Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in *Saccharomyces cerevisiae*

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Abstract The hexose transporter family of *Saccharomyces cerevisiae* comprises 18 proteins (Hxt1–17, Gal2). Here, we demonstrate that all these proteins, except Hxt12, and additionally three members of the maltose transporter family (Agt1, Ydl247, Yjr160) are able to transport hexoses. In a yeast strain deleted for *HXT1–17*, *GAL2*, *AGT1*, *YDL247w* and *YJR160c*, glucose consumption and transport activity were completely abolished. However, as additional deletion of the glucose sensor gene *SNF3* partially restored growth on hexoses, our data indicate the existence of even more proteins able to transport hexoses in yeast.

Key words: Functional analysis; Gene family; Sugar transport; Multiple deletion; Yeast; Gene redundancy

1. Introduction

The preferred carbon sources for most prokaryotic and eukaryotic cells are carbohydrates, above all the monosaccharide glucose. This biomolecule makes up most of the organic matter on earth. An obligatory and essential step in the utilization of glucose is its transport across the plasma membrane into cells, a process that is achieved by transporter proteins. Different mechanisms have evolved in various organisms mediating the transport of glucose, i.e. proton symport systems, Na^+-glucose cotransporters, binding protein-dependent systems, phosphotransferase systems and facilitated diffusion systems [1–3]. One of the most prominent glucose utilizing organisms is the yeast *Saccharomyces cerevisiae*. The yeast sugar permease family consists of 34 proteins, 18 of which comprise the hexose transporter subfamily [4–6] (Fig. 1). As transport across the plasma membrane is an important step in controlling the rate of glucose metabolism, the elucidation of the functions of this multitude of glucose transporter-related proteins is a fundamentally important question. Moreover, it might provide insights into the general principles of glucose transport in other organisms also.

2. Materials and methods

2.1. Yeast strains and growth conditions

Yeast strains were as described in Table 1. Media and growth conditions were as described in [7]. Growth properties were determined on synthetic medium (YNB+$(NH_4)_2SO_4$+carbon source) only supplemented for auxotrophic requirements.

2.2. Construction of multiple deletion mutants

Strains lacking multiple sugar transporter genes were constructed with the *loxP::kanMX::loxP*/Cre recombinase system and the 'short flanking homology PCR' technology [8]. The primers used for the construction of the replacement PCR constructs (obtained from MWG-Biotech) are listed in Table 2. For the deletion of gene pairs *HXT15* and *HXT16*, *HXT13* and *HXT17*, *YDL247w* and *YJR160c*, the same primer pairs could be used, respectively. As induction of the galactose-inducible, glucose-repressible Cre recombinase by galactose appeared to have deleterious effects on cells containing several *loxP* sites, we routinely used maltose (which has a weaker repressive effect than glucose) to induce/derepress *loxP*-Cre recombination. The complete *GAL2* coding region together with about 390 bp of its 5′ promoter region and 125 bp of the 3′ region was replaced with a *URA3* gene which was flanked by 58 bp direct repeats, using plasmid pAK83 (a kind gift of A. Kruckeberg). The *URA3* gene was excised with FOA selection. Yeast transformation was as described [9]. The sugar transporter genes were deleted successively in the following sequence: *HXT13*, to *HXT15, HXT16* (*HXT17*), *HXT14*, *HXT12*, *HXT9*, *HXT11*, *HXT10*, *HXT8*, *HXT5-1-4*, *HXT2*, *HXT3-6-7, GAL2, STL1, AGT1, YDL247w, YJR160c*, *SNF3* and *RGT2*, selecting for G418 resistance on yeast extract-peptone medium with 2% maltose. As *HXT5-HXT1-HXT4* and *HXT3-HXT6-HXT7* are arranged as two clusters of transporter genes, in both cases, the complete clusters were deleted. The correct replacements were confirmed by PCR, phenotype, and in some cases Southern or Northern analysis.

2.3. Construction of sugar transporter overexpression plasmids

The sugar transporter genes were amplified from strain FY1679 or CEN.PK2-1C with the GENEPAIRS primers obtained from Research Genetics, using whole-cell PCR with the Expand High Fidelity PCR system of Boehringer Mannheim for 10–15 amplification cycles. PCR products were further amplified for 20 cycles with primers T1-ORFs (5′-**GTAATACAGG GTCGTCAGAT ACATAGATAC AATTCTATTA CCCCCATCCA TAC***GGAATTC CAGCTGACCA CC*-3′) and T2-ORFs (5′-**GGGGGAGGGC GTGAATGTAA GCGTGACATA ACTAATTACA TGACTCGAG***G ATCCCCGGGA ATTGCCATG*-3′). At their 3′ ends, these primers are homolog to the common sequences added to the 5′ ends of the GENEPAIRS primers (italic); at their 5′ ends, they are homolog to the sequences flanking the multiple cloning site of vector p426MET25 (bold) [10]. p426MET25 was linearized with *Eco*RI and *Sal*I and transformed into the *hxt1–17 gal2 stl1 agt1* mutant strain, together with the PCR-amplified sugar transporter genes, selecting for uracil prototrophy on a 2% maltose medium after homologous recombination in yeast. For most genes, two or more independently generated PCR products were used. Plasmids were re-isolated as described [11], amplified in *Escherichia coli* strain SURE (Stratagene), analyzed by restriction enzyme mapping or sequencing (Seqlab), and re-transformed into the *hxt1–17 gal2* deletion strain for complementation analysis. Plasmids carrying *HXT15* and *HXT16* could not be amplified in *E. coli*.

2.4. Other molecular biology techniques

Genomic replacement of the *AGT1* coding regions by a PCR-amplified *lacZ-kanMX* reporter cassette was used to fuse the promoter and the first 48 nucleotides of the truncated *AGT1* ORF to the *E. coli lacZ* gene [12]. For PCR amplification of the *lacZ-kanMX* reporter

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cassette, plasmid pUG6lacZ [12] and the primers listed in Table 2 were used. DNA and RNA were prepared and manipulated according to published procedures [13,14].

2.5. Sugar uptake assays and fermentation studies

Sugar uptake assays and fermentation studies were as described [15]. Fermentation studies were done in 50 mM potassium phosphate buffer, pH 6.3, supplemented with 1% glucose. Glucose transport was measured as uptake of D-[U-^{14}C]glucose (Amersham). Glucose uptake activity in wild-type and in *hxt* mutant cells was determined at glucose concentrations of 10 or 50 mM for 15 or 60 s.

3. Results

3.1. Deletion of all hexose transporter genes

As the sequence of the yeast genome was being completed, altogether 18 different genes, *HXT1–HXT17* and *GAL2*, were identified that encode proteins belonging to the yeast hexose transporter family (Fig. 1) [5,16]. Only seven of the hexose transporter proteins, Hxt1–4, 6, 7 and Gal2, have been functionally characterized so far (reviewed in [16]). However, nothing is known about the actual functions of Hxt5 and Hxt8–Hxt17.

In order to dissect the function of the complete hexose transporter family of *S. cerevisiae*, we deleted all 18 members of the family in the CEN.PK2-1 yeast strain (Table 1) by succession of one-step gene deletions using a *loxP-kanMX-loxP* resistance marker designed for repeated use [8]. Additionally, some of the deletions were made in strain EBY.FY100B derived from FY1679 [17]. Southern analysis revealed that, in contrast to the FY1679 strain, the CEN.PK2-1C wild-type strain does not contain the *HXT17* gene (data not shown). The hexose transporter genes were deleted successively starting with *HXT17/16*. Deletion of all the genes from *HXT17/16* to *HXT8* plus *HXT5*, *HXT4*, *HXT2* and *HXT1* in strains EBY.FY100B and CEN.PK2-1C yielded the mutant strains EBY.FY367$^+$ and EBY.VW367$^+$, respectively. These strains, which still contained *HXT3*, *HXT6* and *HXT7*, did not exhibit any growth defects on glucose-, fructose- or mannose-containing media as compared to the corresponding wild-type strains (Fig. 2).

HXT3, *HXT6* and *HXT7* comprise a cluster of three hexose transporter genes on chromosome IV. When this cluster was deleted in strain EBY.VW367$^+$, the cells could no longer grow on glucose, fructose or mannose as the sole carbon sources. However, growth on maltose, which is taken up by a different uptake system, was indistinguishable from that of the wild-type strain (Fig. 2). Deletion of the *GAL2* gene in the *hxt1–17* mutant or the CEN.PK2-1C wild-type strain strongly reduced growth on galactose (Fig. 2). The growth properties of the *hxt1–17 gal2* strain were characterized in detail on synthetic solid media with various hexoses (2% glucose, fructose, mannose, galactose) as well as 2% maltose, sucrose, raffinose, ethanol or glycerol, and also on media containing various concentrations (0.2%, 2%, 20%) of glucose, at 15°C, 30°C and 37°C. Under all conditions tested, even after 1–2 weeks of incubation, no growth could be observed on glucose, fructose, mannose, sucrose or raffinose, growth on galactose was very slow, whereas the growth rates on maltose, ethanol or glycerol were identical to those of wild-type cells (Fig. 2 and data not shown). Moreover, the hexose transporter-less strain was not defective in mating or sporulation, in the homozygous mutant diploid form, which was obtained after transformation with a plasmid carrying the HO endonuclease gene.

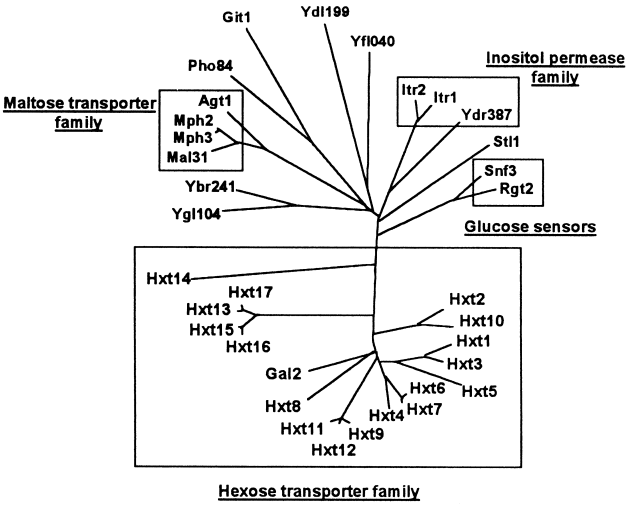

Fig. 1. The yeast sugar transporter homologues.

3.2. Overproduction of all hexose transporters

It has already been shown that Hxt1–Hxt4, Hxt6 and Hxt7 are the major hexose transporters in yeast and can transport glucose, fructose and mannose [15,18]. To investigate whether all of the other hexose transporter-related proteins, if present in sufficient amounts, have the intrinsic capacity to transport glucose or one of the other hexoses, each of the other *HXT* genes was overexpressed from multicopy plasmids under control of the strong *MET25* promoter in the *hxt1–17 gal2* deletion strain. We found that all of the Hxt proteins but Hxt12 are able to transport at least one of the different hexoses and

Table 1
Yeast strains

Strain	Genotype	Source
CEN.PK2-1C (=VW1A)	*MATα leu2-3,112 ura3-52 trp1-289 his3-Δ1 MAL2-8^c SUC2 hxt17Δ*	K.-D. Entian
EBY.VW367$^+$	CEN.PK2-1C *hxt13Δ::loxP hxt15Δ::loxP hxt16Δ::loxP hxt14Δ::loxP hxt12Δ::loxP hxt9Δ::loxP hxt11Δ::loxP hxt10Δ::loxP hxt8Δ::loxP hxt514Δ::loxP hxt2Δ::loxP*	This study
EBY.VW1000	EBY.VW367$^+$ *hxt367Δ::loxP gal2Δ*	This study
EBY.VW2000	EBY.VW1000 *snf3Δ::loxP*	This study
EBY.VW4000	EBY.VW1000 *stl1Δ::loxP agt1Δ::loxP ydl247wΔ::loxP yjr160cΔ::loxP*	This study
EBY.VW5000	EBY.VW4000 *snf3Δ::loxP rgt2Δ::loxP*	This study
EBY.FY100B	*MATα ura3-52 trp1Δ63 his3Δ200* (derived from FY1679)	This study
EBY.FY367$^+$	EBY.FY100B *hxt13Δ::loxP hxt15Δ::loxP hxt16Δ::loxP hxt17Δ::loxP hxt14Δ::loxP hxt12Δ::loxP hxt9Δ::loxP hxt11Δ::loxP hxt10Δ::loxP hxt8Δ::loxP hxt514Δ::loxP hxt2Δ::loxP*	This study

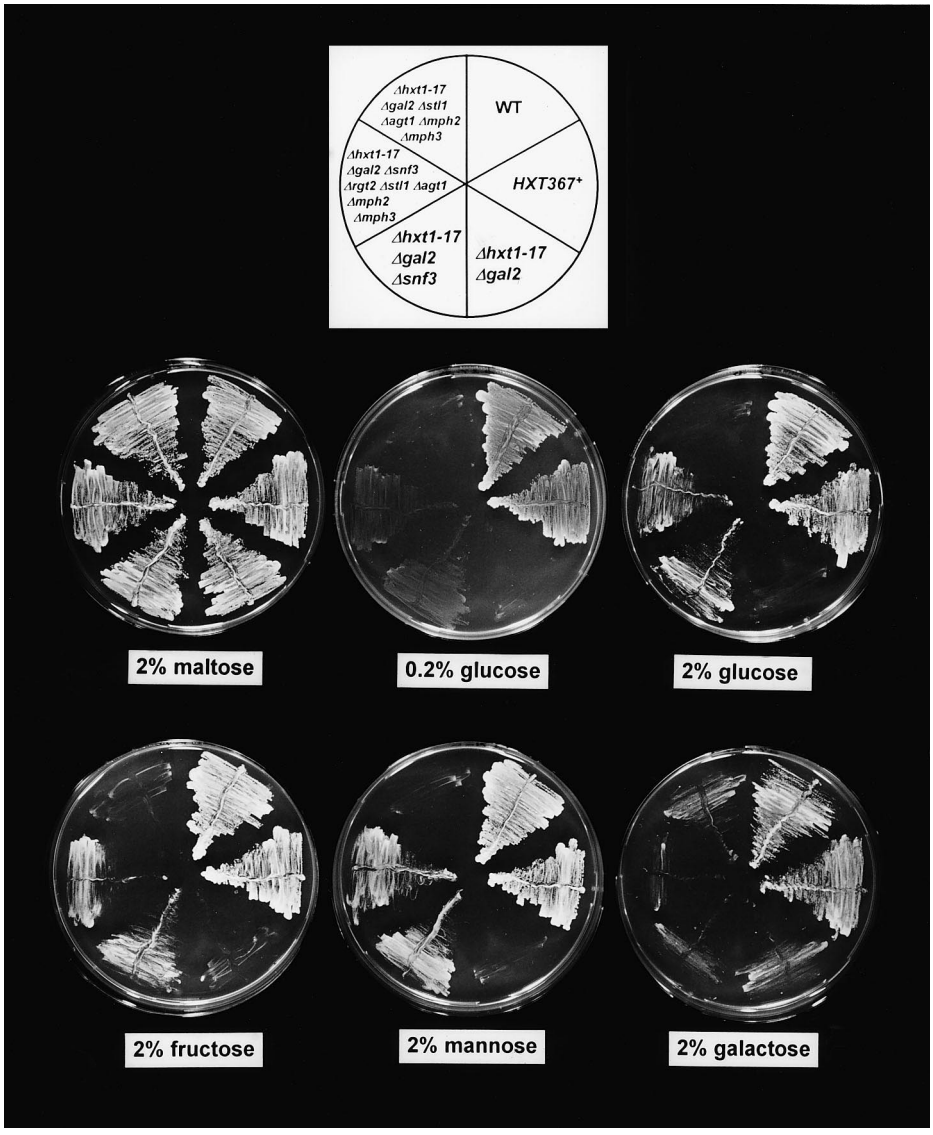

Fig. 2. Growth of wild-type yeast strain CEN.PK2-1C and of various sugar transporter deletion mutants on different sugars. The cells were pregrown on 1% yeast extract-2% peptone medium with 2% maltose, and streaked on solid media consisting of 0.67% Difco yeast nitrogen base with ammonium sulfate, supplemented with leucine, uracil, tryptophan and histidine and various sugars. The plates were incubated at 30°C for 2 days (2% maltose) or 3 days (other sugars). The strains are: EBY.VW367 (*HXT367*$^+$), EBY.VW1000 (*Δhxt1–17 Δgal2*), EBY.VW2000 (*Δhxt1–17 Δgal2 Δsnf3*), EBY.VW5000 (*Δhxt1–17 Δgal2 Δsnf3 Δrgt2 Δstl1 Δagt1 Δmph2 Δmph3*), EBY.VW4000 (*Δhxt1–17 Δgal2 Δstl1 Δagt1 Δmph2 Δmph3*).

permit growth of the *hxt1–17 gal2* deletion strain, although with different substrate specificities (Fig. 3). We were not able to functionally express Hxt12, supporting the view that *HXT12* is a pseudogene [4,5].

3.3. Cloning and deletion of additional glucose transporters

The glucose uptake activity of the *hxt1–17 gal2* mutant cells was below 2 nmol/min/mg dry weight, in contrast to the corresponding wild-type cells where glucose uptake activity reached about 13–15 nmol/min/mg dry weight. However, determination of glucose consumption revealed that after growth on maltose, the *hxt1–17 gal2* strain still exhibits a residual capacity to utilize glucose (Fig. 4). In contrast to the wild-type strain, the glucose consumption rate of the multiple mutant slowly decreased and ceased altogether after about 15 h when about half of the glucose had been consumed, suggesting that the proteins responsible for transport become repressed by glucose. To identify the protein(s) that mediate residual glucose uptake, all of the remaining 16 sugar transporter homologues of yeast (Fig. 1) were overexpressed in the *hxt1–17 gal2* strain from multicopy plasmids controlled by the *MET25* promoter. Overproduction of three of the proteins belonging to the maltose permease subfamily, Agt1, Ydl247 (= Mph2) and Yjr160 (= Mph3), but not Mal31, mediated growth of the cells on glucose, but not on fructose, mannose or galactose (data not shown). None of the other 12 proteins tested, including the glucose sensors Snf3 and Rgt2, was able to support growth on any of the different hexoses.

To analyze transcriptional regulation of the *AGT1* gene, which encodes a general α-glucoside transporter [19,20], the *AGT1* coding region in strain CEN.PK2-1C was replaced by a *lacZ-kanMX* reporter cassette. Measuring of β-galactosidase

activities revealed that the *AGT1* gene is strongly induced by maltose (1382 mU/mg protein) but repressed by glucose (23 mU/mg protein). The results indicate that Agt1 and probably also the other maltose permease homologues, Mph2 and Mph3, mediate residual glucose uptake by the *hxt1–17 gal2* strain after prior growth on maltose, but become repressed by glucose leading to cessation of glucose consumption and growth. In agreement with these observations, deletion of all three genes, *AGT1*, *YDL247w*/*MPH2* and *YJR160c*/*MPH3*, in the *hxt1–17 gal2* mutant strain completely abolished residual glucose consumption (Fig. 4).

3.4. Additional deletion of the glucose sensor genes

SNF3 and *RGT2* encode glucose sensors that generate a glucose signal in response to low and high concentrations of glucose, respectively, ultimately leading to transcriptional induction of several *HXT* genes (reviewed in [21]). Surprisingly, after deletion of the *SNF3* gene, but not the *RGT2* gene, in the *hxt1–17 gal2* or the *hxt1–17 gal2 agt1 mph2 mph3* mutant strain, growth of the cells on glucose, fructose, mannose and sucrose was partially restored (Fig. 2). Also, the *hxt1–17 gal2*

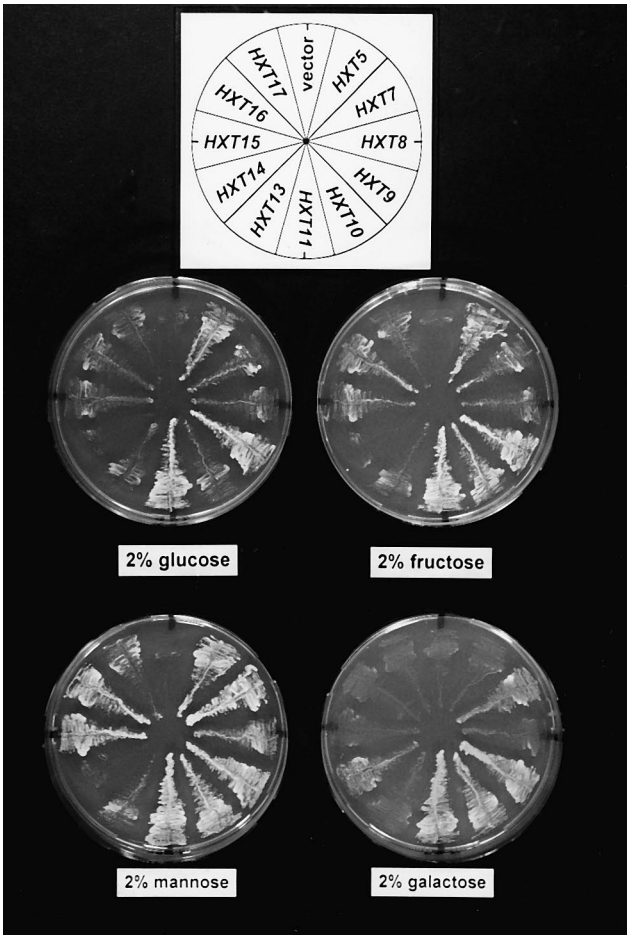

Fig. 3. Growth of *hxt* transformants overexpressing individual *HXT* genes. The *hxt1–17 gal2* deletion strain EBY.VW1000 was transformed with p426MET25-derived multicopy plasmids expressing the different *HXT* genes under control of the *MET25* promoter. The transformants were pregrown on media consisting of 0.67% Difco yeast nitrogen base with ammonium sulfate, leucine, tryptophan and histidine, and with 2% maltose as the carbon source, and streaked on the same solid medium with different sugars as carbon sources. The plates were incubated at 30°C for 3 days.

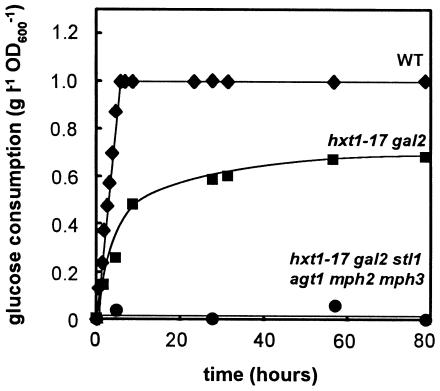

Fig. 4. Glucose consumption in *hxt* mutant strains. Yeast cells were grown in 1% yeast extract-2% peptone medium with 2% maltose into the exponential growth phase. Cells were then washed once and resuspended in 50 mM KP_i buffer, pH 6.3, supplemented with 1% glucose at 30°C. Samples were removed at different times and assayed for glucose concentration. The initial glucose consumption rate of the wild-type strain was about 0.2 g/l/OD_{600}, that of the *hxt1–17 gal2* mutant strain reached about 0.06 g/l/OD_{600}, and no glucose consumption was detectable in the *hxt1–17 gal2 stl1 agt1 mph2 mph3* mutant strain. ♦ CEN.PK2-1C (wild-type), ■ EBY.VW1000 (*hxt1–17 gal2*), ● EBY.VW4000 (*hxt1–17 gal2 stl1 agt1 mph2 mph3*).

agt1 mph2 mph3 snf3 rgt2 deletion strain could still grow on these hexoses. These results indicate a repressing function of Snf3 on an additional protein that is able to transport hexoses. Indeed, we found that the *STL1* gene became derepressed after deletion of *SNF3* in the *hxt1–17* mutant strain (data not shown). However, deletion of the *STL1* gene in the *hxt1–17 gal2 agt1 mph2 mph3 snf3 rgt2* mutant strain did not prevent residual growth on hexoses (Fig. 2), nor did its overexpression restore growth of the *hxt1–17 gal2* strain on hexoses.

4. Discussion

Here, we have demonstrated that more than 20 different proteins in yeast are able to transport hexoses. Previous results had shown that deletion of only *HXT1–HXT7* in the yeast MC996A strain prevented growth of the mutant cells on glucose, suggesting that the other *HXT* genes are not expressed or are not functional [18]. In contrast, an *hxt1–7* deletion mutant constructed in the CEN.PK strain background which was used in this work was still able to grow on glucose, although slowly (unpublished data). We suggest that these differences can be attributed to the higher respiratory capacity of the CEN.PK strain [22]. The higher respiration rate obviously enables CEN.PK cells to catabolize glucose even at very low uptake rates. Likewise, deletion of *HXT1–4* and *HXT6/7* in another laboratory strain [23] did not completely eliminate glucose transport. Inhibition of mitochondrial electron transport/oxidative phosphorylation was necessary to prevent residual growth on glucose, suggesting that minor glucose transporters were still active.

The hexose transporter family of *S. cerevisiae* provides an intriguing example of a family of proteins with strongly overlapping but nevertheless distinct functions. No one of the hexose transporters is essential for viability. Instead, each transporter plays a more or less highly specialized role. On the one hand, the different hexose transporters seem to have

Table 2
Primers used to disrupt the sugar transporter genes

Δhxt13/Δhxt17	5′-**CGCACCACCCGTGGAAAAAGAGTGGTCAGATGGATTTGATGACAA***CAGCTGAAGCTTCGTACGC*-3′
	5′-**TTGAGAACTTCAAAAATTTCTTCCAATCCTTCTTCTCGGTCTTAC***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt15/Δhxt16	5′-**ATGGCAAGCGAACAGTCCTCACCAGAAATTAATGCAGATAATCTAA***CAGCTGAAGCTTCGTACGC*-3′
	5′-**AGCATGTTTATCAAGCGCGCATATTGATCAATTAAAACTCTTTGGGAA***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt14	5′-**GGCAGGGGAAGGGATTATAATGTAACCATTAAGTATCTAGATGAT***CAGCTGAAGCTTCGTACGC*-3′
	5′-**TCCTAGTTTGAAATCAATTTTGGATACAATCATTGGTGTTAAAAG***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt12	5′-**ATCTTTGGTTGGGATACCGGTACCATTTCTGGTTTTGTTAACCTTTC***CAGCTGAAGCTTCGTACGC*-3′
	5′-**GGACAAAGAAAAAGACATAAAAGTATGCAAAAACCAGACAGCCTAAGA***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt11	5′-**ATGTCAGGTGTTAATAATACATCCGCAAATGAGTTATCTACTACCATG***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**GGACAAAGAAAAAGACATAAAAGTATGCAAAAACCAGACAGCCTAAGA***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt10	5′-**ATGGTTAGTTCAAGTGTTTCCATTTTGGGGACTAGCGCCAAGGCATCC***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**AATTATTTACTATCAACAATAACTAATGGTGTACTGCTTGTTGGTTGTGG***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt9	5′-**GTAGCAAATGCACCATCTGTAAAAACTGAGCATAATGACTCTAAAAAC***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**GGACAAAGAAAAAGACATAAAAGTATGCAAAAACCAGACAGCCTAAGA***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt8	5′-**ATGACTGATCGTAAAACCAACTTGCCAGAAGAACCGATTTTCGAAGAG***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**GGTCTAAAACATTCTTTTGTAGAAGGGTTTCTCGTCATGCTGTAATTT***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt5-hxt1-hxt4	5′-**ATGTCGGAACTTGAAAACGCTCATCAAGGCCCCTTGGAAGGGTCTGCTACT***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**TTAGATCATCAGCGTTGTAGTCAGTACCTCTCTTGTTTGGTGGAACCAAG***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt2	5′-**ATGTCTGAATTCGCTACTAGCCGCGTTGAAAGTGGCTCTCAACAAACT***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**TATTCCTCGGAAACTCTTTTTTCTTTTGAGATCCAGCTACCAGAT***GCATAGGCCACTAGTGGATCTG*-3′
Δhxt3-hxt6-hxt7	5′-**ATGAATTCAACTCCAGATTTAATATCTCCACAAAAGTCAAGTGAGAATTCG***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**AAGTTTCTTTGTCTCCGTCCCACTCAACTTTCTGAGAACAAATGATC***GCATAGGCCACTAGTGGATCTG*-3′
Δstl1	5′-**ATGAAGGATTTAAAATTATCGAATTTCAAAGGCAAATTTATAAGCAGA***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**ACCCTCAAAATTTGCTTTATCGTTCACTGTATCTTCATTTTTGATGTT***GCATAGGCCACTAGTGGATCTG*-3′
Δagt1/Δagt1::lacZ	5′-**ATGAAAAATATCATTTCATTGGTAAGCAAGAAGAAGGCTGCCTCAAAA***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**TAATTCTCGCTGTTTTATGCTTGAGGACTGACTGATACTCTCATCAGC***GCATAGGCCACTAGTGGATCTG*-3′
Δydl247w/Δyjr160c	5′-**ATGAAAAACTTATCTTTTCTCATAAACAGAAGAAAGGAAAATACAAGT***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**TTCCTCTGCAATGGAAGCTTCGATATCTCCCTTGGGGTCGTTATG***GCATAGGCCACTAGTGGATCTG*-3′
Δsnf3	5′-**ATGGATCCTAATAGTAACAGTTCTAGCGAAACATTACGCCAAGAGAAA***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**TATTTCAAATCATTATTTTCATTTACAGGTTGATTAGTGGCGTTTTC***GCATAGGCCACTAGTGGATCTG*-3′
Δrgt2	5′-**ATGAACGATAGCCAAAACTGCCTACGACAGAGGGAAGAAAATAGTCAT***TTCGTACGCTGCAGGTCGAC*-3′
	5′-**GGGGGAAGTGTATTGGCTGTGCTTACTAGCGAGGTCACTCGTCCAATT***GCATAGGCCACTAGTGGATCTG*-3′

Homology to the sugar transporter genes, bold; homology to *kanMX* cassette, italic.

different specificities for various hexoses (Fig. 3) [15]. Whereas Hxt5, 8, 13, 15, 16 and 17 restored growth of the *hxt1–17 gal2* deletion strain on glucose, fructose and mannose but not on galactose, Hxt9, 10 and 11 did also transport galactose. The Hxt14 protein seemed to be rather specific for galactose transport. On the other hand, expression of most of the hexose transporters is tightly regulated by a variety of conditions like glucose concentration, starvation, osmotic pressure or the physiological state of the cells [24–29]. In contrast to higher organisms, where different transporters are expressed in different cell types or tissues, the multitude of hexose transporter proteins in the unicellular yeast seems to reflect an adaptation to the variety of environmental conditions to which yeast cells are exposed. The availability of yeast strains expressing single hexose transporters will help to further characterize the role of the individual transporters under specific conditions. Moreover, the availability of a yeast strain unable to take up hexoses will be helpful for the cloning and characterization of hexose transporters from other organisms.

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