A Glucose Sensor in *Candida albicans*†

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The Hgt4 protein of *Candida albicans* (orf19.5962) is orthologous to the Snf3 and Rgt2 glucose sensors of *Saccharomyces cerevisiae* that govern sugar acquisition by regulating the expression of genes encoding hexose transporters. We found that *HGT4* is required for glucose induction of the expression of *HGT12*, *HXT10*, and *HGT7*, which encode apparent hexose transporters in *C. albicans*. An *hgt4Δ* mutant is defective for growth on fermentable sugars, which is consistent with the idea that Hgt4 is a sensor of glucose and similar sugars. Hgt4 appears to be sensitive to glucose levels similar to those in human serum (~5 mM). *HGT4* expression is repressed by high levels of glucose, which is consistent with the idea that it encodes a high-affinity sugar sensor. Glucose sensing through Hgt4 affects the yeast-to-hyphal morphological switch of *C. albicans* cells: *hgt4Δ* mutants are hypofilamented, and a constitutively signaling form of Hgt4 confers hyperfilamentation of cells. The *hgt4Δ* mutant is less virulent than wild-type cells in a mouse model of disseminated candidiasis. These results suggest that Hgt4 is a high-affinity glucose sensor that contributes to the virulence of *C. albicans*.

Candida albicans is responsible for most yeast infections in humans. In addition to causing ordinary vaginal and oral (thrush) infections, this fungus is a serious threat to immunocompromised patients, for whom disseminated, invasive candidiasis is common and often fatal (31, 56). The fungus is normally maintained as a harmless commensal on skin, on mucosa, and in the gut (23) but becomes an opportunistic pathogen upon entry into the bloodstream of immunocompromised (particularly neutropenic) individuals (25, 45). Its "virulence" is due to its robust ability to access and thrive in various niches within its host, which provide an abundant source of microbes available to cause disease (1, 25, 26). Within the host, *C. albicans* must efficiently compete for nutrients with host cells and an extensive repertoire of resident microbes (3).

Glucose acts as a morphogen for *C. albicans*, triggering the yeast-to-hyphal transition that is a vital determinant of virulence (15). Mutant *C. albicans* strains that are "frozen" in either phase cause less mortality in mouse models of disseminated infection, underscoring the importance of morphological plasticity for virulence (47). The yeast form is thought to disseminate easily via body fluids, and the hyphal form can extravasate into tissues or form mycelial biofilms (6, 52).

Because glucose plays a central role as a carbon and energy source, glucose sensing and response is highly evolved and closely regulated in most organisms. The importance of glucose to yeast cells is illustrated by the large number of hexose transporters they possess: *Saccharomyces cerevisiae* has at least 17 different hexose transporters that are expressed under different conditions; *C. albicans* appears to have over 20 (11). *S.*

cerevisiae is thought to require this large number of hexose transporters because it prefers to ferment glucose, a lifestyle that demands a high influx of glucose because it yields only two molecules of ATP for each molecule of glucose metabolized. It is somewhat surprising that *C. albicans*, which is thought to prefer respiration and therefore can garner up to 38 ATPs per glucose molecule metabolized, also has many hexose transporters (9). This may reflect the varied niches in which *C. albicans* thrives, which likely offer many different sugars as carbon sources.

Little is known about how *C. albicans* senses and responds to sugars. A sugar-sensing and signaling pathway that primarily regulates glucose transport has been elucidated in the yeast *S. cerevisiae* (13, 46). This pathway uses two glucose sensors in the cell membrane—Snf3 and Rgt2—that govern sugar acquisition by regulating the expression of genes encoding hexose transporters (*HXT*s) (21). The glucose sensors are orthologues of transporters but have long cytoplasmic tails involved in intracellular signaling that are missing from glucose transporters. Sensors cannot transport sugars. Rather, they generate intracellular signals in response to glucose that induce the expression of genes encoding hexose transporters (39). A single missense mutation in the *S. cerevisiae* glucose sensors illustrates this well: the Snf3(R229K) or Rgt2(R231K) mutations cause constitutive expression of genes encoding hexose transporters, presumably because these mutations convert the sensors into their glucose-bound (signaling) conformations (40).

C. albicans possesses an orthologue of the Snf3 and Rgt2 glucose sensors: Hgt4 (orf19.5962). We present evidence that Hgt4 is a high-affinity glucose sensor that generates an intracellular signal to induce expression of certain *HGT* genes encoding hexose transporters and that this is required for growth on fermentable carbon sources, for filamentation, and for optimal virulence.

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MATERIALS AND METHODS

Yeast strains. A wild-type *C. albicans* strain SC5314 (12) was purchased from ATCC (catalogue number MYA-2876). The *C. albicans* parental strain we used

for all gene knockouts is BWP17 (*ura3::imm434/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG*) (55); the DAY286 strain is BWP17 that was rendered Arg$^+$Uri$^+$ (44); both strains were gifts from Aaron P. Mitchell (Columbia University). The *C. albicans* GPR1 parental strain, *GPR1/gpr1* heterozygote, *gpr1/gpr1*-null mutant (*gpr1Δ*), and *gpa2/gpa2*-null mutant (*gpa2Δ*) have been described previously (34), and were obtained from Joseph Heitman (Duke University). The *S. cerevisiae snf3Δ rgt2Δ* mutant strain (YM6870, similar to YM6370 [22]) was generated by Aneta Kaniak (unpublished data).

Plasmids. The UAU1 cassette (pBME101) and *HIS1*-complementing plasmid pDDB78 were gifts from Aaron Mitchell (Columbia University) (10, 51). The *HXT1-lacZ* reporter (BM3212) was generated by Sabire Ozcan (41). The *SAT1* flipper cassette (pSFS2A) was a gift from Joachim Morschhauser (Institute Fur Molekulare Infektions Biologie) (43).

Gene deletions. (i) hgt4Δ. The UAU1 cassette replaced the *HGT4* open reading frame (ORF) as follows. *HGT4*, including 0.9 kb of DNA upstream and downstream of the ORF, was amplified by the PCR (primers OM5073 and OM5076) and cloned via "gap-repair" (38) into pRS316 (CEN, *URA3* (49) in *S. cerevisiae*. The resulting *HGT4* plasmid (BM4913) was linearized with BsrGI, and the PCR-amplified UAU1 cassette (primers OM6040 and OM6042) was inserted by gap repair of pBM4914 into *S. cerevisiae*, selecting for Ura$^+$ (vector) and Arg$^+$ (full UAU1 insert). The *hgt4*::UAU1 fragment was released from the plasmid by digesting it with NotI-SalI and used to transform strain BWP17. Arg$^+$ colonies were screened for the Uri$^+$ phenotype. Three independent clones were verified by PCR for the absence of the *HGT4* ORF and the presence of UAU1 at *hgt4*.

(ii) hgt12Δ. The strategy described above was used with the following modifications. Primers OM5075 and OM5078 amplified the *HGT12* ORF plus 0.9 kb of flanking sequence; primers OM6044 and OM6046 amplified the UAU1 cassette. Cloned *HGT12* was linearized with HpaI to replace its ORF with UAU1. The *hgt12*::UAU1 fragment was recovered by digestion of the plasmid with KpnI-SacI and introduced into BWP17 cells.

(iii) Complementing clones. The *HGT4* ORF (primers OM6132 and OM6133) or the *HGT4-1* allele (R167K) (primers OM6132 and OM6133, along with OM6134 and OM6135, which encode the R167K mutation) and 1 kb of upstream flanking sequence ("promoter") and 0.5 kb of downstream flanking sequence (3′-untranslated region [3′UTR]) were amplified by PCR using high-fidelity pHusion polymerase (New England Biolabs). The products (one fragment for *HGT4* and two fragments for *HGT4-1*) were cloned by gap repair into pDDB78 (51) that had been linearized with NotI-SpeI. The clones were verified by determining their DNA sequence, and the resulting plasmids were linearized with NruI for transformation into *hgt4Δ* strains, selecting for His$^+$ colonies. Controls were the pDDB78 empty vector ("vector") or pDDB78 containing only the 1 kb upstream and 0.5 kb downstream (relative to the coding ORF) DNA sequences but lacking any coding ORF ("UTRs"). Since the *HIS1* locus is on chromosome 5, and BWP17 is heterozygous in this region, all complemented strains were genotyped to determine complementation site. All insertions complemented the wild-type (full-length) chromosome 5a. Two of the three confirmed *hgt4Δ* mutants were complemented each with five independent wild-type *HGT4* clones. Similar results were observed at 30 and 37°C. We investigated whether the *HGT4* locus was heterozygous by DNA alignment of orf19.5962 and its allele orf19.13383 (2). Alignment of the 2.2470-kb coding region, including 1 kb upstream and 1 kb downstream of the ORF showed the "alleles" to be identical.

(iv) rgt1Δ. the *SAT1* cassette was used to replace both copies of the *RGT1* ORF as follows. Two clones containing either 0.5 kb of DNA from the 5′ and 3′UTRs of *RGT1* (clone pBM4707) or 0.5 kb of DNA internal to the *RGT1* ORF (0.5 kb from the 5′ end of the gene and 0.5 kb from the 3′ end) (clone pBM4864) were constructed as described below. In each clone, DNA fragments from the *RGT1* locus flanked the *SAT1* flipper cassette (43) and contained KpnI and SacI restriction sites enabling release of the *RGT1* knockout cassettes by KpnI/SacI digest. To create an *RGT1/rgt1* heterozygous strain in an *hgt4Δ* background, strain CM9 was transformed with ~1 μg of KpnI/SacI-digested pBM4707, and integration of the knockout cassette was selected for by plating transformants on yeast extract-peptone-dextrose (YPD) containing 100 mg of nourseothricin (Nat)/liter. NatR colonies were purified and then grown in YP broth containing 2% maltose to induce loss of the *SAT1* cassette through recombination (43). Cells were then plated on YPD, screened for Nat sensitivity, colony purified, and genotyped by PCR to confirm loss of one copy of *RGT1*. Next, the *hgt4Δ*, *RGT1/rgt1* strain was transformed with ~1 μg of KpnI/SacI-digested pBM4864 and processed as just described to obtain an *hgt4Δ rgt1Δ* double-knockout strain. This strain was genotyped by PCR to confirm the presence of both *rgt1* deletions and the absence of the wild-type *RGT1* gene.

Media. Standard YPD medium (10 g of yeast extract, 20 g of peptone, 20 g of glucose, and 18 g of Difco agar per liter) was supplemented with 80 to 200 mg of uridine/liter. For SAT1-flipper-mediated gene deletions, 100 mg of nourseothri-
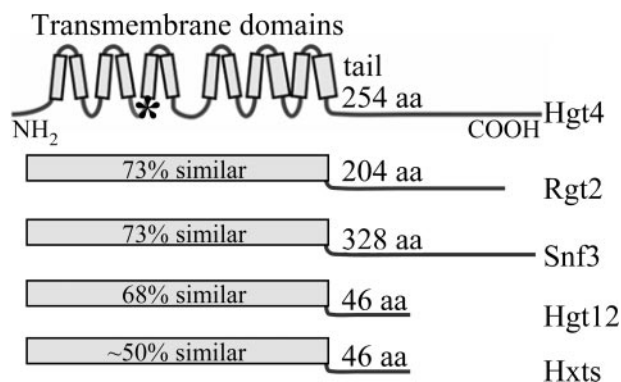

FIG. 1. Glucose sensors and transporters. The *C. albicans* Hgt4 and the *S. cerevisiae* Snf3 and Rgt2 glucose sensors are illustrated. For comparison, the *C. albicans* putative hexose transporter Hgt12 and a composite representation of the *S. cerevisiae* hexose transporter family (Hxts) are shown. The 12-transmembrane glucose-binding domain and the C-terminal cytoplasmic tails are diagrammed. The percent values indicate similarity to Hgt4 throughout the transmembrane domain. An asterisk indicates the position of the arginine-to-lysine mutation that causes constitutive (sugar-independent) signaling of Snf3(R229K), Rgt2(R231K), or Hgt4(R167K) (40).

cin/liter was added to the YPD as needed (35). Standard synthetic complete medium (1.7 g of yeast nitrogen base, 5 g of ammonium sulfate, 2 g of −Leu amino acid dropout mix [U.S. Biologicals D9525], 5 ml of 200× leucine, and 18 of g Difco agar/liter) was supplemented with arginine (80 mg/liter), uridine (80 mg/liter), and histidine (40 mg/liter). Spider medium was prepared as previously described (29) and supplemented with arginine, uridine, and histidine (final concentrations as described above). Antimycin A (50 to 75 μl of stock: 1 mg/ml in ethanol) was spread onto petri plates with 30 ml of YPD-uridine (YPD+uri) solid medium.

Yeast culture conditions. For observing growth on solid medium containing various carbon sources, fresh colonies harvested from YPD+uri plates were resuspended in liquid YPD+uri and grown for 5 h at 30°C, reaching an optical density at 600 nm (OD$_{600}$) of 2 to 3. Cells were diluted to an OD$_{600}$ of 0.0001 (ca. 1 CFU/μl) in sterile water, and 20 μl was spotted onto the plates. For liquid cultures (sugar inductions), cells were grown to log phase in synthetic complete medium with 5% glycerol as a carbon source and then diluted back to an OD$_{600}$ of 0.2 to 0.3 in synthetic complete medium with the indicated carbon sources.

RT-PCR. Cells grown to log phase in synthetic complete medium were diluted to an OD$_{600}$ of 0.2 to 0.3 in synthetic complete medium with the indicated carbon sources and grown for 2.5 h at 30°C. Cell pellets were snap-frozen in liquid nitrogen and stored at −80°C. For total RNA purification, cells were resuspended in 400 μl of Tris-EDTA plus 0.5% sodium dodecyl sulfate and 500 μl of hot acid-phenol (65°C) and kept at 65°C for 1 h, with vortexing for 10 s every 10 min. The RNA was phenol-chloroform extracted and ethanol precipitated. A total of 5 μg of RNA was treated with DNase (Ambion DNA-free, catalogue no. 1907), and 500 ng was used in a 20-μl reverse transcription (RT) reaction (Superscript II; Invitrogen). One microliter of cDNA was used in each 20-μl PCR; the number of amplification cycles ranged from 18 (*ACT1*, *HGT7*) to 22 (*HGT12*, *HXT10*) to 24 (*HGT4*).

Microarray analysis. DNA oligonucleotide microarrays were generated that represent 6,346 of the 6,354 predicted ORFs in the annotated *C. albicans* genome assembly 19 (8). Each ORF is represented by a specific 70-mer oligonucleotide, and one genome equivalent was spotted three times per slide (i.e., *n* = 3 for each hybridization). Oligonucleotide sequences were selected by using an ArrayOligoSelector (7) and were obtained from Illumina (San Diego, CA). *HGT4-1* (CM36) or *hgt4Δ* (CM32) cells were grown to log phase in synthetic complete medium plus 5% glycerol. Total RNA was prepared as described above. DNA contamination was removed by using RNeasy columns (QIAGEN), and purified RNA was quantified by using a Nanodrop spectrophotometer. The quality was confirmed by using an Agilent 2100 bioanalyzer. A two-step protocol was used for hybridization (3DNA array 350 detection system; Genisphere, Hatfield, PA). First, oligonucleotide arrays were hybridized to the cDNA probes in 2× formamide-based hybridization buffer overnight at 43°C and washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.2% sodium dodecyl
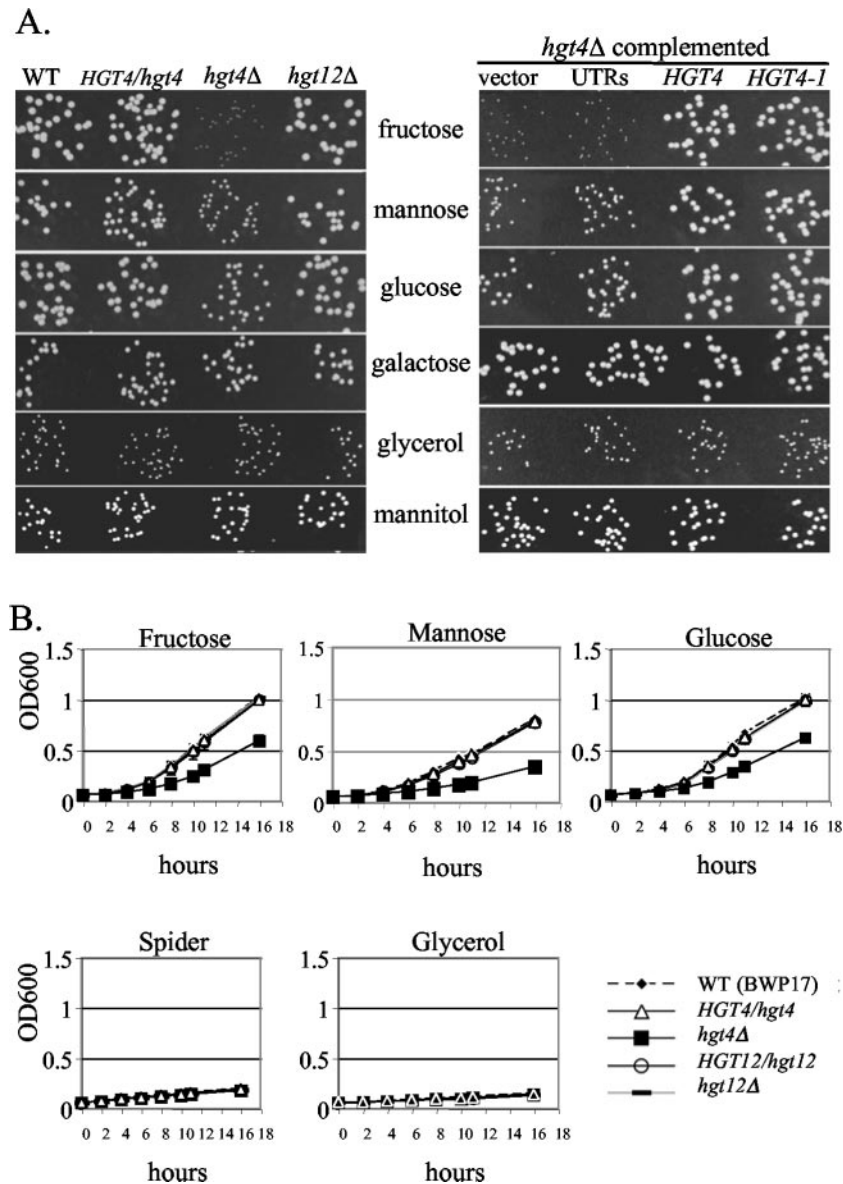
FIG. 2. (A) *HGT4* is required for optimal growth on fermentable carbon sources. *C. albicans* strains were grown 4 to 6 days in 0.2% of various carbon sources at 30°C. In the left panel, WT is the parental strain BWP17 (CM50), *HGT4/hgt4* (CM4) is the sensor heterozygote, *hgt4Δ* is a *hgt4/hgt4* knockout strain (CM9), and *hgt12Δ* is a *hgt12/hgt12* knockout strain (CM64). In the right panel, the *hgt4Δ* strain carrying an empty vector (pDDB78, strain CM32), the "UTRs" only (pDDB78 containing 1 kb of the *HGT4* promoter fused to 0.5 kb of the *HGT4* 3′UTR but lacking any ORF, strain CM137), *HGT4* (pDDB78 containing one copy of the wild-type *HGT4* gene, strain CM87), and *HGT4-1* (pDDB78 containing one copy of the Hgt4 [R167K] allele, strain CM36). (B) *HGT4* is essential for growth of *C. albicans* strains in hypoxic conditions. Log-phase cells prepared in aerated glycerol cultures were inoculated into various 2% carbon sources and grown in static (not shaken) microtiter plates at 30°C. Every 2 h, plates were lightly vortexed, and the optical densities (OD_{600}) of the wells were measured.

sulfate according to the manufacturer's protocol. Fluorescent Cy3- and Cy5-capture reagents were combined in hybridization buffer and added to each array, which were incubated and washed again as described above. Slides were scanned immediately after hybridization on a ScanArray express HT scanner (Perkin-Elmer) to detect Cy3 and Cy5 fluorescence. The laser power was kept constant, and photomultiplier tube (PMT) values were set for optimal intensity with minimal background. An additional scan was done for each slide with the PMT such that <1% of the elements are saturated in order to characterize spots which were saturated at the higher PMT setting. Gridding and analysis of images was performed with ScanArray software express V2.0 (Perkin-Elmer). Significant expression changes were determined as follows: the hybridization intensity values are imported into GeneSpring, and the local background intensity was subtracted. The mean signal and control intensities of the duplicate (or triplicate) spots were calculated. A Lowess curve was fit to the log-intensity versus log-ratio plot. A total of 20.0% of the data was used to calculate the Lowess fit at each point. The resulting curve was used to adjust the control value for each measurement. If the control channel was lower than 10 relative fluorescence units, then 10 was used instead. The mean signals to Lowess-adjusted controlled ratios were calculated. The cross-chip averages were derived from the antilog of the mean of the natural log ratios across the two arrays (original and dye-swap). The data was then filtered as follows. Oligonucleotides that received a "present" call (intensity of >200 relative fluorescence units or local signal-to-background value of >2) by the ScanArray software in two of the four high-PMT scans in either the Cy3 or Cy5 were identified for each condition, and all others were excluded from the analysis. The resulting data set (present genes) was filtered for genes which in either replicate 1 or 2 had (i) a >2-fold change in either the high- or low-PMT
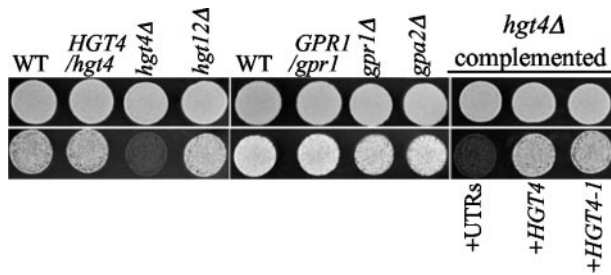
FIG. 3. *HGT4* is essential for the growth of *C. albicans* strains when respiration is inhibited. Cells spotted onto YPD+uri without antimycin A (top row; 3 days growth) or with 2 to 4 μg of antimycin A/ml (bottom row; 5 days of growth). Similar results were observed at 30 and 37°C. For the Hgt4 pathway, WT is the parental strain BWP17 (CM50), *HGT4/hgt4* (CM4) is the sensor heterozygote, *hgt4Δ* is a *hgt4/hgt4* knockout strain (CM9), and *hgt12Δ* is a *hgt12/hgt12* knockout strain (CM64). For the Gpr1 pathway, WT is the parental CM149 strain, *GPR1/gpr1* is the sensor heterozygote (CM152), *gpr1Δ* is a *gpr1/gpr1* knockout strain (CM151), and *gpa2Δ* is a *gpa2/gpa2* knockout strain (CM150). For *hgt4Δ* strain complementation, "UTRs" indicates the noncoding flanking regions of *HGT4* (CM137), *HGT4* indicates one copy of the wild-type *HGT4* gene (CM87), *HGT4-1* and indicates one copy of the constitutively signaling *HGT4* gene (CM35).

scan and (ii) $P < 0.05$ in either the high- or low-PMT scan. Genes that met these criteria in both replicates are shown.

Mouse procedures. All animal experimental procedures were approved by the Washington University Institutional Animal Care and Use Committee and by the Animal Studies Committee. Female 5- to 6-week-old BALB/c mice were pur-

chased from Harlan Bioproducts, Indianapolis, IN, and acclimated (five mice/ cage) in a pathogen-free facility for 4 weeks. Two independent infection experiments were performed with 10- and 12-week-old mice. *HGT4* infections ($n = 11$ and $n = 7$, respectively; strain CM87 genotype *hgt4::ARG4/hgt4::URA3, HIS1::* pDDB78/*HGT4* [including 1 kb upstream and 0.5 kb downstream and the coding ORF]) and *hgt4Δ* infections ($n = 9$ and $n = 8$, respectively [strain CM137 genotype *hgt4::ARG4/hgt4::URA3, HIS1::*pDDB78 carrying only noncoding regions, i.e., 1 kb upstream plus 0.5 kb downstream, of *HGT4*]) were performed by injecting 7.5×10^5 cells into the lateral tail vein. Cells were prepared by growth to log phase in YPD+uri media and washed thoroughly with endotoxin-free phosphate-buffered saline (PBS), and cell densities were measured by cell counts obtained by using a hemacytometer. Cells were diluted to 3.75×10^6 per ml in PBS, and 200 μl of the suspension was used per infection. Mice determined to be moribund were sacrificed by CO_2 asphyxiation, and the death was recorded as the next day. Kidneys were harvested using sterile techniques and Dounce homogenized in 3 ml of sterile PBS, and serial 10-fold dilutions were plated on YPD+uri medium.

RESULTS

A glucose sensor orthologue in *C. albicans*. Among the 21 hexose transporter orthologs encoded in the *C. albicans* genome (11), Hgt4 is most similar to the Snf3 and Rgt2 glucose sensors of *S. cerevisiae* (Fig. 1). Moreover, because it possesses a long C-terminal cytoplasmic tail, a hallmark of glucose sensors, it is a good candidate for a glucose sensor (Fig. 1). Hgt4 is 56% identical and 73% similar to Snf3 and Rgt2 throughout its predicted transmembrane domains (the glucose-binding region), but the sequences of their cytoplasmic C-terminal tails
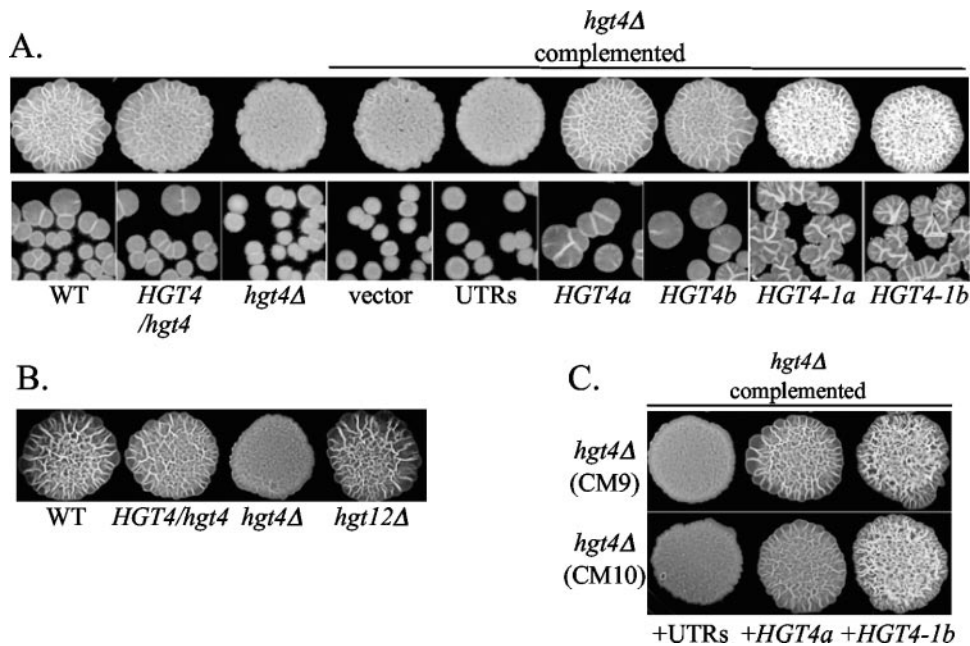

FIG. 4. (A) Filamentation defects of *hgt4Δ* mutants. Cells were grown to log phase in YPD+uri, diluted 50-fold (patches) or 5,000-fold (single cells) in sterile water, spotted onto spider medium, and grown for 4 to 5 days at 37°C. WT is the parental strain BWP17 (CM50), *HGT4/hgt4* (CM4) is the sensor heterozygote, and *hgt4Δ* is a *hgt4/hgt4* knockout strain (CM9). For *hgt4Δ* strain complementation, "vector" indicates the pDDB78 empty vector (CM32), "UTRs" indicates the noncoding flanking regions of *HGT4* (CM137), *HGT4* indicates one copy of the wild-type *HGT4* gene (*a* is CM87 and *b* is CM97), and *HGT4-1* indicates one copy of the constitutively signaling *HGT4* allele (*a* is CM35 and *b* is CM36). (B) Deleting the Hgt12 hexose transporter does not affect filamentation on spider medium. WT is the parental strain BWP17 (CM50), *HGT4/hgt4* (CM4) is the sensor heterozygote, *hgt4Δ* is a *hgt4/hgt4* knockout strain (CM9), and *hgt12Δ* is a *hgt12/hgt12* knockout strain (CM64). (C) The constitutively signaling *HGT4-1* allele causes hyperfilamentation. Cells were grown to log phase in YPD+uri, diluted 50-fold in sterile water, spotted onto spider medium, and grown for 4 to 5 days at 37°C. Two independent *hgt4Δ* mutants (CM9 top row and CM10 bottom row) were complemented: "UTRs" indicates the noncoding flanking regions of *HGT4* (pBM4819), *HGT4* indicates one copy of the wild-type *HGT4* gene (pBM4807), and *HGT4-1* indicates one copy of the constitutively signaling *HGT4* allele (pBM4782). At 30°C, weak, equivalent filamentation was observed for all strains at day 7 or later (data not shown). The same results were observed when using the DAY286 strain (BWP17 rendered Arg⁺ Uri⁺) as the "parent" strain.

are not similar. The next most similar protein to Snf3 and Rgt2 is Hgt12 (orf19.7094 or Ca*SNF32*), which has been dubbed a glucose sensor (32), but its short C-terminal tail suggests that it is a sugar transporter rather than a sensor.

Elucidating the function of the *C. albicans* Hgt4 and Hgt12 proteins. If Hgt4 is indeed a glucose sensor, we would expect it to be required for normal sugar sensing and acquisition; if Hgt12 is indeed simply a glucose transporter, then it is not expected to be required for growth of cells on glucose due to the redundancy of the *HGT* genes. We constructed homozygous deletion mutants of *HGT4* and *HGT12* using the UAU1 disruption cassette (see Materials and Methods) (10). The *hgt12*Δ mutants have no defects in utilization of any of the carbon sources tested, but the *hgt4*Δ mutants have clear growth defects on solid media with fructose as carbon source, and a slight growth defect on medium containing mannose or glucose that is only apparent at low (0.2%) sugar concentrations (Fig. 2A and data not shown). No *hgt4*Δ growth defects are observed in highly aerated liquid cultures with 0.2 or 2.0% sugars (data not shown) but, if the cultures are not aerated by shaking, a condition that demands increased fermentation, then the *hgt4*Δ mutants have a clear growth defect, even at high sugar concentrations (Fig. 2B). Hgt4 is also required for growth on glucose in the presence of antimycin A, which substantially inhibits respiration (Fig. 3) (14, 24). Mutants defective in another glucose sensing pathway triggered by the Gpr1 G-protein coupled receptor and its G protein alpha Gpa2 appear to ferment glucose normally (Fig. 3) (33, 34). These *hgt4*Δ phenotypes are indeed due to the deletion of the *HGT4* ORF because they are complemented by the wild-type *HGT4* gene or by a constitutively signaling version of *HGT4* (*HGT4-1*; see below). Thus, Hgt4 is essential for normal growth on low levels of fermentable carbon sources and is especially important for growth when respiration is reduced. Furthermore, these results imply that *C. albicans* is a facultative anaerobe that can ferment hexoses if necessary, provided that Hgt4 is functional.

Hgt4 contributes to the filamentation of *C. albicans* cells. Yeast-form *C. albicans* cells respond to certain environmental cues such as high pH (>7.0), high temperature (37°C), exposure to serum, engulfment by macrophages, or growth in rich spider medium by forming germ tubes that elongate into branched hyphae or filaments (52). Glucose was recently identified as the main component of serum that is responsible for inducing the filamentation response in *C. albicans* (15). On solid spider medium (which robustly induces germ tubes and hyphae (filamentation), filamented cells form wrinkled colonies, whereas yeast cells form smooth colonies (29). The *hgt4*Δ mutant, but not the *hgt12*Δ mutant, is impaired in its ability to form filaments on spider medium, and filamentation was restored by one copy of the wild-type *HGT4* gene (Fig. 4). Interestingly, complementing the *hgt4*Δ mutant with the constitutively signaling *HGT4-1* allele not only rescued the null phenotype but also caused hyper-filamentation of cells (Fig. 4C). Cells harvested from the spider agar plates and stained with calcofluor white (a fluorescent dye that binds to fungal cell walls) showed that the *hgt4*Δ mutant cells were predominantly yeast form, the *HGT4-1*-expressing cells were predominantly filamented, and the *HGT4*-expressing cells produced a mixture of yeast and filamented cells (data not shown). The *HGT4-1*
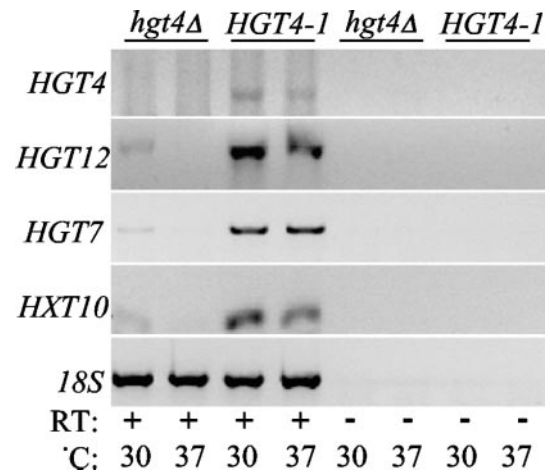

FIG. 5. RT-PCR confirmation of the three genes most differentially expressed in the *HGT4-1* (CM36) versus *hgt4*Δ (CM32) strains, as suggested by the microarray results in Table 2. The RNA used for the microarray analyses was reverse transcribed and amplified by PCR with the gene-specific primers indicated in Table 1.

mutation changes arginine 167 to lysine; the orthologous change in Rgt2 and Snf3 of *S. cerevisiae* causes constitutive glucose signaling and constitutive expression of genes regulated by the glucose sensors (Fig. 1) (40). In contrast to their behavior on spider media, all strains developed germ tubes and filamented equally well in YPD plus 10% calf serum (data not shown).

Identifying target genes regulated by Hgt4. In an attempt to identify genes whose expression is regulated by Hgt4, we profiled the expression of *C. albicans* genes in *HGT4* mutants using an array of DNA oligonucleotides representing nearly all of the ORFs annotated in assembly 19 of the *C. albicans* genome (8). To avoid detecting transcriptional changes caused by differences between the strains in growth rate, glucose repression, or the yeast-to-hyphal transition, we compared gene expression in the *HGT4-1* constitutively signaling mutant to that in an *hgt4*Δ mutant grown on glycerol. These two mutants are isogenic prototrophs, differing only in their *HGT4* allele. Only 35 genes responded differently to the different *HGT4* alleles: expression of 28 genes increased, and expression of 7 genes decreased in the *HGT4-1* strain compared to the *hgt4*Δ strain (see Table 2, genes that differed ≥2-fold; also see the supplemental material). Only three genes, all putative hexose transporters, were dramatically induced (>10-fold) by the *HGT4-1* mutation: *HGT12* (orf19.7094), *HGT7* (orf19.2023), and *HXT10* (orf19.4384). Cells cultured at 30 and 37° gave nearly identical results. These results suggest that this signaling pathway is primarily dedicated to regulating the expression of genes encoding hexose transporters. *HGT4* seems to regulate the expression of only 6 of the 20 *HGT* genes (see Table 2 and the supplemental material). The differential expression of the three most highly regulated *HGT* genes was validated by RT-PCR with gene-specific primers (Fig. 5).

Expression of *HGT4* and of Hgt4-regulated genes. We assessed the expression of *HGT4* and its main target genes
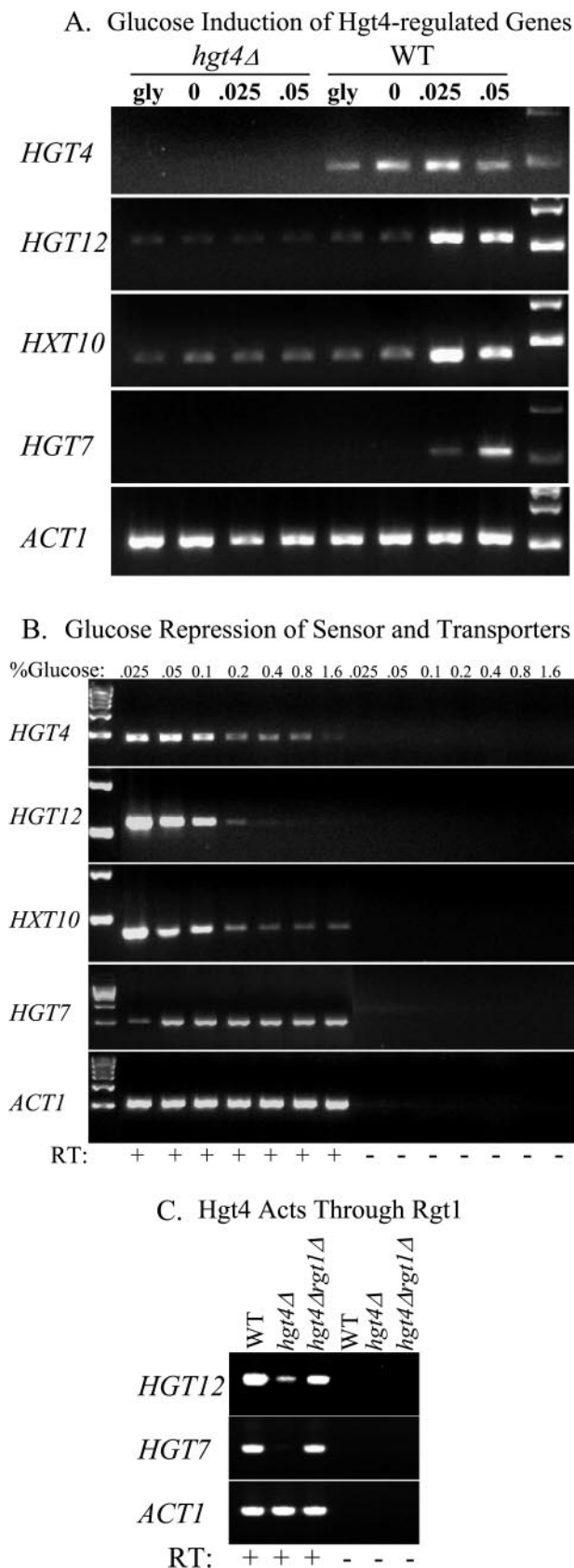
A. Glucose Induction of Hgt4-regulated Genes

B. Glucose Repression of Sensor and Transporters

C. Hgt4 Acts Through Rgt1

FIG. 6. (A) Glucose induction of *HGT12*, *HXT10*, and *HGT7* is dependent on *HGT4*. Cells were grown to log phase (OD_{600} = 0.5 to

HGT12, *HXT10*, and *HGT7* in wild-type and *hgt4*Δ strains (Fig. 6A). *HGT4* is expressed in cells grown on a nonfermentable carbon source (glycerol) or on low levels of glucose (Fig. 6A), but its expression is repressed when glucose levels are high (Fig. 6B). The expression of *HGT12* and *HXT10* transporters is induced by low (physiologic) levels of glucose and repressed by high levels of glucose, suggesting that they encode high-affinity hexose transporters (Fig. 6A and B). *HGT12* and *HXT10* expression is repressed by high levels of sugar in both wild-type cells and *hgt4*Δ mutants. In wild-type cells, this occurs via the Mig1 transcriptional repressor, whose expression is induced by the Hgt4 signal (Table 2 and Fig. 6B; also J. A. Sexton et al., unpublished data). In *hgt4*Δ mutants, *HGT12* and *HXT10* expression is constitutively repressed via the Rgt1 transcriptional repressor (Fig. 6A and 9; also, data not shown).

In contrast, in wild-type cells the expression of *HGT7* is induced by glucose but is not repressed by high levels of sugar. Our gene expression profiling experiments also indicate that *HGT7* expression is induced in response to high levels of many sugars including glucose, fructose, maltose, and galactose (data not shown). In the *hgt4*Δ mutants, *HGT7* is constitutively repressed (Fig. 6A to 6C and data not shown). Glucose induction of *HGT12*, *HXT10*, and *HGT7* expression by low levels of glucose (0.025 to 0.1%) requires *HGT4*, which is consistent with the idea that *HGT4* is a high-affinity glucose sensor that controls the expression of downstream genes.

In *S. cerevisiae*, the intracellular signal generated by the glucose sensors leads to inactivation of the transcriptional repressor Rgt1, resulting in depression of its target genes (see Fig. 9). Deleting the *C. albicans RGT1* orthologue (orf19.6173) in the *hgt4*Δ mutant restored the expression of *HGT12*, *HGT10*, and *HGT7* to wild-type levels and in fact causes *HGT* gene expression to be constitutive (Fig. 6C and data not shown). These results are consistent with the view that CaRgt1 is a transcriptional repressor that mediates Hgt4 regulation of the *HGT* genes.

Determining the glucose responsiveness of Hgt4. We compared the functionality of Hgt4 to that of the glucose sensors of *S. cerevisiae* by testing its signaling ability in *S. cerevisiae*. Because the amino acid sequence of the cytoplasmic (C-terminal) tail of Hgt4 is not similar to that of the tails of the two glucose sensors of *S. cerevisiae*, we surmised that we needed to provide

1.0) and then incubated for 2.5 h in 5% glycerol (gly) or with the indicated levels (%) of glucose. Cells were harvested, total RNA was prepared, and RT-PCR was performed on the indicated targets (see Materials and Methods). The strains used were wild-type CM49 and *hgt4*Δ CM137. Control reactions lacking reverse transcriptase produced no PCR products (data not shown). (B) High levels of glucose repress the expression of *HGT4*, *HGT12*, and *HXT10* but not *HGT7*. Cells (CM49) were incubated for 2.5 h with the indicated level of glucose (%). RT-PCR analysis of the indicated target genes is shown. (C) Hgt4 acts through the transcriptional repressor Rgt1. Deleting the *RGT1* gene restored the expression of *HGT12* and *HGT7* in the *hgt4*Δ mutant. RT-PCR analysis compared the expression of Hgt4-target genes in wild-type DAY286 cells (induced expression) versus *hgt4*Δ mutant CM9 cells (uninduced expression) versus *hgt4*Δ *rgt1*Δ double mutant CM170 cells (constitutive expression). Cells were induced for 2 h in 0.05% glucose. Total RNA was extracted, and RT-PCR was performed as described in the text.

TABLE 1. Reagents used in this study

Strain, plasmid, or primer	Genotype or sequence	Source or reference
Strains		
CM49	SC5314 wild-type (blood isolate)	12
CM50	BWP17, *ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::his*	55
DAY286	BWP17, *ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::his/ARG4::URA3*	44
CM149	*ura3::imm434/ura3::imm434 TRP1/TRP1::URA3*	34
CM152	*ura3::imm434/ura3::imm434 GPR1/gpr1::hisG TRP1/TRP1::URA3*	34
CM151	*ura3::imm434/ura3::imm434 gpr1::hisG/gpr1::hisG TRP1/TRP1::URA3*	34
CM150	*ura3::imm434/ura3::imm434 gpa2::hisG/gpa2::hisG TRP1/TRP1::URA3*	34
CM4	BWP17, *HGT4/hgt4::ARG4*	This study
CM9	BWP17, *hgt4::ARG4/hgt4::URA3*	This study
CM10	BWP17, *hgt4::ARG4/hgt4::URA3* (independently generated null mutant)	This study
CM63	BWP17, *HGT12/hgt12::ARG4*	This study
CM64	BWP17, *hgt12::ARG4/hgt12::URA3*	This study
CM32	CM9, *his1::hisG/HIS1*::pDDB78	This study
CM137	CM9, *his1::hisG/HIS1*::pDDB78 +*HGT4*UTRs only (no coding ORF)	This study
CM140	CM9, *his1::hisG/HIS1*::pDDB78 +*HGT4*UTRs only (independent transformant)	This study
CM87	CM9, *his1::hisG/HIS1*::pDDB78 +wt*HGT4*(*a*)	This study
CM97	CM9, *his1::hisG/HIS1*::pDDB78 +wt*HGT4*(*b*) independent transformant	This study
CM35	CM9, *his1::hisG/HIS1*::pDDB78 +*HGT4-1*(*a*)	This study
CM36	CM9, *his1::hisG/HIS1*::pDDB78 +*HGT4-1*(*b*) independent transformant	This study
CM170	CM9, *rgt1Δ::FRT/rgt1Δ::*FRT	This study
YM6870	*MATa his3Δ leu2Δ ura3Δ met15Δ LYS2 rgt2::kanMX::natMX snf3::kanMX*	22
YM7309	YM6870, plasmid pRS316 (CEN, *URA3*)	This study
YM7311	YM6870, plasmid pBM3272 (pRS316+*RGT2*)	This study
YM7312	YM6870, plasmid pBM3272 (pRS316+*SNF3*)	This study
YM7314	YM6870, plasmid pBM4840 (pRS316+*HGT4-RGT2*tail)	This study
Plasmids		
pBM3212	pYEp367R + HXT1 promoter-*lacZ* fusion (2μ ori, *LEU2* selection)	40
pBM4665	pBME101 (UAU1 cassette)	10
pBM4671	pDDB78 (*HIS1* complementation, *TRP1* selection)	51
pBM4819	pDDB78 + *HGT4* UTRs only (no coding ORF)	This study
pBM4807	pDDB78 + wild-type *HGT4*(*a*)	This study
pBM4809	pDDB78 + wild-type *HGT4*(*b*) independent clone	This study
pBM4780	pDDB78 + constitutively signaling (R167K) *HGT4-1*(*a*)	This study
pBM4782	pDDB78 + constitutively signaling (R167K) *HGT4-1*(*b*) independent clone	This study
Primers[a]		
hgt4::UAU1		
OM5073	5′-AACTAGTGGATCCCCCGGGCTGCAGGAATTCtaaattttgttgacagtttttagcattg	This study
OM5076	5′-CGGTATCGATAAGCTTGATATCGAATTCatgtaatgggtgtatacccttttatc	This study
OM6040	5′-CTCGTATAATGTACGATGCTTCATTGGAAGACGAGTATTAggttttcccagtcacgacgt	This study
OM6042	5′-ACCCCTTCTTGTTCCACCACCACTTCCTCTTCCTCTTCATtgtggaattgtgagcggata	This study
hgt12::UAU1		
OM5075	5′-AACTAGTGGATCCCCCGGGCTGCAGGAATTCtccagggattaggtgattatttcag	This study
OM5078	5′-CGGTATCGATAAGCTTGATATCGAATTCttagcggcctgtaacgggactgaatac	This study
OM6331	5′-TGAGTGCAAATATCCAAGCTCTtagaaggaccacctttgattgtaaatag	This study
OM6332	5′-ACTAAAAACTATAAACTGTCATTAgggatttggatggtataaacggaaac	This study
hgt4Δ complementation		
OM6132	5′-gatatcgaattcctgcagcccggggggatccactagtatgatagtgccacttgtcatcatc	This study
OM6133	5′-atagggcgaattggagctccaccgcggtggcggccgcaactgcccaatgtaatggagttc	This study
OM6134[b]	5′-gtcgccaaaatggctgaAaggttcagtggtgtttac	This study
OM6135[b]	5′-gtaaacaccactgaacctTtcagccattttggcgac	This study
RT-PCR		
OM6296	5′-cattgtataatggcgaagatcag (*HGT4* forward)	This study
OM6297	5′-cagaatcacttggaggatgagc (*HGT4* reverse)	This study
OM6298	5′-tatggtactcaattcttcaagcg (*HGT12* forward)	This study
OM6299	5′-tcatctctaaatgcgtggacac (*HGT12* reverse)	This study
OM6474	5′-gacgttgatgggcagaatcg (*HXT10* forward)	This study
OM6475	5′-ctgtgtatgtcatggccacc (*HXT10* reverse)	This study
OM6292	5′-aaccaagaatctttgaaagggttg (*HGT7* forward)	This study
OM6295	5′-gaaaactaaacatcccataaagacg (HGT7 reverse)	This study
OM6306	5′-tgatttggctggtagagacttg (*ACT1* forward)	This study
OM6307	5′-tttgtggtgaacaatggatggac (*ACT1* reverse)	This study

[a] Uppercase letters indicate sequences used during gap-repair cloning. Lowercase letters indicate sequences used during PCR amplification.
[b] The uppercase letter indicates the mutation encoding the R167K missense.
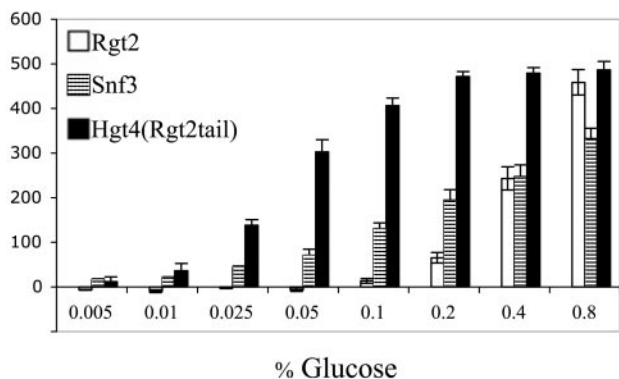
FIG. 7. Signaling by glucose sensors in *S. cerevisiae*. The *snf3Δ rgt2Δ* sensor-deficient *S. cerevisiae* mutant strain (YM6870) carrying a P_{HXT1}-*lacZ* reporter plasmid (pBM3712) was transformed with constructs expressing wild-type *S. cerevisiae* Rgt2 (YM7311), wild-type *S. cerevisiae* Snf3 (YM7312), *C. albicans* Hgt4 glucose-binding domain fused to the Rgt2 cytoplasmic tail (Hgt4-Rgt2 tail, YM7314), or vector only (YM7309). Values were normalized to cells harboring vector only (baseline). The β-galactosidase activity is indicated in Miller units.

Hgt4 with a tail that can interact with the components of the glucose-signaling pathway (Mth1 and Std1), so we replaced the C-terminal tail of Hgt4 with the tail of the Rgt2 sensor. The resulting protein (codon optimized for expression in *S. cerevisiae*) was expressed in a *snf3Δ rgt2Δ* mutant that carries the *HXT1-lacZ* gene fusion to report the glucose signaling activity (Table 1). The Hgt4-Rgt2 chimera mediates induction of *HXT1* expression in response to low levels of glucose, like the low-glucose (high-affinity) sensor Snf3 (Fig. 7). This is unlikely to simply be due to attaching the Rgt2 tail to Hgt4, because attaching the identical tail to glucose transporters does not bestow upon them the ability to generate a glucose signal (35). This suggests that Hgt4 is a high-affinity glucose receptor. It is notable that the Hgt4-Rgt2tail protein efficiently senses 0.05 to 0.1% glucose, which corresponds to the normal glucose concentration in human blood (0.09% or 5 mM).

***HGT4* contributes to *C. albicans* virulence.** Since Hgt4 is required for filamentation of *C. albicans* cells and for normal growth on low levels of glucose, we suspected it may be necessary for virulence in vivo. However, since *C. albicans* favors respiration over fermentation and since growth of the *hgt4Δ* mutant on glucose is impaired only under conditions in which fermentation is important, it was possible that *HGT4* would be irrelevant to virulence. To investigate this, we assessed the colonization and virulence of the *hgt4Δ* mutant (Fig. 8) (50). We used isogenic, prototrophic strains of *C. albicans* that differed only in the presence or absence of the *HGT4* ORF. Cells used for the infections were prepared from log-phase cultures grown under conditions in which there is no growth rate difference between these strains (Fig. 8A) (36). Cells were injected into the lateral tail veins of female mice, which were monitored for morbidity and mortality. Ninety percent mortality was observed within 5 days for mice infected with the wild-type *HGT4* strain but was not observed until 14 days postinfection in mice infected with the *hgt4Δ* mutant (Fig. 8B). The fungal burden in the kidneys of the mice was determined throughout the course of infection and was similar to that reported in other studies (Fig. 8C) (50). The fungal burdens in

hgt4Δ-infected mice were similar to the burdens in mice infected with wild-type cells but, by day 6, 90% of the wild-type-infected mice had died and 90% of the *hgt4Δ*-infected mice were still alive. This suggests that *HGT4* is not necessary for colonization of the kidney. Perhaps the delayed virulence of the *hgt4Δ* mutant is due to slower organ invasion or to a filamentation defect in vivo.

The sudden onset of mortality seen in the *hgt4Δ* infection at days 6 to 7 postinfection could have been due to genetic "revertants" in the in vivo yeast population. However, when *C. albicans* colonies rescued postmortem from the kidneys in two *hgt4Δ*-infected mice (days 6 to 7 postinfection) and from two *HGT4*-infected mice (day 2 postinfection) were compared, the *hgt4Δ* strains had not reverted to the wild-type phenotypes for filamentation on spider medium, growth on YPD plus antimycin A, or growth on 0.2% fructose (Fig. 8D and E). Thus, the *hgt4Δ* mutants are able to kill mice, albeit more slowly than wild-type cells.

DISCUSSION

C. albicans is a significant contributor to morbidity and mortality for hospitalized and immunocompromised people, in part because of its ability to access and thrive in varied environments. The primary requirement for fitness in any environment is the ability to garner nutrients such as glucose. Previous work has indicated that genes for the *HGT4* sensor and the *HGT12* transporter increase in expression when *C. albicans* cells are phagocytosed by macrophages (32, 42) and that these genes are regulated during filamentation, biofilm formation, and in response to antifungal drugs (5, 30), which implies a vital function for these proteins in the host environment.

We have provided evidence that Hgt4 is a sensor of sugar in *C. albicans*. The Hgt4 sensor appears to sense fructose and mannose, as well as glucose, like the Snf3 and Rgt2 sensors in *S. cerevisiae* (19, 27, 54). Hgt4 seems to be functionally orthologous to Snf3 of *S. cerevisiae*, a sensor of low levels of glucose. Like *SNF3*, *HGT4* is expressed when glucose is scarce but not when glucose is abundant, which suggests that both of these proteins are high-affinity glucose receptors. As a high-affinity glucose receptor, *C. albicans* Hgt4 appears to sense lower glucose concentrations than are sensed by *S. cerevisiae* Snf3 (Fig. 7). This likely reflects adaptation of these two species to different niches.

The Hgt4 sugar sensor is required for optimal growth on low concentrations of fructose, mannose, and glucose and for utilization of glucose by apparent fermentation. We do not yet understand the reason for the more severe growth defect of the *hgt4Δ* mutant on fructose compared to mannose and glucose. Hgt4 regulates only 6 of the 20 predicted hexose transporters, and these may be critical for fructose transport, whereas mannose and glucose may be transported by other (constitutively expressed) Hgts. In addition, the first step of hexose metabolism is its phosphorylation catalyzed by hexokinases. *C. albicans* possesses two hexokinases (*Hxk1* and *Hxk2*) and four glucokinases (*Glk1*, *Glk2*, *Glk3*, and *Glk4*), only one of which (*HXK2*) is regulated by Hgt4. Fructose phosphorylation may require Hxk2.

Growth on galactose plates was not affected by the *hgt4Δ* mutation (Fig. 2A), as expected; in *S. cerevisiae*, genes encod-
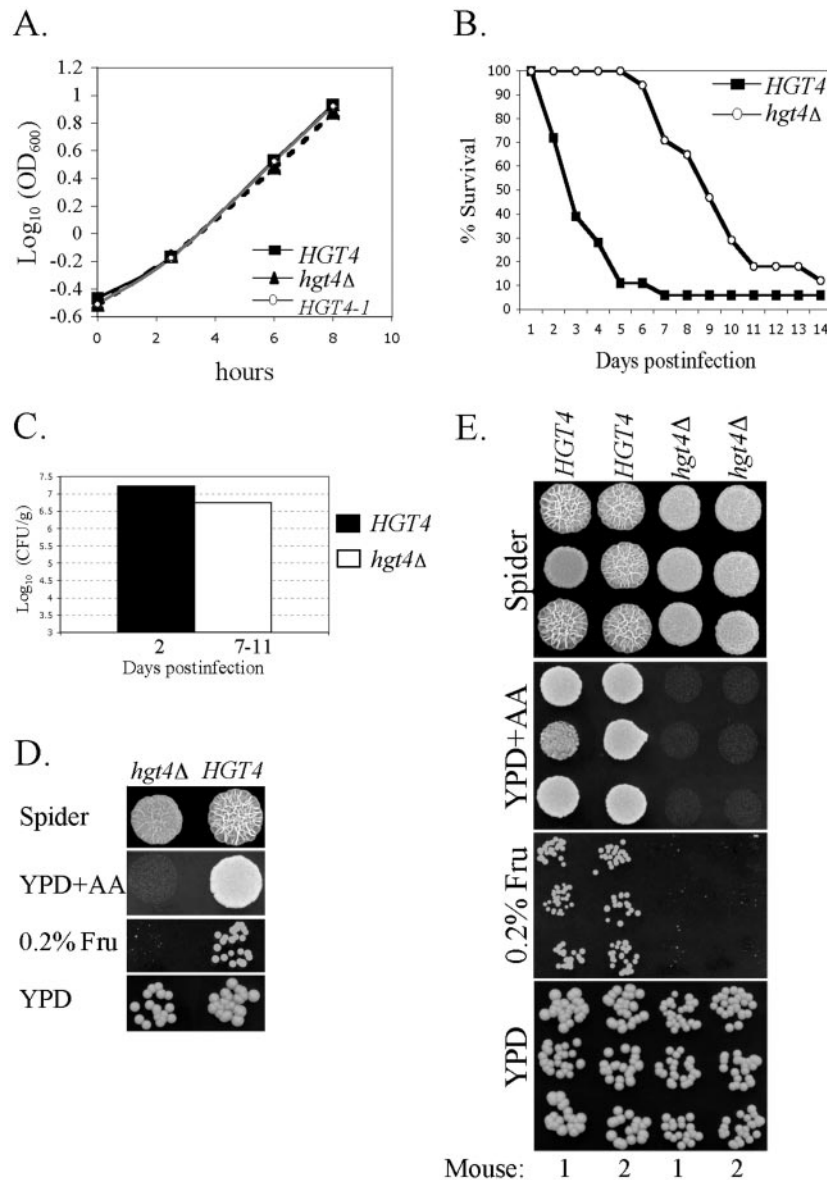
FIG. 8. *HGT4* is required for optimal virulence in vivo. (A) Growth curves for isogenic *C. albicans* strains. HGT4 (wild type: CM87 and CM97), *hgt4Δ* (null mutants: CM137 and CM140), and *HGT4-1* (constitutive mutants: CM35 and CM36) cells were grown to log phase in liquid YPD aerated by shaking at 30°C. All standard deviations were <3% of the OD_{600} values. These *C. albicans* strains are isogenic and prototrophs, differing only in the *HGT4* allele. (B) *HGT4* affects virulence during disseminated candidiasis. Mice were injected in the lateral tail vein with 7.5×10^5 cells and monitored for morbidity and mortality. *HGT4* indicates the *hgt4Δ* CM9 complemented with one copy of the wild-type gene, strain CM87 ($n = 18$), and *hgt4Δ* indicates the *hgt4Δ* CM9 complemented with the vector plus noncoding flanking regions of *HGT4* (UTRs), strain CM137 ($n = 17$). (C) The fungal burden was determined for three mice infected with the *HGT4* strain (day 2) and for three mice infected with the *hgt4Δ* strain (one mouse from day 7 and two mice from day 11). The variation between mice was 6×10^6 CFU/g (*HGT4* strain) and 6×10^5 CFU/g (*hgt4Δ* strain). (D) Phenotypes for the original strains used to infect mice in the disseminated *Candidiasis* experiments in panel C: *HGT4* (CM87) and *hgt4Δ* (CM137). (E) Phenotypes of individual clones recovered postmortem from the kidneys. *C. albicans* isolates were recovered from two mice infected with the *HGT4* strain (day 2) and from two mice infected with the *hgt4Δ* strain (days 6 and 7). Three independent colonies from each mouse were tested for filamentation on spider medium, for growth on YPD-antimycin A, and for growth on synthetic complete medium with 0.2% fructose.

ing enzymes for galactose uptake and utilization are induced by a different signaling pathway (19, 20, 54). A slight growth defect was found for the *hgt4Δ* mutants in some galactose-based media under certain conditions, but the effect was subtle and inconsistent and may have been due to variable, minute amounts of glucose that often contaminate galactose prepara-

tions. It is not surprising that *HGT4* is required for fermentation because fermenting cells demand increased glucose transport to achieve the high glucose flux necessary to obtain sufficient energy from that relatively inefficient mode of metabolism. It is not clear to us why the *hgt4Δ* strains grow more slowly on fructose agar plates when oxygen is abundant and

TABLE 2. Genes regulated by Hgt4 (*HGT4-1* versus *hgt4*Δ[ρ])[a]

ORF no.	Fold change		Gene	GO annotation
	30°C	37°C		
orf19.5962	28.6	65.4	HGT4/SNF3	High-affinity glucose transport protein; response to glucose stimulus
orf19.7094	27.9	53	HGT12/SNF32	Glucose sensor or transporter protein; response to glucose stimulus
orf19.2023	14.8	29.5	HGT7/HXT3	Hexose transporter; fructose, mannose, glucose transport
orf19.4384	13.6	29.2	HXT10	Fructose symporter; galactose, glucose, mannose transport
orf19.508	5.83	5.5	QDR1	Multidrug resistance transporter
orf19.5713	3.48	3.11	NDE1	NADH dehydrogenase; cytochrome *c* oxidase, ethanol fermentation
orf19.5730	3.14	5.28	orf19.13152	3-Polyprenyl-4-hydroxybenzoate decarboxylase
orf19.5337	3.03	3.65	UBC15	E2 ubiquitin-conjugating enzyme; ubiquitination, DNA repair
orf19.4527	2.98	2.98	HGT1	Hexose transporter
orf19.1048	2.93	2.39	IFD1	Benzyl or aryl-alcohol dehydrogenase; aldehyde metabolism
orf19.999	2.73	5.62	GCA1	Glucoamylase; alpha glucosidase, cell wall biosynthesis
orf19.5911	2.71	4.8	CMK1	Ca^{2+}/calmodulin-dependent protein kinase; signal transduction
orf19.3670	2.64	2.24	GAL1	Galactokinase; galactose metabolism, transcription regulation
orf19.3392	2.61	3.04	DOG1	2-Deoxyglucose-6-phosphate phosphatase; glucose metabolism
orf19.1509	2.47	3.37	ROD1	Calcium and zinc resistance protein
orf19.2244	2.46	2.78	orf19.9785	Aldoketo reductase
orf19.2020	2.45	3.48	HGT6/HXT6	Hexose transporter; fructose, mannose, glucose transport
orf19.4773	2.42	3.97	AOX2	Alternative oxidase II
orf19.4941	2.38	2.15	TYE7	Transcription factor; positive regulator of glycolysis
orf19.6803	2.36	2.74	HUT1	UDP-galactose transporter, endoplasmic reticulum translocation, galactosylation
orf19.4318	2.3	2.37	MIG1	Transcriptional regulator; transcription corepression, glucose metabolism
orf19.542	2.26	3.32	HXK2	Hexokinase II, fructose metabolism
orf19.6420	2.06	2.19	PGA13	GPI anchor, similar to mucins, regulated by Tsa1, Cyr1, Nrg1, Tup1, Rlm1
orf19.5816	2.02	2.14	EBP7	NADPH dehydrogenase
orf19.2990	2	2.3	XOG1	Glucan 1,3-β-glucosidase; cell wall biogenesis/organization, glucan metabolism
orf19.1237	1.96	1.95	ARO9	Aromatic amino acid aminotransferase II; amino acid metabolism
orf19.3668	1.92	4.15	HGT2	Hexose transporter
orf19.5392	1.91	4.19	orf19.12847	Conserved protein; iron homeostasis siderochrome-iron transport
orf19.6993	0.44	0.47	GAP2	General amino acid permease regulated by Nrg1 and Tup1
orf19.5503	0.44	0.46	Putative ORF	Hypothetical protein
orf19.5975	0.42	0.5	orf19.13396	Zinc-finger transcription factor
orf19.4568	0.4	0.22	ZCF2	Zn(2)-Cys(6) transcription factor
orf19.2292	0.37	0.48	OPT4	Oligopeptide transporter protein
orf19.3902	0.31	0.42	orf19.11383	Hypothetical protein
orf19.3904	0.3	0.4	orf19.11385	Hypothetical protein

[a] The *HGT4-1* (CM36) and *hgt4*Δ[ρ] null (CM32) strains were grown to log phase in synthetic complete medium with glycerol at 30°C (n = 2 each strain) and 37°C (n = 2 each strain). Total RNA was prepared and used to probe 70-mer DNA oligonucleotide microarrays representing all of the ORFs annotated in the *C. albicans* genome assembly 19. Genes changed by >2-fold at 30 and 37°C are shown.

respiration is preferred (Fig. 2A). This could be because (i) colonies on solid media may encounter a microhypoxic environment; (ii) *C. albicans*, like *S. cerevisiae*, may ferment sugars even when oxygen is available (37); or (iii) the Hgt4 sensor may regulate genes required for respiration. The third possibility gains some support from our observation that expression of two genes that may influence respiration—the NADH dehydrogenase encoded by Ca*NDE1/YMX6* (orf19.5713), which transfers electrons from NADH in the cytoplasm to ubiquinone, and orf19.5730, encoding a putative 3-octaprenyl-4-hydroxybenzoate carboxy-lyase involved in ubiquinone biosynthesis—is slightly increased by the *HGT4-1* mutation.

HGT4 appears to be important for fermentation because it is required for growth in the presence of antimycin A, which substantially inhibits respiration by blocking electron transfer between cytochromes *b* and *c* (14). Wild-type *C. albicans* is able to carry out some respiration in the presence of antimycin A because it has the alternative oxidases Aox1 (encoded by orf19.4774) and Aox2 (encoded by orf19.4773) that function when cytochromes *b* and *c* are inhibited (24) and *AOX2* expression is induced by antimycin A (16). *AOX2* expression is

also induced by the glucose signal generated by Hgt4 (Table 2), and this may account for the severe growth defect of the *hgt4*Δ mutant on glucose plus antimycin A. The coregulation of fermentative and respiratory pathways via Hgt4 would represent yet another significant transcriptional rewiring that occurred since the divergence of *C. albicans* and *S. cerevisiae* (17, 48, 53).

Hgt12 (orf19.7094) is homologous to both Hgt4 and Snf3. It was suggested to be a glucose sensor because it was shown that *hgt12* mutations have effects on filamentation of cells (32). We detected no growth or filamentation abnormalities in our *hgt12*Δ mutants and found that *hgt12*Δ mutants exhibit normal regulation of the *HGT4* target genes *HXT10* and *HGT7* (data not shown). Thus, we suggest that Hgt12 is likely a hexose transporter rather than a glucose sensor. Another *HGT* gene (*HGT3*, orf19.4356) encoding a putative hexose transporter is predicted to possess a C-terminal extension of 177 amino acids, which opens the possibility that another sugar sensor exists in *C. albicans*.

From these data we propose a model for glucose sensing through Hgt4 in *C. albicans* that is based on our understanding of the orthologous pathway in *S. cerevisiae* (Fig. 9). Hgt4 is
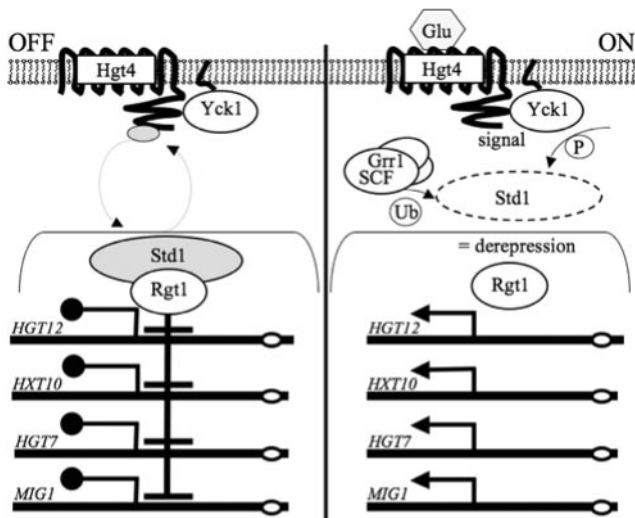
FIG. 9. Model for glucose sensing in *C. albicans*. The Hgt4 glucose sensor resides in the plasma membrane of cells. The C-terminal tail of Hgt4 associates with Std1, a transcriptional corepressor that also associates with the Rgt1 DNA-binding protein. Std1, together with Rgt1, represses expression of the *HGT* genes. Upon stimulation of the sensor by physiologic levels of glucose, Std1 is phosphorylated by Yck1, then ubiquitinated by SCF^{Grr1}, and finally degraded by the proteasome. Without Std1, Rgt1 is unable to repress transcription, leading to derepression of target genes such as those identified in Table 2.

expressed on the surface of cells growing on nonfermentable carbon sources or at physiologic (relatively low) levels of glucose. Hgt4 likely associates with CaStd1 (orf19.6173), the orthologue of the Std1 and Mth1 transcriptional corepressors of *S. cerevisiae*. The CaStd1 corepressor probably also associates with CaRgt1 (orf19.2747), a nuclear DNA-binding protein that binds to the promoters of *HGT* genes encoding glucose transporters and effects their repression. The Hgt4-associated and Rgt1-associated Std1 protein presumably shuttles between the cytoplasm and the nucleus. Upon stimulation of the Hgt4 sensor by glucose, cytoplasmic Std1 becomes phosphorylated by Yck1 (orf19.7001 or orf19.2222), is then ubiquitinated by SCF^{Grr1}(orf19.3944), and is subsequently degraded in the proteasome. Without Std1, the Rgt1 transcriptional repressor is inactive, resulting in derepression of a handful of target genes (identified in Table 2). This model for the *C. albicans* glucose-sensing and signaling pathway predicts that deletion of *RGT1* will suppress the *hgt4Δ* mutant phenotypes, and that is indeed the case (Fig. 6C and data not shown). The *C. albicans* and *S. cerevisiae* glucose-sensing pathways are remarkably similar, with the exception that the Hgt4 glucose signal in *C. albicans* may affect genes in additional pathways such as drug resistance (*QDR1*), respiration (*NDE1*), alternative oxidation (*AOX2*), and galactose metabolism (*GAL1*) (Table 2).

At high glucose levels, expression of the genes encoding the high-affinity sensor *HGT4* and the transporters *HGT12* and *HXT10* is repressed. In *S. cerevisiae*, glucose repression of *SNF3* and the transporters *HXT2* and *HXT4*, mediated by the Mig1 and Mig2 repressors, shuts down the high-affinity sensor and transporters when they are not needed. In *C. albicans*, expression of *MIG1* (orthologous to *MIG1* and *MIG2* in *S. cerevisiae*) is induced by glucose via the Hgt4 glucose sensor,

implying that a feedback repression mechanism exists in *C. albicans* as well (Table 2). *C. albicans* genes *HGT12* and *HXT10* expression mimics *S. cerevisiae* genes *HXT2* and *HXT4* (induced by low levels of glucose; repressed by high levels of glucose), and *C. albicans* gene *HGT7* expression behaves like *S. cerevisiae* gene *HXT3* (induced by low and high levels of glucose). Thus, this glucose-sensing pathway functions to ensure that the appropriate glucose transporters are expressed in response to the amount of glucose available.

The most surprising aspect of our results is that *HGT4* is essential for optimal virulence in the mouse. This could be simply due to the fact that *HGT4* is required for optimal growth in the relatively low concentrations of sugars present in the mammalian bloodstream (<0.2%). We do not favor this idea because aerated cultures of cells exhibit no differences in growth between the *hgt4Δ*, *HGT4*, and *HGT4-1* strains (growth curves of cells growing on YP, YP plus 5 mM glucose, or synthetic complete media with 0.2% glucose are identical to Fig. 8A [data not shown]). Also, we suspect that *C. albicans* cells infecting the host spend little time in the bloodstream and thus have little opportunity for growth there.

Oxygen, which is required for respiration, is unlikely to be available to *C. albicans* cells in the bloodstream because it is sequestered in hemoglobin (18), and it is unclear how available oxygen is to *C. albicans* cells colonizing the organs of an infected host. We suggest that *C. albicans* depends at least in part on fermentation during host infection (4, 28); thus, knocking out the Hgt4 sensor reduces virulence.

One of the major reservoirs for *C. albicans* is the (largely anaerobic) mammalian gastrointestinal tract, a niche where fermentation is undoubtedly important for survival. We have shown that *hgt4Δ* mutants are severely inhibited for growth in conditions that favor fermentation, even when sugar concentrations are high. Therefore, shutting down fermentation in *C. albicans* (by inactivating Hgt4) may destroy the ability of this opportunistic pathogen to colonize the human gut. This along with the cell surface location of the glucose sensor makes it an attractive potential drug target.

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