The ability to sense and rapidly respond to available nutrients in the environment is crucial to free-living microorganisms (1). To maximize the use of available environmental nutrients for fast growth and proliferation, different microbes have adapted in different ways to their environments. For example, glucose is the primary fuel and the preferred carbon source for the yeast *Saccharomyces cerevisiae* (2). As such, glucose is used as an important signaling molecule to regulate many biological programs, including the entry to and exit from the cell cycle and the ability to undergo morphological transitions (3). A number of physiological and biological processes are thus activated in a coordinated manner to facilitate cell growth and proliferation (4, 5).

When cultured in water with glucose (and in the absence of other nutrients needed for growth), glucose acts as a false signal for a nutrient-rich condition, which “tricks” stationary-phase cells of *S. cerevisiae* into entering a state of active metabolism for a nutrient-rich condition, which “tricks” stationary-phase cells of *S. cerevisiae* into entering a state of active metabolism for growth and division. At first, the cells undergo morphological and physiological changes characteristic of mitotic cell division, but then they rapidly lose viability due to the lack of other nutrients required for cell growth (5). This phenomenon, called sugar-induced cell death (SICD), has characteristics of programmed cell death (apoptosis), including nuclear DNA fragmentation, cell membrane damage, and the production of reactive oxygen species (ROS) (6). Although the role of programmed cell death in microbes is not known with certainty, it has been suggested to be an altruistic community behavior, providing released substances to promote viability of healthy cells and thus conferring a “group selection” advantage (7). The human fungal pathogen *Candida albicans* also undergoes what seems to be programmed cell death in response to environmental stresses, pheromone gradients, and certain antifungal agents (8, 9). *C. albicans* and *S. cerevisiae* last shared a common ancestor.
about 300 million years ago (10). The natural ecological niche for \textit{C. albicans}, which is inside mammalian hosts, is very different from that of \textit{S. cerevisiae}. In particular, \textit{C. albicans} is primarily associated with the gastrointestinal (GI) tract of humans (11), where glucose is often limiting. In principle, alternative carbon sources such as N-acetylglucosamine (GlcNAc) and other polysaccharides broken down from the intestinal mucosa or bacterial cell wall components could be used as primary energy and carbon sources (12, 13). In this study, we report that GlcNAc induces cell death in \textit{C. albicans} in a way analogous to that of SICD in \textit{S. cerevisiae}. We propose that GlcNAc serves as a primary carbon source for \textit{C. albicans} within a mammalian host and acts as a signaling molecule regulating multiple cellular programs in a coordinated manner, thereby maximizing the efficiency of nutrient use.

**RESULTS**

GlcNAc, but not glucose, induces cell death in \textit{C. albicans}. Consistent with previous studies (5, 6), in the absence of additional nutrients for growth, 2% glucose induced rapid cell death in \textit{S. cerevisiae} (Fig. 1A). However, GlcNAc-induced cell death in \textit{C. albicans} at 30°C (Fig. 1B) and at 25°C, 30°C, and 37°C (Fig. 1C). We tested whether \textit{C. albicans} cells also undergo sugar-induced cell death (SICD). As shown in Fig. 1B, neither glucose nor sorbitol alone in water induced cell death in \textit{C. albicans} at 30°C. However, GlcNAc-induced cell death (GICD) occurred rapidly in \textit{C. albicans} cells incubated in the presence of water plus 2% GlcNAc at 30°C (Fig. 1B). We tested the effect of culture temperature on GICD. Similar to the 30°C culture, almost all cells (over 99.9%) died after 5 days of incubation in the GlcNAc culture at both 25°C and 37°C, although the percentages of viable cells at 25°C were higher than those at 30°C within the first 3 days (Fig. 1C). We further found that standard yeast laboratory media (Lee’s and yeast nitrogen base [YNB]) with GlcNAc as the sole added carbon source could also induce cell death in \textit{C. albicans} (Fig. 1D). This could be due to GICD after the essential nutritional components were depleted in the medium. These results suggest that GlcNAc, but not glucose, can induce cell death in \textit{C. albicans}.

In all of the subsequent cell death assays, we cultured \textit{C. albicans} in water plus 2% GlcNAc (or 0.2% for the \textit{ngt1/ngt1} mutant as explained below) at 30°C.

**Cells undergoing GICD exhibit both apoptotic and necrotic features.** Necrosis is a form of cell death, morphologically characterized by an increase in cell volume and plasma membrane rupture followed by loss of organized intracellular structures (14). Transmission electron microscopy (TEM) assays indicated that cells treated with water or sorbitol for 24 to 72 h appeared normal, while a part or all of the cells exhibited necrotic features after

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**FIG 1** Sugar-induced cell death in \textit{S. cerevisiae} and \textit{C. albicans}. Stationary-phase cells of \textit{S. cerevisiae} and \textit{C. albicans} were incubated in water (H2O), 2% sorbitol, 2% glucose, or 2% GlcNAc. Cell viabilities at different time points were determined using plating assays as described in Materials and Methods. (A) Glucose-induced cell death in \textit{S. cerevisiae} at 30°C. (B) GlcNAc-induced cell death in \textit{C. albicans} at 30°C. (C) GlcNAc-induced cell death in \textit{C. albicans} at 25°C, 30°C, and 37°C. (D) Transmission electron micrographs of \textit{C. albicans} cells. Cells were used for TEM assays after incubation in H2O, 2% sorbitol, 2% glucose, or 2% GlcNAc at 30°C for 3 days.
induced cell death assays. Cell viabilities at different time points were determined using plating assays as described in Materials and Methods. The WT (SC5314) and the WT RAS1V13 (overexpression of the activating form of Ras1) strain. Cells grown to stationary phase in liquid YPD medium were used for GlcNAc interference contrast.

Production by dying cells of C. albicans induces in dying cells. Cells were incubated in H2O, 2% sorbitol, 2% glucose, or 2% GlcNAc at 30°C for 2 days prior to TUNEL assays and 3 days prior to ROS assays. (A) TUNEL reaction of H2O- or sugar-treated cells. (B) ROS production by dying cells of C. albicans. BF, bright field; DIC, differential interference contrast.

FIG 2 Nuclear DNA fragmentation and reactive oxygen species (ROS) production in dying cells. Cells were incubated in H2O, 2% sorbitol, 2% glucose, or 2% GlcNAc at 30°C for 2 days prior to TUNEL assays and 3 days prior to ROS assays. (A) TUNEL reaction of H2O- or sugar-treated cells. (B) ROS production by dying cells of C. albicans. BF, bright field; DIC, differential interference contrast.

incubation in GlcNAc for 24 to 72 h (Fig. 1D; see also Fig. S3 in the supplemental material). Cells treated with glucose appeared healthy except for their remarkably thickened cell wall and large vacuoles (Fig. 1D; see also Fig. S3). Unlike glucose-induced cell death in S. cerevisiae (6), scanning electron microscopy (SEM) assays demonstrated that GICD in C. albicans did not cause cell shrinkage (data not shown). The surface appearance of GlcNAc-treated cells was not notably different from that of water-, sorbitol-, or glucose-treated cells.

Annexin V affinity and TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assays to assess cell death demonstrated that at least a proportion (~25%) of the cells treated with GlcNAc for 48 h exhibited apoptotic features (phosphatidylserine [PS] exposure and nuclear DNA fragmentation indicated by positive annexin V and TUNEL staining [Fig. 2A; see also Fig. S1B in the supplemental material]).

As expected, almost all cells treated with water, sorbitol, or glucose were negatively stained by both annexin V and TUNEL assays (Fig. 2A; see also Fig. S1B). However, after incubation in GlcNAc for 72 h, over 90% of cells were PI positive (not shown). These results indicate that GlcNAc-induced cell death initially exhibits apoptotic features and subsequently becomes necrotic. Over 90% of cells lost viability between 24 and 72 h (Fig. 1B). This rapid process of losing cell viability could lead to the apoptotic-necrotic transition of cell death mechanisms. The percentages of apoptotic and necrotic types of cell death were measured after treatment with GlcNAc for 48 and 72 h (see Fig. S1C).

Cells undergoing GICD accumulate high levels of ROS. ROS play a critical role in causing cell death in S. cerevisiae and mammalian cells (14). 2’,7’-Dichlorofluorescein diacetate (DCFDA) staining to detect cellular ROS demonstrated that GlcNAc-treated cells exhibited remarkably elevated ROS levels, while no fluorescence signal was detected in the water, sorbitol, or glucose treatments (Fig. 2B). In addition, N-acetylcysteine (NAC), a scavenger of ROS, could delay GICD in 0.2% GlcNAc (not shown), suggesting that ROS are the causative agent of cell death.

Roles of the Ras1-cAMP signaling pathway in GICD. Phillips et al. have reported that the Ras1-cyclic AMP (cAMP) signaling pathway regulates hydrogen peroxide (H2O2)- and acetic acid-induced cell death in C. albicans (15). We therefore tested the hypothesis that this pathway also regulates GICD. When treated with GlcNAc, over 99% of cells of both the ras1/ras1 mutant and wild-type (WT) control died within 3 days (Fig. 3), suggesting that deletion of RAS1 has no obvious effect on GICD. However, about 50% of cells of the strain carrying an activating form of Ras1 (Ras1V13) died after only 1 day and all cells (100%) died after 2 days of incubation in GlcNAc (Fig. 3). Deletion of PDE2, encoding a high-affinity cyclic nucleotide phosphodiesterase in C. albicans, also accelerated GICD (Fig. 3). Consistent with these observations, deletion of the single adenylyl cyclase gene CYR1 in C. albicans notably delayed cell death induced by GlcNAc; over 50% of cells maintained viability after 7 days of incubation in GlcNAc (Fig. 3). We further observed that inactivation of the Ras1-cAMP signaling pathway by deletion of CYR1 decreased the production of ROS, while activation of this pathway by deletion of PDE2 or overexpression of RAS1V13 increased the production of ROS (see Fig. S4 in the supplemental material).

The GlcNAc catabolic pathway and mitochondrial protein Muc1 regulate GICD in C. albicans. The pathway allowing C. albicans to use GlcNAc as a carbon source appears absent in

FIG 3 The Ras1-cAMP signaling pathway regulates GlcNAc-induced cell death in C. albicans. Strains used were the ras1/ras1, cyr1/cyr1, and pde2/pde2 mutants and the WT+RAS1V13 (overexpression of the activating form of Ras1) strain. Cells grown to stationary phase in liquid YPD medium were used for GlcNAc-induced cell death assays. Cell viabilities at different time points were determined using plating assays as described in Materials and Methods. The WT (SC5314) served as the control. An asterisk indicates a significant difference (P < 0.05, Student’s t test) between the survival rates of the WT and the mutants.
S. cerevisiae (16). Ngt1 is a GlcNAc-specific transporter that is induced by GlcNAc (17). Since GlcNAc uptake can occur via either an Ngt1-dependent (high-affinity) or an Ngt1-independent (low-affinity) process in C. albicans (17), we performed GICD assays at both low and high concentrations of GlcNAc. As shown in Fig. 4A and B, deletion of NGT1 remarkably delayed cell death when cells were incubated in 0.2% GlcNAc but did not affect cell death in 2% GlcNAc. This result is consistent with a previous study showing that NGT1 is not required for GlcNAc uptake in the presence of high levels of GlcNAc (17). Ngt1 facilitates the high-affinity transport of GlcNAc. Under high GlcNAc concentrations, GlcNAc can also be transported by an unidentified low-affinity transporter into the cell. Hxk1, a GlcNAc kinase, converts GlcNAc to GlcNAc-6-PO4, and Dac1, a deacetylase, converts GlcNAc-6-PO4 to glucosamine-6-PO4. Then, the glucosamine-6-phosphate deaminase, Nag1, converts this compound to fructose-6-PO4 (18, 19). Deletion of HXK1, DAC1, or NAG1 also notably delayed GICD in the 2% GlcNAc cultures (Fig. 4C). As expected, cell death induced by GlcNAc was further delayed in the dac1/dac1 hxlk1/hxlk1 nag1/nag1 triple mutant, where we observed that about 50% of cells still maintained viability after 20 days of incubation in GlcNAc (Fig. 4C). The mitochondrial protein Mcu1 is essential for utilization of GlcNAc as the sole carbon source for growth of C. albicans (20). Deletion of MCU1 in C. albicans also remarkably delayed cell death induced by GlcNAc (Fig. 4D). As expected, cells of the MCU1-; HXXK1-; NAG1-; and HXXK1-, DAC1-, and NAG1-reconstituted strains underwent GICD in a manner similar to that of the WT control (data not shown). These results indicate that the GlcNAc catabolism pathway plays a critical role in the regulation of GICD in C. albicans.

The copper-sensing transcription factor Mac1 regulates GICD in C. albicans. To further explore the regulatory mechanism, we screened a library of 165 transcription factor mutants for mutants defective in GICD (21). As shown in Fig. 5 and in Data Set S1 in the supplemental material, we found 16 mutants with survival rates over 5% after 5 days of culture in 2% GlcNAc at 30°C, compared to the parental strain rate of <0.5%. The survival rate of the mac1/mac1 mutant was the highest (75%), while that of the others was about 5 to 10%. Mac1 regulates copper metabolism and filamentation in C. albicans (21–23). In all, six of the 16 mutants (MAC1, LEU3, UGA33, SUC1, UPC2, and MIG1) are associated with metabolism, while 11 (SEL1, ZCF21, MAC1, SUC1, UPC2, CZF1, RIM101, ZNC1, AAF1, CAP1, and MIG1) are associated with adhesion or biofilm formation (note that there is some overlap within these categories). ORF19.2476 is involved in histone demethylation, while the function of ORF19.3928 is unknown.

The library strains are arginine autotrophic; we next assessed whether the lack of ARG4 was related to GICD. As shown in Fig. 5B, deletion of ARG4 alone had no obvious effect on GICD. However, we did observe that, while the mac1/mac1 and mac1/mac1 arg4/arg4 double mutants showed delayed cell death, the survival rate of the latter was significantly higher (Fig. 5B and C). These results suggest that Arg4 and Mac1 have a synergistic effect on GICD.

Compared to the WT control, the mutants of mac1/mac1 and GlcNAc catabolic pathways produced much less ROS (see Fig. S5 in the supplemental material) when treated with GlcNAc. Mitochondrial metabolism is the major source of ROS. These results...
further support the idea that mitochondrial metabolism and ROS play a critical role in GICD.

Quantitative reverse transcription-PCR (RT-PCR) assays demonstrate that the expression levels of MAC1 in the ras1/ras1 and cyr1/cyr1 mutants were clearly lower than that in the WT control (especially in the ras1/ras1 mutant [Fig. 5D]), suggesting that Ras1 plays a critical role in the regulation of the expression of MAC1 in a direct or indirect manner. However, deletion of PDE2 did not significantly affect the transcriptional level of MAC1. Compared to that of the WT, the expression levels of a number of genes involved in oxidative metabolism and the tricarboxylic acid (TCA) cycle were decreased in the mac1/mac1 mutant (Fig. 6A). These genes include CIT1 (encoding a citrate synthase), ACO1 (encoding an aconitase), KGD1 (encoding a putative 2-oxoglutarate dehydrogenase), KGD2 (encoding a putative dihydrolipoamide S-succinyltransferase), MDH1-1 (encoding a malate dehydrogenase, Fe-S subunit), and SDH2 (encoding a succinate dehydrogenase, Fe-S subunit). We next examined whether the TCA cycle regulates GICD in C. albicans. As shown in Fig. 6B, deletion of CIT1, ACO1, KGD1, KGD2, MDH1-1, or SDH2 significantly delayed GICD in C. albicans, suggesting that the TCA cycle plays a critical role in the regulation of GICD.

GICD is independent of Cst20 and Mca1. The Ste20 kinase, a member of the p21-activated kinase family, regulates H₂O₂-induced apoptosis and glucose-induced cell death in S. cerevisiae (24, 25), and yeast caspase 1 (Yca1) is a caspase-related protease regulating apoptosis in S. cerevisiae (26). We next examined the roles of Cst20, the C. albicans ortholog of Ste20 (27), and Mca1, the C. albicans ortholog of Yca1 in S. cerevisiae, in GICD. Deletion of CST20 or MCA1 in C. albicans had no significant effect on GICD. Similar to the WT control, almost all cells of the two mutants died within the first 3 days of incubation (data not shown), suggesting that GICD is independent of the regulation of Cst20 and Mca1.

Protein synthesis and cellular metabolism genes are constitutively active in GlcNAc-treated cells. Cells of C. albicans undergo one to two divisions in the 2% glucose and 2% sorbitol cultures (Fig. 1B), suggesting that cells are metabolically active in the first several hours. We performed a comparative transcriptome sequencing (RNA-Seq) analysis of C. albicans cells treated with GlcNAc, glucose, or sorbitol for 5 or 24 h (Fig. 7; see also Data Sets S2, S3, and S4 in the supplemental material). The results demonstrated the following. (i) The expression of GlcNAc catabolism-related genes, including HXK1, DAC1, and NAG1, was upregulated in GlcNAc-treated cells at both 5 and 24 h. (ii) With almost no exceptions, the expression of genes encoding cytoplasmic protein synthesis, ribosomal biogenesis-related proteins, and mitochondrial ribosomal proteins was upregulated in cells treated with GlcNAc for 24 h, compared to that in cells treated with glucose and sorbitol. Moreover, these genes were upregulated at 24 h of GICD exposure relative to 5 h, illustrating that the response is gradual. These results are consistent with previous work showing...
the strong correlation between the expression of cytoplasmic ribosomal protein genes and that of mitochondrial ribosomal protein genes (28). (iii) Many genes encoding stationary-phase-enriched proteins (29) were downregulated in GlcNAc-treated cells and upregulated in glucose- or sorbitol-treated cells for 24 h (see Data Set S4). The experiments demonstrated that GlcNAc-treated cells continued with high levels of metabolic activity, while glucose- or sorbitol-treated cells entered stationary phase after 24 h of incubation. A comparative analysis of differentially expressed genes between sorbitol, glucose, and GlcNAc treatments at both 5 and 24 h was performed. Details are presented in the supplemental material (see Text S1 and Data Sets S2, S3, and S4).

DISCUSSION

The ability to adapt to changing ecological niches is central to the survival of microorganisms. In this study, we report that C. albicans utilizes GlcNAc, a niche-specific carbon source, as a signaling molecule for the availability of external nutrients and to coordinate a series of biological processes for cell growth. In the absence of additional nutrients, GlcNAc acts as a false signal for high nutrient conditions, which leads to an increase in cellular metabolism, failure to enter a quiescent (G0) state (or exit from the cell cycle), and rapid cell death due to the accumulation of intracellular ROS. Consistent with this observation, protein synthesis- and ribosomal biogenesis-related genes are highly activated, while the expression of stationary-phase-related genes (which would allow survival) is repressed in C. albicans cells after 24 h of GlcNAc treatment (Fig. 7; also see Data Sets S2, S3, and S4 in the supplemental material).

Glucose is often limiting in the natural habitats of C. albicans, such as the GI tract (30), and alternatives such as GlcNAc may serve as the major carbon source in host environments (12, 13). Therefore, it is reasonable that GlcNAc, rather than glucose, serves as an extracellular nutritional signal in C. albicans. Pande et al. have recently reported that genes of the GlcNAc catabolic pathway are upregulated during the “gut-induced transition” undergone by C. albicans when it enters the gut (31). All these results suggest that the ability of C. albicans to respond to GlcNAc contributes to its ability to colonize the gut.

Cell death triggered by GlcNAc in C. albicans initially exhibits apoptotic features and rapidly becomes necrotic over extended incubation times (Fig. 1 and 2; see also Fig. S1 and S3 in the supplemental material). This cell death process appears analogous to that observed in glucose-induced cell death in S. cerevisiae (6).

As summarized in Fig. 8, we demonstrate that multiple pathways, including the GlcNAc catabolic, Ras1-cAMP signaling, and TCA pathways, are involved in the regulation of GICD in C. albicans. Mitochondria play a central role in this regulation. All these pathways are involved in the regulation of respiratory metabolism and the production of ROS.

Given the nutrient-limiting conditions of the gut, the ability of carbon source sensing and utilization by C. albicans likely contributes to the ability of C. albicans to colonize this niche. We suggest that C. albicans may use the amino sugar GlcNAc, which is an important component of bacterial cell wall peptidoglycan, fungal cell wall chitin, and animal cell extracellular matrix, as a signal to trigger maximal utilization of environmental nutrients, thus promoting its growth and survival in the host when nutrients are abundant. Microorganisms living in different ecological niches are likely to possess distinct responses to specific nutritional signaling molecules. Despite the divergence of these signaling molecules themselves and their respective sensing pathways, the outputs (such as coordination of growth rate and the cell cycle, optimal use of environmental nutrients, reprogramming of transcriptional and metabolic profiles, and cell death) could be similar. Despite the divergence of ecological niches and metabolic pathways between S. cerevisiae and C. albicans, the response to primary carbon sources (glucose in S. cerevisiae and GlcNAc in C. albicans) and the induction of cell death by these carbon sources have similar outputs. Finally, given that C. albicans is also a human pathogen, the pathways involved in the regulation of this
type of cell death could be explored as possible therapeutic targets to specifically induce cell death.

**MATERIALS AND METHODS**

**Culture conditions, strains, and plasmids.** The strains and primers used in this study are listed in Text S1 in the supplemental material. Yeast extract-peptone-dextrose (YPD) (20 g/liter glucose, 20 g/liter peptone, 10 g/liter yeast extract; 20 g/liter agar added for solid medium) and synthetic defined (SD) medium were used for routine growth of yeast cells. The open reading frame (ORF) region of the activating form of \(RAS1\), \(RAS1V13\), was amplified by PCR from the plasmid pCaEXP-RAS1V13 (32) and digested with EcoRV and HindIII. The digested \(RAS1V13\) fragments were inserted into the plasmid pACT1 (32), generating pACT-RAS1V13. The plasmid was digested with AscI and transformed into the WT strain (CAI4) to generate the RAS1V13 overexpression strain (WT/H11001RAS1V13).

The two alleles of \(MCA1\) were deleted in SN152 using the fusion PCR strategy as described previously (33), to generate the \(mca1/mca1\) mutant. \(HIS1\) and \(LEU2\) were used as markers for selection. A copy of the \(C. albicans\) ARG4 gene was reintegrated into its original locus in the mutant to create prototrophic strains (33).

**GICD and viability assays.** \(C. albicans\) and \(S. cerevisiae\) cells were grown to stationary phase in liquid YPD medium at 30°C, collected by centrifugation, and washed three times with double-distilled water (ddH\(_2\)O). Cells (6 × 10\(^7\) per ml) were used for GlcNAc-induced cell death (GICD) assays. Water (ddH\(_2\)O), 2% GlcNAc (TCI Inc., Japan), 2% sorbitol (Sigma-Aldrich; grade of 99%), and 2% glucose (Sigma-Aldrich; grade of 98%) media were used for cell treatments. The last three media contain water with 2% GlcNAc, 2% sorbitol, or 2% glucose, in the absence of additional nutrients for cell growth. Cells were incubated in 20 ml of different media with shaking and replated onto YPD plates at the time points indicated above. After 2 to 3 days of growth at 37°C, the numbers of CFU were determined to indicate percentages of viable cells. GICD assays were performed in several WT strains (including SC5314, SN250, DIC185, and P37005). All the strains underwent GICD in a similar manner (not shown). The results from SC5314 were used as a control for most experiments. The average numbers of percentages and standard deviations (SD) are presented.

**PI and annexin V staining, ROS, and TUNEL assays.** Propidium iodide (PI; Sigma-Aldrich) staining and annexin V (Invitrogen, Inc.) labeling assays were performed as described previously (8, 34). Briefly, cells treated in different media (H\(_2\)O, 2% GlcNAc, 2% sorbitol, or 2% glucose) for 48 h were collected and washed with 1× phosphate-buffered saline (PBS). For PI staining, PBS-PI buffer (containing 2 μg/ml of PI) was used. For annexin V staining, cells were treated with Zymolyase to form protoplasts at 30°C for 20 min. Zymolyase reaction buffer consisted of 0.02 mg/ml Zymolyase 20T in PPB (potassium phosphate buffer; 0.5 ml of 50 mM K\(_2\)HPO\(_4\), 5 mM EDTA, 50 mM dithiothreitol [DTT], 50 mM

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**FIG 7**

Global gene expression analysis of sorbitol-, glucose-, and GlcNAc-treated \(C. albicans\) cells. (A and B) Venn diagrams of differentially expressed genes comparing sorbitol versus glucose, sorbitol versus GlcNAc, and glucose versus GlcNAc and sorbitol-, glucose-, and GlcNAc-induced genes. Cells were incubated at 30°C for 5 (A) or 24 (B) hours. In panel A, the total numbers of sorbitol-, glucose-, and GlcNAc-induced genes are shown in their corresponding colors; the numbers of genes transcriptionally upregulated in both the 5-h treatment and 24-h treatment are indicated in parentheses. (C) Venn diagrams of differentially expressed genes comparing 5- versus 24-h cultures. A 4-fold cutoff was used. In each comparison, genes with values for reads per kilobase per million of mapped reads lower than 50 in all treatments were excluded.
Electron microscopy. Cells treated with 2% GlcNAc, 2% glucose, 2% sorbitol, or water for 24 to 72 h were used for transmission electron microscopy (TEM) assays. TEM assays were performed according to the previously reported protocol (35). Briefly, cells were fixed in 0.5% polyoxymethylene and 2.5% glutaraldehyde in a buffer solution (0.2 M PIPES [piperazine-N,N′-bis(2-ethanesulfonic acid)], pH 6.8, 1 mM MgCl₂, 1 mM CaCl₂, 0.1 M sorbitol) and then washed three times with deionized water. Dehydration was performed postfixing with increasing concentrated acetone solutions (50, 75, 85, 95, 100, 100%). Cells were then infiltrated and embedded in Spurr resin.

RNA-Seq analysis. Cells of *C. albicans* SC5314 treated with 2% GlcNAc, 2% sorbitol, or 2% glucose for 5 h or 24 h were used for RNA-Seq analysis. Total RNA was extracted as described previously (36) using the GeneJET RNA purification kit according to the manufacturer’s instructions. RNA-Seq analysis was performed by the company BGI-Shenzhen according to the company’s protocol (37).

Quantitative real-time PCR (Q-RT-PCR) assays. Quantitative PCR was performed according to our previous publication with modifications (38). Cells of *C. albicans* were grown in liquid Lee’s glucose medium for 24 h and used for RNA extraction. Briefly, total RNA was used to synthesize cDNA with RevertAid H Minus reverse transcriptase (Thermo Scientific). Quantification of transcripts was performed in a Bio-Rad CFX96 real-time PCR detection system using SYBR Green. The signal from each experimental sample was normalized to expression of the *ACT1* gene.

### Nucleotide sequence accession number.

The detailed RNA-Seq data set has been deposited in the NCBI Gene Expression Omnibus (GEO) portal (accession number GSE46459).

### SUPPLEMENTAL MATERIAL


Text S1, DOC file, 0.1 MB.

Data Set S1, XLS file, 0.1 MB.

Data Set S2, XLS file, 1.5 MB.

Data Set S3, XLS file, 0.6 MB.

Data Set S4, XLSX file, 0.2 MB.

Figure S1, TIF file, 1.4 MB.

Figure S2, TIF file, 0.1 MB.

Figure S3, PDF file, 0.9 MB.

Figure S4, TIF file, 2.1 MB.

Figure S5, TIF file, 1.7 MB.

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