesrq mmrek...kv
 II  l..t.Gilva a.v.ygt..y .n....r.pl gLq.aw.lfl aig.fllPES Pr.lvekgk. eear.sl.k. rk.p..d.lv d.El.eIkat ie.e.s.g..
III  lai..Gi.la ...n.gda.. .....WR.Mf ....iPAlL. ..l....PeS PRwL...G.. e.Ae.iLrk. .........v ...i.Ei... ...g......

     301                                                              400
Hxt2 SWGELFSNKG AILPRVIMGI MIQSLQQLTG NNYFFYYGTT IFNAVGMKDS FQTSIVLGIV NFASTFVALY TVDKFGRRKC LLGGSASMAI CFVIFSTVGV
  I  tilelfrs.p ayrq.iliav vLqlsQQlSG INavFYYSTs IFekAGvqqP vYATIGsGiV NtaFTvVSlf .VErAGRRtL hliGlaGMag C...m.tial
 II  .wd..fstks ...q.lltgi .lqwfqQfsg .n.i.yYg.v ff..vg.dns .lvsi...av nvastf..l. f..kfGRrkl ll.g.agmai .lfila.vg.
III  ....lf.... .....v.iGm .Lsa.QQFvG iNvi.YYap. .fk..Gftt. ll.T.ivG.i n...T..Ai. tVDKfGRKPl l.iG..gMai g...Lg..f.

     401                                                              500
Hxt2 TSLYPNGKDQ PSSKAAGNVM IVFTCLFIFF FAISWAPIAY VIVAESYPLR VKNRAMAIAV GANWIWGFLI GFFTPFITSA IGFSYGYVFM GCLVFSFFYV
  I  alL.....eq lpwmsylsiv aifg..FVaF FE.GPGPIPW FiVAElFSQg PRPAamAvAg fSNWTsNFiv gmcFqyv.ql cGpYVFiiFt vlLvFfiF.f
 II  ........t. pkkk.a.ngm .vficlfif. f..tw.pvvw vis.E.f.lr .rskctala. .anwv.qfvi afatpyi.s. iky...ff.g gflvamiv.y
III  .....ng... t...a.gi.a l.....yva. famSwgPvvW vLlsEifpnk iR..al..a. ..qW.aN.ii s.TFp.Lld. .g.g..y.y. t...ia..fi

     501                                  541
Hxt2 FFFVCETKGL TLEEVNEMYV EGVKPWKSGS WISKEKRVSE E
  I  ..kVPETkGr tFdeIasgFr qgg..... .asqsdktpe e
 II  .ff.pETkG. .leeiqel.. e.v.prks.. ..a.irr..n a
III  w.fvpETKgk TLE..E.... ...K..... TL..... .
```

FIG. 3. Homology of the HXT2 protein with a large family of procaryotic and eucaryotic sugar transporters. The HXT2 amino acid sequence is aligned with the consensus sequences from three subsets of this family. Subset I includes the four human glucose transporters, GLUT1 (41), GLUT2 (23), GLUT3 (33), and GLUT4 (22), the mouse 1a and 2a glucose transporters (32), and the rabbit brain (1) and rat brain (4) glucose transporters. Subset II includes the *S. cerevisiae* GAL2 galactose permease (53), SNF3 high-affinity glucose transporter (12), and MAL61 maltose permease (15), the *Kluyveromyces lactis* LAC12 lactose permease (13), and the *Chlorella kessleri* H^+-hexose cotransporter (49). Subset III includes the *E. coli xylE* H^+-xylose permease and *araE* H^+-arabinose permease (38) and the *Synechocystis* strain PCC6803 *glcP* glucose transporter (58). Each amino acid sequence was aligned individually with HXT2 by the GAP program (19), and then the sequences in each subset were aligned to HXT2 with gaps as a group. The consensus sequence for each subset was determined by the LINEUP program (19) and aligned with HXT2. The consensus sequences were edited to remove leading and trailing sequences and regions aligned with gaps in the HXT2 sequence. In the consensus sequences, capital letters indicate that all sequences in the subset have that residue at that position, lowercase letters indicate that the majority of sequences in the subset have that residue, and a dot indicates that no consensus residue occurs at that position.

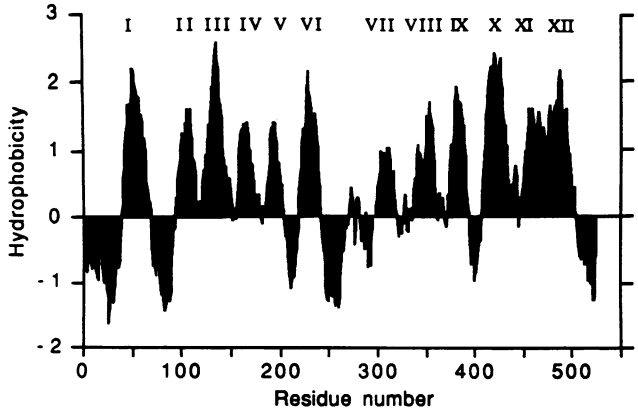

FIG. 4. Hydropathy analysis of HXT2. The amino acid sequence of HXT2 was provided to the PEPPLOT program (19), and the Kyte-Doolittle (36) hydropathy profile of the protein was calculated by using a window of 20 residues. Roman numerals indicate hydrophobic regions that are hypothesized to be TMDs (shaded boxes in Fig. 2).

SNF3 probe, and washed under identical low-stringency conditions. Only a single band was observed in each genomic DNA lane, at a size corresponding to that predicted for the *SNF3* gene, even after prolonged film exposure (data not shown); positive control DNA for *SNF3*, but not *HXT2* or *HXT1*, yielded an autoradiographic signal.

Disruption of the *HXT2* gene. To characterize the physiological role of HXT2, the *HXT2* gene was disrupted by transformation of YPH499 and YPH501 with the 4.2-kb *Mlu*I-*Xba*I fragment from pAK9a, which contains the *LEU2* gene inserted at codon 122 of *HXT2*. Leucine prototrophs were recovered from both the haploid and the diploid strain upon plating of the transformants on YNB high-glucose plates lacking leucine. One haploid Leu$^+$ strain, LBY406, was selected for detailed analysis. The Leu$^+$ phenotype was mitotically stable in this strain (data not shown). Genomic DNA was isolated from LBY406, double-digested with *Eco*RI and *Hin*dIII, and separated by agarose gel electrophoresis. Southern hybridization with *HXT2* and *LEU2* probes clearly showed that the *HXT2* gene in LBY406 was disrupted by the *LEU2* gene (data not shown). This result suggests that the *HXT2* gene is not essential for cell viability. In the cross of LBY404 (*HXT2 snf3::HIS3*) × LBY406 (*hxt2::LEU2 SNF3*), the Leu$^+$ phenotype segregated 2:2 among the spores (n = 23 tetrads), which confirms that the *hxt2::LEU2* DNA fragment had inserted at a single site in the LBY406 genome. This cross also showed that *HXT2* and *SNF3* are unlinked (5 parental ditype, 2 nonparental ditype, 16 tetratype; $0.7 > P > 0.5$).

Growth phenotypes of *HXT2* and *SNF3* alleles. Figure 7 shows growth curves of four strains derived from a single tetrad, which carry wild-type or null alleles of *HXT2* and *SNF3*. The exponential-phase growth rates of the strains with defective glucose transporters were indistinguishable from the rate of the wild-type strain, LBY410. The lag phase of the *hxt2 snf3* double null strain LBY416 was prolonged by about one generation, before the cells commenced exponential growth. All strains achieved a similar final cell density, but the strains with null alleles of these high-affinity glucose transporters approached this density more slowly than did the wild type. This was particularly apparent for LBY413 (*hxt2 SNF3*). On low-glucose plates, LBY413 developed colonies smaller than wild type, whereas LBY411 and LBY416 were unable to grow (data not shown).

Glucose transport phenotypes of *HXT2* and *SNF3* alleles. The glucose transport kinetics of four strains, derived from a single tetrad and carrying the different combinations of *HXT2* and *SNF3* wild-type and null alleles, were determined by short-term [^{14}C]glucose uptake assays (Fig. 8). When LBY410 (*HXT2 SNF3*; Fig. 8A) was grown on high glucose, only low-affinity transport was apparent, whereas a significant high-affinity transport system was evident after the cells were shifted to low glucose for 90 min, as expected. Transport by LBY413 (*hxt2 SNF3*; Fig. 8B) was not significantly

```
A.  HXT2      1767 ACTGATCAAATCTTACGGACTCGACGTTAAAAAGTTCCTACATACGTCTGGTACT.TGAAACGCTGC
    rholl-1    132 ACaacTagAgTgagAtctcaTCGACGcgtgAAtagTagTACATAtagagaGTAaTGTacAgtaaTGt

    HXT2                TTCGAGGTATTGACACTATAAGAATACGATCCAAATACTTACACCGCATGTAAAAATATGCCGAC 1897
    rholl-1             gTaGAatgAaTcAtAaTAcccatAatCacaCgAggTAaaatagCacCATGTgAgAATAacgCacC 1

B.  HXT2      1026 CAACGCCGTCGGTATGAAAGATTCTTTCCAAACTTCCATCGTTTTAGGTATAGTCAACT
    rholl-2    168 CAACGC.GTCG.TATGAAAGAT.CTT.CCAAACT.CCATCGTTT.AG.TATAGTCAACT

    HXT2                TCGCA..TCCACTTTCGTGGCCTTATACACTGTTGATAAATTTGGTCGTCGTAAGTGTC
    rholl-2             TCGCCAATC.ACTTTCGTGGCCTTATACACTGTTGATAAATTTGGTCGTCGTAAGTGTC

    HXT2                TATTGGGTGGTTCTGCTTCCATGGCCATTTGTTTTGTTATCTTCTCTACTGTCGGTGTC  1194
    rholl-2             TATTGGGTGGTTCTGCTTCCATGGCCATTTGTTTTGTTATCTTCTCTACTGTCG.TGTC  1
```

FIG. 5. Sequences of the *rholl* (45) cDNA fragments optimally aligned to the nucleotide sequence of *HXT2* by the GAP program (19). Gaps were introduced by GAP, and additional gaps were added by eye in panel B. Lowercase letters represent nucleotides in the *rholl* sequence which do not match the sequence of *HXT2*. Note that the complementary strands of the *rholl* cDNAs are presented. (A) Alignment of *HXT2* from nucleotides 1767 to 1897 with the *rholl-1* cDNA fragment; (B) alignment of *HXT2* from nucleotides 1026 to 1194 with the *rholl-2* cDNA fragment.

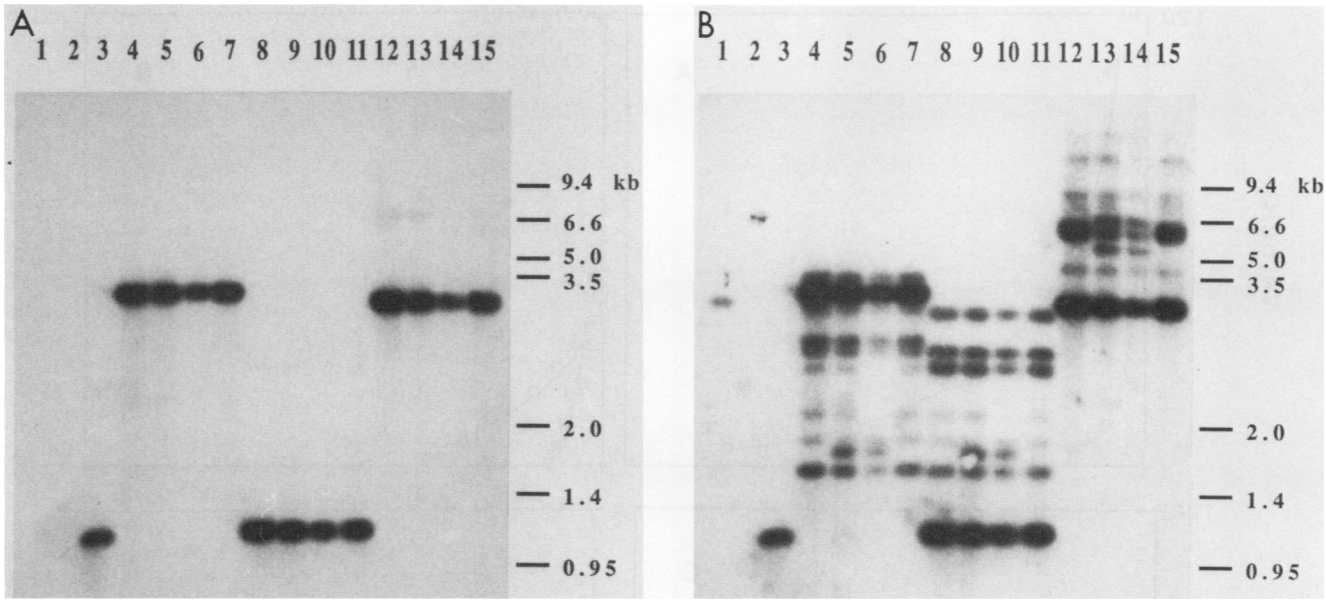

FIG. 6. Southern blot analysis of the *HXT2* gene. Genomic DNA of strains X2180-1A, DFY1, MCY1407, and YPH500 from 8 A_{580} units of cells were digested with the indicated restriction enzymes, electrophoresed in a 1% agarose gel, and transferred to Nytran nylon membranes. The 1.1-kb *Eco*RI-*Hin*dIII fragment of *HXT2* was radiolabeled by random priming (21) and hybridized to filter-bound DNA in 6× SSPE at 65°C for 14 h as prescribed by Schleicher & Schuell. Lanes: 1, 300 pg of pDAL1, containing the *Eco*RI-*Xba*I fragment of *HXT1*; 2, 300 pg of pLN19 (44), containing *SNF3*, digested with *Eco*RI; 3, 300 pg of the 1.1-kb *Eco*RI-*Hin*dIII fragment of *HXT2*; 4 to 7, genomic DNAs digested with *Hin*dIII; 8 to 11, *Hin*dIII-*Eco*RI double digest; 12 to 15, *Eco*RI digest; 4, 8, and 12, MCY1407; 5, 9, and 13, DFY1; 6, 10, and 14, X2180-1A; 7, 11, and 15, YPH500. (A) High-stringency wash. The blot was washed as for panel B, with a further wash of 0.1× SSPE–0.5% sodium dodecyl sulfate (SDS) at 65°C for 30 min. (B) Low-stringency wash. The blot was washed twice with 6× SSPE–0.5% SDS for 15 min at room temperature and then twice with 2× SSPE–0.5% SDS for 15 min at 37°C.

different from that by the wild type grown on high glucose. However, this strain lacked a major component of the high-affinity system after being shifted to low glucose. LBY411 (*HXT2 snf3*; Fig. 8C) was also deficient in a component of the high-affinity system when shifted to low glucose, as previously reported (8). Furthermore, the residual high-affinity system of LBY411 was not significantly repressed in cells from high-glucose medium. In the double null strain LBY416 (Fig. 8D), high-affinity transport was severely diminished. The presence of residual high-affinity transport in cells from high glucose was also seen in this strain. Patterns of transport kinetics identical to the pattern for LBY413 were observed for LBY406 (the original *hxt2*::*LEU2* disruptant) and for LBY414, which is isogenic to LBY413. Similarly, other wild-type and *snf3* strains gave results identical to those for the strains of the genotypes depicted in Fig. 8.

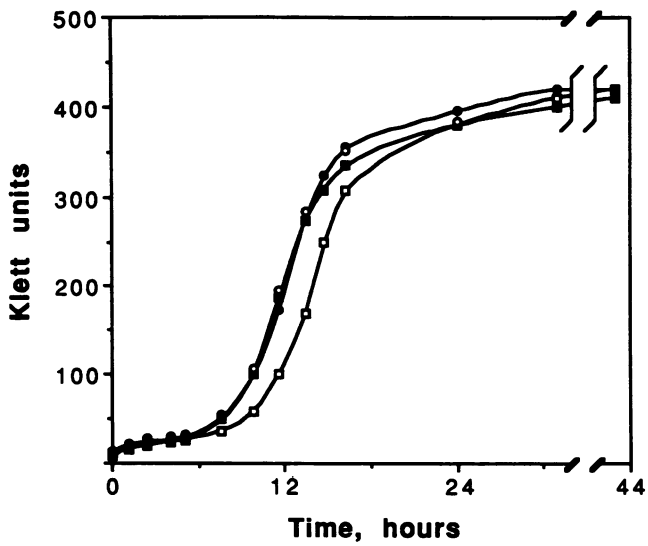

FIG. 7. Growth curves of wild-type, *hxt2*, and *snf3* strains. Cultures were grown in side-arm culture flasks, and the optical density was measured at the times indicated with a Klett-Summerson photometer fitted with a green filter. Symbols: ●, LBY410 (*HXT2 SNF3*); ○, LBY411 (*HXT2 snf3*::*HIS3*); ■, LBY413 (*hxt2*::*LEU2 SNF3*); □, LBY416 (*hxt2*::*LEU2 snf3*::*HIS3*).

DISCUSSION

The long open reading frame in the SC2 DNA fragment, designated *HXT2*, is inferred to be an active gene: it is preceded by sequences which are likely to promote transcription in a carbon source or physiological state-dependent fashion, and the environment of the putative start codon is similar to that of actual yeast translational start sites. Preliminary RNA blot experiments have indicated that a transcript of the appropriate sense and length to encode HXT2 is more abundant in cells grown on high glucose than on low glucose (unpublished data).

On the basis of our observations, we propose that *HXT2* encodes a high-affinity glucose transporter: (i) deletion of either end of the *HXT2* region results in loss of the ability to restore growth on low glucose to a *snf3* null mutant; (ii) the HXT2 protein is highly homologous to a number of other sugar transporters, including yeast and mammalian glucose transporters; (iii) growth of *hxt2* null mutants is impaired in media which have been depleted of glucose; and (iv) *hxt2*

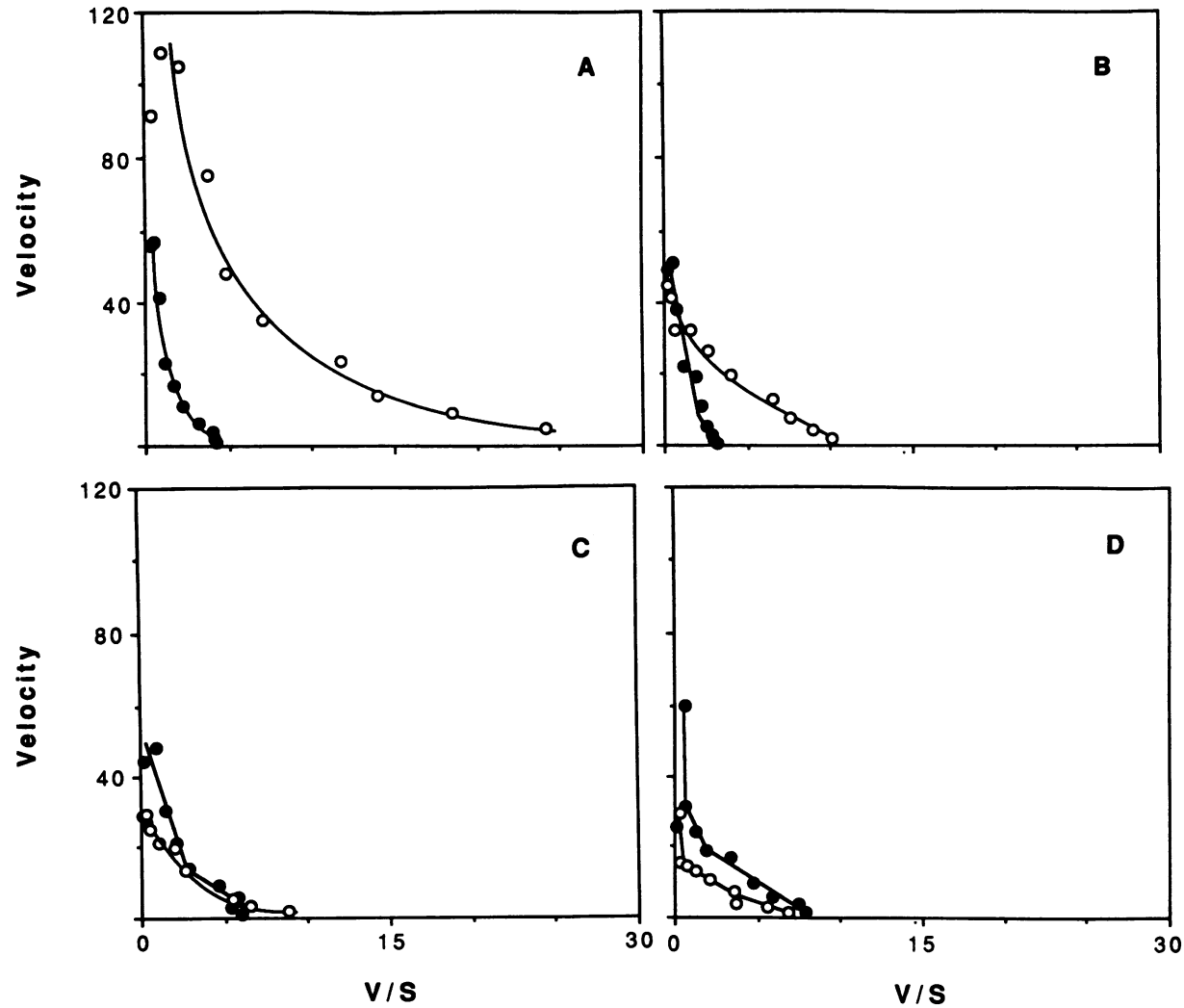

FIG. 8. Eadie-Hofstee plots of glucose uptake by wild-type, *hxt2*, and *snf3* strains. Velocity is expressed as nanomoles of glucose per minute per milligram (dry weight); V/S is expressed as velocity per millimolar concentration. Symbols: ○, cells shifted to low glucose for 90 min; ●, cells shifted to high glucose for 150 min. (A) Strain LBY410 (*HXT2 SNF3*); (B) strain LBY413 (*hxt2::LEU2 SNF3*); (C) strain LBY411 (*HXT2 snf3::HIS3*); (D) strain LBY416 (*hxt2::LEU2 snf3::HIS3*). The glucose concentration remaining in the medium at the end of the shift period averaged 0.02% (low glucose) or 0.34% (high glucose).

null mutants are deficient in a significant component of high-affinity glucose transport.

The diminished high-affinity glucose transport capability displayed by the *hxt2* null strain LBY413 demonstrates that *HXT2* is necessary for some component of high-affinity glucose transport in a *SNF3* genetic background. A very similar phenotype of partial loss of high-affinity uptake is observed in *snf3* null strains that are wild type at the *HXT2* locus. Thus, loss of either *HXT2* or *SNF3* leads to a deficiency in high-affinity glucose uptake. Null *snf3* mutants in this genetic background are unable to grow on low-glucose plates. The *hxt2* null strains, on the other hand, are only partially defective in growth on low glucose.

A number of sequences were found to cross-hybridize with *HXT2* in Southern blots washed at low stringency, suggesting that other transporterlike proteins may also be encoded in the yeast genome. It is of interest that *SNF3* was not among these cross-hybridizing genes, though it has significant amino acid and functional homology with *HXT2*. A low level of high-affinity glucose uptake was detectable in the double null (*hxt2::LEU2 snf3::HIS3*) strain and may be the consequence of the expression and activity of one or more of the other homologs. It is important to note that *snf3* null mutations do not confer growth defects on low glucose in all genetic backgrounds. Point or null *snf3* mutations in strain DFY1 display no discernible phenotype (5). The differences in severity of growth defects resulting from loss of one or more of the glucose transporter genes in different genetic backgrounds is understandable in light of the observation that *S. cerevisiae* has many genes closely related to the *HXT2* gene. Furthermore, strains DFY1 and X2180-1B display a prominent cross-hybridizing band in Fig. 6B that is not observable in YPH500 or MCY1407, two related strains that are isogenic to S288C.

It is noteworthy that in the conditions used for this study, some high-affinity transport was expressed in the *snf3* and *snf3 hxt2* null strains when grown on high glucose (Fig. 8C and D). This has not been previously observed and may reflect differences in the growth medium or culture conditions. In previous studies (e.g., reference 8), YP high-

glucose medium was not supplemented with adenine or uracil, and very dilute inocula underwent 15 generations to reach early log phase before assays of glucose transport were performed. In the work described here, cells were grown for four to six generations before transport assays were done. Possibly strains containing *snf3* or *hxt2* alleles express low levels of some other, stable high-affinity glucose transport system under these conditions.

The potential asparaginyl glycosylation site at residue 82 (Asn-Gln-Thr) of HXT2 occurs between the first and second transmembrane domains. The identical tripeptide sequence is found in the corresponding topological position of the human erythrocyte GLUT1 glucose transporter (residue 44; 41). All other mammalian facilitated diffusion glucose transporters which have been sequenced have glycosylation sites in this region as well. However, the other yeast sugar transporters whose sequences are known do not have glycosylation sites in this region. The GLUT1 transporter has been shown by proteolytic and endoglycosidic digestion to be glycosylated at residue 44; the hydrophilic region containing the glycosylation site has been mapped to the extracellular side of the plasma membrane (11, 18, 41, 42).

The leucine zipper motif in *HXT2* is one of the conserved features of the sugar transport family. It is found in GAL2 (53), SNF3 (12), and a number of vertebrate glucose transporters (57). Leucine zippers are able to form coiled coil structures, which result in strong hydrophobic interactions between two proteins surfaces. The leucine zippers of glucose transporters have been proposed to mediate oligomer formation (57). Furthermore, Janoshazi and Solomon (30) have shown that the anion, cation, and glucose transporters of the human erythrocyte plasma membrane interact directly, perhaps forming a transport protein complex. The anion-exchange protein has also been shown to bind cytosolic glycolytic enzymes (31). Our analysis of the erythrocyte anion-exchange protein sequence has identified numerous leucine zipper motifs. In view of the dependence of high-affinity glucose transport on glucose kinase activity, and on both the HXT2 and SNF3 proteins, complexes of membrane transport and cytosolic proteins may occur in yeast cells, and the leucine zippers of HXT2 and SNF3 may mediate some of the protein-protein contacts.

Two types of regulatory motifs were found in the upstream region of *HXT2*. One type, consisting of a pair of CT-rich direct repeats (CT blocks) and the sequences TTCCA and CAAG, have been found in transcriptional regulatory regions of glycolytic genes (9, 20, 50).

The other regulatory motif conforms to the extended critical promoter of the mitochondrial RNA polymerase and was defined by identifying nuclear genes which have a consensus mitochondrial promoter in their 5′ untranscribed regions and are transcribed in vitro by the mitochondrial RNA polymerase (40). It has been proposed that transcription of certain nuclear and mitochondrial genes is coordinated by a common *trans*-acting factor which acts through the extended critical promoter (40).

Furthermore, the *HXT2* gene was found to have high homology to a gene, *RHO11* (45), whose expression is controlled by mitochondrial genotype. The *rho11* mRNA is expressed at elevated levels in a yeast strain with a hypersuppressive *rho* mitochondrial genotype (i.e., which is impaired in respiration and lacks most of the mitochondrial genome). The *rho11* cDNA is not detectable in a congenic respiration-deficient *mit* strain (45). The *rho11* gene is proposed to be repressed in respiring cells and to be transcriptionally regulated by a mitochondrially encoded factor (45). *HXT2* may be allelic to *rho11* and thus may be under similar transcriptional control. Alternatively, *rho11* and *HXT2* may be related by a recent gene duplication and could have diverged in their patterns of regulation.

HXT2 has two consensus cyclic AMP-dependent protein kinase (cAPK) phosphorylation sites. We have not yet assessed whether these sites are phosphorylated in vivo or in vitro. However, the cAPK site at residue 266 is conserved between GAL2 (53) and MAL61 (15), and the site at residue 539 may be homologous to a cAPK site in the C terminus of the human hepatic glucose transporter GLUT2 (23). Ramos and Cirillo (46) have shown by genetic means that both the galactose and high-affinity glucose transport systems are catabolite inactivated (29) and that inactivation is dependent on cAPK activity. Busturia and Lagunas (10) have also shown that glucose transport is catabolite inactivated in response to the metabolic status of the cell. In both of these studies, high-affinity glucose transport kinetics were measured and shown to disappear in a manner consistent with catabolite inactivation of the transport proteins. Perhaps catabolite inactivation of high-affinity glucose transport involves phosphorylation of HXT2 by cAPK, followed by its sequestration, degradation, or both. Studies are currently under way to test this hypothesis.

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