## Cloning of the *Candida albicans* Homolog of *Saccharomyces cerevisiae* GSC1/FKS1 and Its Involvement in $\beta$ -1,3-Glucan Synthesis

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Saccharomyces cerevisiae GSC1 (also called FKS1) and GSC2 (also called FKS2) have been identified as the genes for putative catalytic subunits of  $\beta$ -1,3-glucan synthase. We have cloned three *Candida albicans* genes, GSC1, GSL1, and GSL2, that have significant sequence homologies with S. cerevisiae GSC1/FKS1, GSC2/FKS2, and the recently identified FKSA of Aspergillus nidulans at both nucleotide and amino acid levels. Like S. cerevisiae Gsc/Fks proteins, none of the predicted products of C. albicans GSC1, GSL1, or GSL2 displayed obvious signal sequences at their N-terminal ends, but each product possessed 10 to 16 potential transmembrane helices with a relatively long cytoplasmic domain in the middle of the protein. Northern blotting demonstrated that C. albicans GSC1 and GSL1 but not GSL2 mRNAs were expressed in the growing yeastphase cells. Three copies of GSC1 were found in the diploid genome of C. albicans CAI4. Although we could not establish the null mutation of C. albicans GSC1, disruption of two of the three GSC1 alleles decreased both GSC1 mRNA and cell wall β-glucan levels by about 50%. The purified C. albicans β-1,3-glucan synthase was a 210-kDa protein as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and all sequences determined with peptides obtained by lysyl endopeptidase digestion of the 210-kDa protein were found in the deduced amino acid sequence of C. albicans Gsc1p. Furthermore, the monoclonal antibody raised against the purified  $\beta$ -1,3-glucan synthase specifically reacted with the 210-kDa protein and could immunoprecipitate  $\beta$ -1,3-glucan synthase activity. These results demonstrate that C. albicans GSC1 is the gene for a subunit of  $\beta$ -1,3-glucan synthase.

β-1,3-Glucan synthase (UDP-glucose; 1,3-β-D-glucan 3-β-Dglucosyltransferase; EC 2.4.1.34) catalyzes the formation of a  $\beta$ -1,3-glucan polymer that is a major component of the fungal cell wall (34). Biochemical studies with Saccharomyces cerevisiae revealed that the enzyme utilizes UDP-glucose as the substrate and consists of at least two components, a catalytic subunit and a regulatory subunit (14, 25). The gene for a possible catalytic subunit of  $\beta$ -1,3-glucan synthase was identified by several approaches (4, 6, 7, 10–12, 22, 30). A gene called FKS1 was isolated by functional complementation of an S. cerevisiae mutation that conferred hypersensitivity to FK506 (6, 10), while the same gene termed GSC1 was obtained following microsequencing of the partially purified enzyme (12). The facts that the disruption of GSC1/FKS1 was not lethal and that residual  $\beta$ -1,3-glucan synthase activity was present in gsc1 $\Delta$ /  $fks1\Delta$  null mutants led to the identification of another gene, GSC2/FKS2 (12, 22). Although Gsc1p/Fks1p and Gsc2p/Fks2p have 88% sequence identity, their expression is controlled by different mechanisms. FKS1 mRNA is accumulated periodically during the cell cycle (22), whereas FKS2 expression is induced by mating pheromone in a calcineurin-dependent manner in the presence of glucose (22). The recent finding of an Aspergillus nidulans gene, FKSA, that is highly homologous to S. cerevisiae GSC/FKS suggests that the GSC/FKS genes are widely conserved in both yeasts and mycelial fungi (17). Finally, GTP-bound active Rho1p is physically associated with Gsc/Fks protein and is essential for  $\beta$ -1,3-glucan synthase ac-

\* Corresponding author. Mailing address: Department of Mycology, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247, Japan. Phone: 81-467-47-2242. Fax: 81-467-46-5320. E-mail: toshiyuki.mio@roche.com. tivity, demonstrating that Rho1p is a regulatory subunit of the enzyme (8, 28).

There is a linkage between  $\beta$ -1,3-glucan and another cell wall component. In *S. cerevisiae*, the nonreducing end of a  $\beta$ -1,3-glucan chain binds to the terminal reducing residue of a chitin chain by a  $\beta$ -1,4-linkage (18). *CHS3*, one of the three chitin synthase genes, is required for the formation of the linkage between  $\beta$ -1,3-glucan and chitin (18). This  $\beta$ -glucan-chitin linkage may be important to retain the rigidness of the cell wall (33).

Candida albicans is a dimorphic fungus that is often found in human deep mycosis, and both yeast and mycelial forms are detected in patients with *C. albicans* infection (26). Although the *C. albicans* cell wall has not been as well characterized as that of *S. cerevisiae*, genes for cell wall biosynthesis, such as the chitin synthase genes and the mannosyltransferase genes, are well conserved in these two organisms (2, 5, 36). In addition, the facts that GTP-dependent  $\beta$ -1,3-glucan synthase activity is present in the membrane fraction of *C. albicans* (27) and that *C. albicans*  $\beta$ -1,3-glucan synthase activity is sensitive to echinocandin B (32) strongly suggest that *S. cerevisiae* and *C. albicans* have similar mechanisms of  $\beta$ -1,3-glucan synthesis. Here we report the cloning and characterization of the *C. albicans* homolog of *GSC1/FKS1*.

### MATERIALS AND METHODS

**Cloning of the** *C. albicans* **homolog of** *GSC1/FKS1*. To generate a *C. albicans* genomic DNA library, *C. albicans* genomic DNA was isolated from strain ATCC 10231 by disrupting cells with glass beads and subsequent extraction with phenol (20). DNA was precipitated with ethanol, partially digested with *Sau3AI*, and fractionated by agarose gel electrophoresis. DNA fragments whose sizes were between 5 and 15 kb were eluted from a gel and ligated at the *Bam*HI cleavage site of YEp24 (3) or lambda ZAPII vector (31, 35). Screening of the *GSC1* 



FIG. 1. Restriction maps of C. albicans GSC1 (CaGSC1), GSL1 (CaGSL1), and GSL2 (CaGSL2). Regions of probes used for Southern and Northern blottings are also indicated. Black bars represent ORFs of C. albicans GSC1, GSL1, and GSL2.

homolog was carried out under low-stringency conditions in a buffer containing 0.25 M sodium phosphate (pH 7.2),  $2 \times$  SSC (1 $\times$  SSC is 150 mM NaCl plus 15 mM sodium citrate), 1% (wt/vol) bovine serum albumin, 1 mM EDTA, and 7% (wt/vol) sodium dodecyl sulfate (SDS) at 66°C for 4 h by using a 3.5-kb *Eco*RI fragment of *S. cerevisiae GSC1* as a probe. Clones that hybridized with the probe DNA were isolated and subjected to further screening. The insert DNA that gave a significant signal after the third screening was excised from the vector, and nucleotide sequences were determined as described elsewhere (31).

Southern and Northern blot analyses. Genomic DNA and  $poly(A)^+$  RNA were prepared as previously described (15). Twenty-five micrograms of genomic DNA digested with *Eco*RI, *Sal*I, and *Bst*PI and 10 µg of  $poly(A)^+$  RNA were fractionated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized with the indicated probes. Hybridization was carried out under stringent conditions in a buffer containing 50 mM sodium phosphate (pH 6.5), 5× SSC, 5× Denhardt's solution, 50% (vol/vol) formamide, 0.25 mg of salmon sperm DNA per ml, and 0.1% (wt/vol) SDS at 42°C for 18 h.

**Disruption of the** *C. albicans GSC1* gene. Gene disruption was carried out according to the *ura* blaster protocol (1, 23). To disrupt the *C. albicans GSC1* gene, pCA1 was constructed by cloning the 4,673-bp *Eco*RI-*Eco*RI fragment of *C. albicans GSC1* into pUC19. After digestion of pCA1 with *Bal*I, the resulting DNA fragment was ligated with a 3.8-kb *Bam*HI-*Bg*/II fragment carrying *hisG*-*URA3-hisG* to generate pCA1U. Thus, 1.2-kb *Bal*I-*Bal*I region of *C. albicans GSC1* was replaced by the *hisG-URA3-hisG* in pCA1U. After digestion of pCA1 with *AccI*, 100 µg of the DNA was transfected into *C. albicans* CA14 (*ura3*Δ:*:imm434*) cells by the lithium acetate method (13). Before the second round of transformation or characterization of the mutants, the *URA3* gene that had been integrated into the *C. albicans* genome was excised by 5-fluoroorotic acid (20). Mutant and parental CA14 cells were cultured in YPD medium (1% peptone, 2% yeast extract, 2% dextrose) at 30°C.

**Determination of cell wall \beta-glucan.** *C. albicans* cells that were grown to mid-logarithmic phase were harvested, washed with H<sub>2</sub>O, and used for extracting cell wall polysaccharides. The amount of cell wall  $\beta$ -glucan was determined by fractionating the cell wall polysaccharides as described previously (16).

Purification of  $\beta$ -1,3-glucan synthase by product entrapment. One hundred grams of *C. albicans* cells was disrupted by using glass beads and a Bead-Beater (Biospec Product, Bartlesville, Okla.).  $\beta$ -1,3-Glucan synthase was extracted from membranes with 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS)-cholesteryl hemisuccinate detergent mixture, purified by product en-

trapment, and used for raising a monoclonal antibody as described previously (12).

Amino acid sequence determination of peptide fragments of the 210-kDa protein. After being stained by Coomassie brilliant blue R-250, the 210-kDa protein in an SDS-polyacrylamide gel was digested with lysyl endopeptidase. The resulting peptides were separated by reverse-phase high-pressure liquid chromatography (Hitachi L-6200 Intelligent pump and L0600 pump) with a Lichrosorb RP-8 column (4 by 250 mm). Amino acid sequences of the specific peptide peaks derived from the 210-kDa protein were determined with 470A protein sequencer (Applied Biosystems). Immunodetection of the 210-kDa protein was carried out by Western blotting by using the monoclonal antibody raised against the 210-kDa protein together with alkali phosphatase-conjugated anti-mouse immunoglobulin G as the second antibody as described previously (31).

**Nucleotide sequence accession numbers.** DNA sequences of *C. albicans GSC1*, *GSL1*, and *GSL2* are available in GenBank, EMBL, and DDBJ databases under accession no. D88815, D88816, and AB001077, respectively.

### RESULTS

**Cloning of the** *C. albicans GSC1/FKS1* **homolog.** To identify the gene for  $\beta$ -1,3-glucan synthase of *C. albicans*, we performed Southern hybridization of the *C. albicans* genomic DNA with the 3.5-kb *Eco*RI fragment of *S. cerevisiae GSC1* DNA under low-stringency conditions. Since this analysis revealed the presence of several discrete bands (data not shown), we screened a *C. albicans* genomic DNA library with *S. cerevisiae GSC1* DNA under the same hybridization conditions and isolated one clone, pCA1. Sequencing of the insert DNA in pCA1 identified a long open reading frame (ORF) that was highly homologous to *S. cerevisiae GSC1/FKS1*, and it was designated *C. albicans GSC1* ( $\beta$ -1,3-glucan synthase catalytic subunit 1). Since there was no translational termination codon, the coding sequence could extend further downstream. We obtained a clone harboring the missing 3' end of the ORF by A

1	ATGTCGTATAACGATAATAATCATTATTACGACCCTAATCAACAGGGCGGTATGCCACCTCATCAAGGAGGAGAAGGGGTATTACCAACAACAAGTATGATGATGATGATGATGATGATGATGATGATGATG	40
121	CACCAACAAGATTATTACGATCCAAATGCTCAATATCAACAACAACCAATATGACATGGATAGGATATCAAGACCAAGCCAACTATGGTGGTGGTGACCCAAGGATGAATGCCCAGGGTTATAATGCT H Q Q D Y Y D P N A Q Y Q Q Q P Y D M D G Y Q D Q A N Y G G Q P M N A Q G Y N A	80
241	GACCCAGAAGCCTTTTCTGACTTTAGTTATGGTGGTGATACTCCTGGAACTCCTGGTATGAATCAATACGGTACTCAATACACCCCATCTCAATGAGTATGGTGGTGATCCAAGATCT D P E A F S D F S Y G G Q T P G T P G Y D Q Y G T Q Y T P S Q M S Y G G D P R S	120
361	TCTGGTGCTTCAACACCAATTTATGGTGGTCAAGGTCAAGGTTACGATCCAATCCAATTCAATATGTCATCGAACTTGGCATATCCAAGCTTGGTCTGCTGATCCTCAAGCTCCAAGTTAAG S G A S T P I Y G G Q G Q G Y D P T Q F N M S S N L P Y P A W S A D P Q A P I K	160
481	ATTGAACACATCGAAGATATTTTCATTGATTAGATATATTGGTTTCCAAAGAGAATTCTATGAGAAAACATGTTTGATTACTTTATGACATTGTTGGACTCGAGATCTTACCGAGATCTTACGACATGTTGGACATGGTGGACTCGAGACTCCGAGATCTATG I E H I E D I F I D L T N K F G F Q R D S M R N M F D Y F M T L L D S R S S R M	200
601	TCACCAGGCCTAGGCCTTGTTGAGTTTACATGCGATATATAGTGGGGGGACAATGCCAATTAGAAAATGGGATTTTGTCACAACAAGATTTGGATGATGGTGATGGTTATGGTAAT S P A Q A L L S L H A D Y I G G D N A N Y R K <u>W Y F S S O O D L D D S L G F A N</u>	240
721	ATGACTTTAGGTAAAATGGTAGAAAAGCCAGAAAAGCTTCCAAGAATCCAAAAAAGCTAGAAAAGGTGCTGAAGAACATGGTCAAGATGTCGATGCTCTATGAAATAGAAGGT M T L G K I G R K A R K A S K K S K K A R K <u>A A E E H G O D V D A L A N E L E G</u>	280
841	GATTATTCATTGAAGCCGCTGAAATCAGATGGAAGCCAAGACAAGAAGAAGAGAAGAGAGAG	320
961	CGTITTACTCCTGAATGTTTGTGTTACAATTTACAAATCTGCCACGAATTATTTAAATTCTCCATTGTGTCAACAAGAACCAGGAACCAGGGCGTGATGACTGGAACGGGGTTACTTGAACGGGGTGATC R F T P E C L C Y I Y K S A T D Y L N S P L C Q Q R Q E P V P E G D Y L N R V I	360
1081	ACTCCACTTTACAGATCACAGATCTCAAGATCTCAAGATTTATGATGGAAGATTTGTCAAGCGTGAAAAAGACCACAACAAGGTCATGGTTATGATGATGATGATGATGATGTTATGATGTTTTGG T P L Y R F I R S Q V Y E I Y D G R F V K R E K D H N K V I G Y D D V N Q L F W	400
1201	TACCCAGAAGGTATTTCCAGAATTATTTTTGAAGATGGAACCAGATGGTATGGTATGGTATGGATGG	440
1321	TATAAGGAAATCAGGAACTGGATGCAATTGGATATGGAATCTGGATAACGATGGATG	480
1441	TATGTCCARACCATARATCAACAACAACCACTTGGTCATAGATGGGGGGGTGTTGGGGGGTGTTGGTGGTGTTGTTCAAATTGTGGCACACTTTTGGAATGGATTTTCGG Y V Q T I N Q Q P L A S S R W A A C A I G G V L A S F I Q I L A T L F E W I F V	520
1561	CCTAGAGAATGGGCCGGTGCTCAACATTTGAGTGCGTGTAGCGATTTTTTGGGTGTAATTTTCTTACTCAAATTGGGTGCACCAGTTTATACAATTGGAGATTAC P R E W A G A Q H L S R R M L F L V L I F L L N L V P P V Y T F Q I T K L V I Y	560
1681	TCGANATCGGCATATGCTGTGTGGATTGTGGATTGTGGATTTTCATTGCGGCCACTTTAGTATTCTTTGCCGTCATGCCATTGGGGGGGTTTATTCACTCATACATGAACAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAG	600
1801	AGATATATTGCATCACAAAAATTTACEGCCAACTACATTAAATTGAAAGGTTTAGATATCTGGATGTCTTATTGGTTTTGGTTTTGGTTTTCCTTGCCAAATTGGTGAATCTTATTTC R Y I A S Q T F T A N Y I K L K G L D M W M S Y L L W F L V F L A K L V E S Y F	640
1 <b>921</b>	TTCTCGACTTTGTCTTTAAAGAACCTATTAGAAACTTGTCGACCATGACAATGAGATGTGTGGTGGAGATTTGGTAGAAAGTATGTTTGTAGAAACCAAGCCAAGATTGTCTTGGGG F S T L S L R D P I R N L S T M T M R C V G E V W Y K D I V C R N Q A K I V L G	680
2041	TTGATGTATCTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTGTG	720
2161	TGGAGAAACATTTTCACCAGATTGCCAAAGAGAATTTATTCCCAAGATTTTAGCTACCACGGAAATGGAAATGAAATATAAAACCTAAAGTTTTGGAAATCGAAATTGGAATGCCATTGT W R N I F T R L P K R I Y S K I L A T T E M E I K Y K P K V L I S Q I W N A I V	760
2281	ATTTCCATGTACAGAGAACATTGTTAGCCATGATCACGTTCAAAAATTATTGTATCATCAAGTTCCATCTGAAGCAAGAGAACTTGAAGAGAACTTGAAGAGCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAACTTGAAGAGCCAAGAGAGAACTTGAAGAGCCAAGAGAGAACTTGAAGAGCCAAGAGAGAACTTGAAGAGCCAAGAGAGAACTTGAAGAGCCAAGAGAGAG	800
2401	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	840
2521	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	880
2641	TTACATCCAGTTGAATGGGATGGTATTTTGTTAAGGACACCAAGATTTTGGCTGAAGAAACGCTGCTTATGAAAATGGTGATGATTCTGAAAAATTATCTGAAGATGGAATGAAATCCAAG L <u>HPVEWDCFY</u> KDTKILAEETAAYENGGDCSCAAGATTTGGCTGAAGAAACGCGCTGATGAAAATGGTGATGATGTGAAAATTATCTGAAGATGGAATGAAT	920
2761	ATTGATGATTACCATTCTATTGTATTGGTTTCAAGTCTGCCGCCCCGAATATACTTTAAGAACAAGAATTTGGGCTTCATTGAGAACCCCAAACTTGTACAGAACTGTATCTGGGTTT I D D L P F Y C I G F K S A A P E Y T L R T R I W A S L R S Q T L Y R T V S G F	960
2881	ATGAATTATGCCAGAGCCATTAAAATTGTTATACACAGGGGAAAACCCAGAATGGTTCAATATTGGGGGGGG	1000
3001	TTTAGATTTTTGGTTTCTATGCAAAGATTGTCTAAATGCAAAGATGGAAATGGAAAMTGCTGAGTTCTTATTGGGTGCTTACCCTGATTTGCAATGATGATGACTACCTGGATGAAGAACCG F R F L V S M Q R L S K F K D D E M E N A E F L L R A Y P D L Q I A Y L D E E P	1040
3121	GCTTTGAATGAGGACGAGGAACGAGGAACGAGGATATACTCTGCCTTGATTGA	1080
3241	TTGGGTGATGGTAAATCTGATAATCAAAATCATGCGGTTATTTTCCATAGAGGTGAATAATTCAATGATTGAT	1120
3361	GTTTTGGCTGAATTTCAAGAAATGGAATGTGAACATGTTAATCCTAAATGCACCAAATTTGAAATCTGAAGATAATAACACCAAGAAGGATCCAGTGGCATTTTTGGGTGCTAGGAATAT V L A E F E E M N V E H V N P Y A P N L K S E D N N T K K D P V A F L G A R E Y	1160
3481	ATTTTCTCAGAAAATTCTGGTGTTTTGGGTGATGTTGCTGCTGGTAAAGAACAAACTTTTGGAACAATTGTTTGCAAGAACTATTGGGAGGAAATTGGAGGTAAATTGGAGGA	1200
3601	$ \begin{array}{c} Gattittegatgctacattratgetaatgcacattratgetaatgcacattratgatgatgatgatgatgatgatgatgatgatgatgatga$	1240
3721	AAGCATTGTGAATATTATCAATGTGGTAAAGGTAGAGATTTAGGTTTGGATCCATTTGGAATATTCCACCACCAAGATTGGTGGGGGGAGGAACAAATGCTTTCAAGAGAATATTTC K H C E Y Y Q C G K G R D L G F G S I L N F T T K I G A G M G E Q M L S R E Y F	1280
3841	TATTTGGGTACTCAACTTCCATTGGATAGATTTTTGTCATTTACTATGGTCATCCACGTTTCCATATTAATAACTTGTTTATTCAATGCTCTTTACAAGTGTTTACTATTTGGTGTTGGGT Y L G T Q L P L D R F L S F Y Y G H P G F H I N N L F I Q L S L Q V F I L V L G	1320
3961	AACTIGAATTCATTAGCTCATGAAGCTATCATCATCTTCTTACTACAACAAGATGTCCCAGTTACTGATGTGTTTCTATTGGTTGTTACAATATTGCTCCTGCCGTTGGATTAGGA N L N S L A H E A I M C S Y N K D V P V T D V L Y P F G C Y N I A P A V D W I R	1360
4081	CGTTATACTITGCTATTGTATTGTTTTCTTCATTGCTTGTATTCATTGCATGGAAGAGGGGTATGGAAAGGGGTATGGAAAGGGGTATGGTAGAGATTGTTAGACATTTATTT	1400
4201	ATGTCACCATTTTTCGAAGTTTTCGTGCCCAAATTTATTCATCATCGGTTTTCACCGGTTTTGACCGTTGGTGGTGGTGGTGAAATATATTTCCACTGGTAGAGGTTTTGCCACTGCAAAATT M S P F F E V F V A Q I Y S S S V F T D L T V G G A R Y I S T G R G F A T S R I	1440

FIG. 2. Nucleotide and predicted amino acid sequences of *C. albicans GSC1* (A), *GSL1* (B), and *GSL2* (C). Underlines represent the corresponding peptide sequences which have been determined with the purified 210-kDa  $\beta$ -1,3-glucan synthase. According to the report by Manuel et al. (21), the CTG codon is decoded as serine instead of leucine.

screening another *C. albicans* genomic DNA library that was constructed with lambda ZAPII by using *C. albicans GSC1* as a probe. The *C. albicans GSC1* gene contained an ORF of 5,694 bp (Fig. 1 and 2A), and the expected product was a

210-kDa protein that had significant sequence similarity with both *S. cerevisiae* Gsc1p/Fks1p and Gsc2p/Fks2p (72.2% identity with *S. cerevisiae* Gsc1p and 72.8% with Gsc2p) and also with *A. nidulans* FksAp (64.6% identity). *C. albicans* Gsc1p

### FIG. 2-Continued.

3241 AACTCTTGCGCCATTGGTTGGAATATGGTTTAA N S C A I G W N M V \*

4681	AGATCCAATGTTTTGTTTGCTGATTTATTCCAACATTGATTTATATATGCTGGTGCTTTATGTTGCTTATAATGCTCAAACTGGGGTTACTAGTATGCAATGAAATCAAT R S N V L F A D F L P T L I Y T A G L Y V A Y T F I N A Q T G V T S Y P Y E I N	1600
4801	GGATCTACTGATCCACAACCAGTTAATTCTACTTTGAGACTTATTATTTTTGCTTTAGCTCCAGTTGTTATTGATATGGGATGTTTAGGTGTTTGTCTTGCCATGGCATGTGTGGCAGG G S T D P Q P V N S T L R L I I C A L A P V V I D M G C L G V C L A M A C C A G	1640
<b>492</b> 1	CCANTGITAGGATTAIGITGITAAAAAAGACIGGIGGIGGIGGIGGIGGIGGIGGIGGIGGIGGIGGIGG	1680
5041	TTTGCCAGATTAATGTTGGGTATTGGCACCATGATTATGTTCAAGATTATTATTCAAGTTTTGACATTATGTTTCTGACTAGAGAATTTAAGAATGATAAAGCCAATACTGCTTTC F A R L M L G I A T M I Y V Q R L L F K F L T L C F L T R E F K N D K A N T A F	1720
5161	TGGACTGGTANATGGTATAATACTGGTATGGGATGGGTGGTGGTTTACTCAACATCTGGGAATTGGTGCTANAATCATTGAAATGTCGGAATTGGCGGTGATTTGGTTTGG	1760
5281	ATTATATTATTCTGTCAATTACCATTATTGTTTATTCCATTGATGATAGATGGCATTCAATGATGTTATTCTGGTGAAACCATCAAGATTGATT	1800
5401	CAMGCCAGATTAAGAAGAGAATGGTGAGAAAATATTGTGTTTTATATTTGTGCGTGTTGATATTATTTGTGCATTATTGTTGCACCAGCAGTGGTGGTGGTGGGGAGAAATTGGTGTTGAT Q A R L R K R M V R K Y C V L Y F A V L I L F I V I I V A P A V A S G Q I A V D	1840
5521	CANTITECCAATATTEGTEGATCTEGTTCTATTEGTEGATGATATTCCAACCAAGAAATGTCAGTAATAATGATACTGGTAATCATAGACCAAAAACCTACACTTEGAGTTATTEGAGT Q F A N I G G S G S I A D G L F Q P R N V S N N D T G N H R P K T Y T W S Y L S	1880
5641	ACTCGTTTTACTGGAAGTACCACCCCCTTATTCTACAAATCCATTCAGAGTTTAA T R F T G S T T P Y S T N P F R V *	1897
<b>B</b> 1	ATGTCATTCAACTCGGCTTCTTTGTACACACGAAATTATACCCCCCAATAAGTCACCGCAAGTGACACTAGGCCATGACGAATCATCGCGGAATCATTGCCGTCTTAATATCGTG M S F N S P S L Y T P N Y T P N K S P Q V H I R L A I V S I G G I I A V L I S L	40
121	GGTGCCGCAATATCTGATTTTTTTTTTTTTGTTGGGAGAGTGTCCGAAACATTGTTTTGTTATTGATATTGACAGTTGCAAACTCGGGATCTATAGTTTACAATCTAGGGCTTTTGAAATGG G A A I S S F F F V S G S V R N I V L L L I L T V A N S G S I V Y N L G L L K W	80
241	GACAAGTATTCTANANATGGGACAGTGGTGGCAGCTATTCTGATGTGGTGTTCTCACAATTTTTGTTCTTGGCCATCAATCCACCAGGAGTTTCAAAACCGTGTTTTCAAAACGGTGTTTCCAAAT D K Y S K N G T V V A A I L M C L S V L T F L F L A I N P P G S F K T V F S N N	120
361	TTCCCAMARTAMAGTAAGAAGCCGATTATTCTCCATATCGTATTGGGGTATTGGAGCCAAGTATTCAGAGTCATACTTTTTCTTAATATTGTCGTGGAAAGACCCCATTCA F P K L K L R S R L F S I S L W I G V F A A K Y S E S Y F P L I L S L K D P I Q	160
481	ATTCTATCTACCATTGAATTAAACTGTGATAACGGTCATTTTCTATGCCGGTTTCAACGAAAGATTACATTGATATTGTTTATCTCACTGATATTGATATTATTCTTTTGGACACTTAT I L S T I E L N C D N G H F L C R F Q P K I T L I L F Y L T D L I L F F L D T Y	200
601	TTGTGGTATGTGTTTGGATTGTTTATTTAGTGGGACTATCGTTTTCATTAGGTGTGTCTATTTTACCCCTTGGAAAAATATTTTTTCTCGATTACCAGATCGGATTTTAACCAAG L W Y V I C N C L F S V G L S F S L G V S I F T P W K N I F S R L P D R I L T K	240
721	ATATATTATGGGGATTCCACAGAGTTGATATTGGTGATATCACAAATATGGAACAGCATTATCATTATCATGTAAGGGGAGGATGTTCTCTGGGTGAACAAGTTTGCAAGTTAT I Y Y G D S T E L I L V I S Q I W N S I I I S M Y R E H V L S V E Q V C K L I Y	280
841	CAACGAGGAGCTGATGAAAACACTATACGACCACCACTATTTTTGTTTACGAAGATGATGATGATAACAAATTTTATGATTTTATTAAAATCGAAAAGGAATGGGAAAGGAATGGGAAAGGAATCACATTTTTT Q R G A D E N T I R P P L F F V Y E D D N K F Y D F I K I E K E W E R R I T F F	320
961	GCTCAATCGTTATCAAGCCCTTTACCAGAAGCATTTCCAGTAGTTTCTACACCAACATTTACCGTTTGATTCCTCATTATCTCAGAAAAAAAA	360
1081	GAACAAAGCTTTTCAAAACTAACGTTGCTAGATTATTTGAAAAAACTTCATTCGAAAGAAGATGGATTCATTGTTCAAGATAGAT	400
1201	AMGTTTGTACGCGAAAATATGGATGATTGTCCGGTACTACTGGTACGGATCAAAGATTCTCACCAGAAAATGTTTACGAACAAGAATTTGGGCTGCATTAAGATGTCAGACATTGTAT K F V R E N M D D L P Y Y C I G F K D S S P E N V L R T R I W A A L R C Q T L Y	440
1321	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	480
1441	ANATICANTITACTANTIGCTATGCAGANTITICCCGAGANTITIGCCCAAGGCGAGACGAIGCGAGGCGAGGCGAGGCTGAGGTTGCCAAGGTTGCCAATGTCAGATGCGATTTAGAATGTGAT K F N L L I A M Q N F Q N F A P D M R T D A D S L F K A F P N V K V A I L E S D	520
1561	AACGATCAAGACTATTATTCAAACACTATTAGATGTTTCAAAAACGAGATGACAAAAATCAGTATGTTAAAAAATACCGAATCAAGCTATCAGGAAATCCTATTTTAGGTGATGGCAAATCT N D Q D Y Y S T L L D V S K R D D K N Q Y V K K Y R I K L S G N P I L G D G K S	560
1681	GATAATCAAAATAGTGCATTAATATTTTATCGTGGGGAATATATCCAGGTCATAGATTCCAACGAGGATAATTATTGAGGGAATGTCTCAAAATCAAATCTATGAATGA	600
1801	GAGATGAACTTGGACGTAAGTTTTGGATATCAGACGGAGGACGACGCAGAAACTAGTTCTGGGGGGAGAAAAGAGAATTCATATTTTGAGAAAATAGGAATTTTAGGAGACATA E M N L D V S F G Y Q T E H P E T S S V A I V G A R E F I F S Q N I G I L G D I	640
1921	GCTGCTGCTGCTGAAGAACAGACTTTTTGGAAGATATTTGCAAGACAATGGGAGAAATGGGAGTCTAAACTTCATTATGGCCACCCCGATTTGCTCAATGGAATATTTATGACGACAAGAGGT A A A K B Q T F G T L F A R T M G E I G S K L H Y G H P D L L N G I F M T T R G	680
2041	GGAATTTCAAAAGCCCAGAGAGGCTTACATTGAATGAAGACATTATGCAGGGATCACTGCGATGTGGTGGTGGGAAGGATAAAACACTCAGATTACTATGTGGCAAGGGAAGG G I S K A Q R G L H L N E D I Y A G I T A T C R G G R I K H S D Y Y Q C G K G R	720
2161	GATCTIGGSTICCAMICAATIGTGAAATITACAAAGAAAATIGGAICIGGAATGGGCGAACAGCTITIGTCAAGAGAATATTAGGGGGGGGAGATGTAACGATAAATICIIG D L G F Q S I V N F T K K I G S G M G E Q L L S R E Y Y Y L G S M L P I D K F L	760
2281	TCATTITACTATGCTCACGCTGGATTCCATATCACCACACATGTCTATAATGTTGTCGTGGAAAAGCCTTTAGTTTATGATGGGCCTGGGGCATAAACAACGGCACGCGCGGG S F Y Y A H A G F H I N N L S I M L S V K A F M F L L M S L G A L N N G T A A C	800
2401	ACGGAAGATAACCCAACACCTGGCTGCCATAATTTAGTCCCCCGTTCTAAACTGGATTGACCGTTTGGCTTTCAGTATTGGTTTGCTCTTCATATTTGCCTTTGATAATTCAA T E D N P T P G C H N L V P V L N W I D R F V L S V F V C F F I S F L P L I I Q	840
2521	GAGTTATCGAAAAGGGATTACTCAAAGCAATTTTGCGAATATTATTACACATGTTTCGTATTCGACGTTTTTGAGTTTTTGTGTCGCAAGTATTATTCAAGGCGTAAGGGACAAT E F I E K G L L K A I L R I L L H I V S L S P F F E V F V C Q V Y S R A L R D N	880
2641	TITATATITIGAGAAGGTAAATATATTGCTACAGGACGAGGATTTGCTATCGCTAGGTATCATTTGCCACATTGTACTCCCAGTATGCCAGTTATCGATTATCGATGAGGGGGGGG	920
2761	TTITIGGTGATTITGTTIGCTCAATAACTAICIGGGAAAATCITTACTATGGTTGGAACATTATATCCTTAIGGTGGGACCATCAITTICAATCCITAGGTTGGAACACTAICIGGAGAAATCITTAATTT F L V I L F A S I T I W R K S L L W F V I T I I S L C L A P F I F N P H Q F N F	960
2881	GTTGATTCTTTGTCGATATAGGACTATGTCAGATGGTTGACAAGAAGAAAGTGTTCTCTCAAAGAACTGTCCTGGACTAGTAGGAGAGGTCGGTAACTGGAGAA V D F F V D Y R D Y V R W L T R G N S S L K E S S W T H Y T K V R R A R L T G E	1000
3001	AMSTITCATGGCGGGTATGTTCTGCGGGAGAAATACTGCTACATTGTATTGGGGGGGG	1040
3121	TCANGTCCCAAATCTACTGCATTCAGTGGAAAAATCCACTAATAAAGTTAACGCAGTGTGTATTGGCACCTTATTCGCAGGAATCGGCGGTCTTTTCTTCGTAGGGTAATGGCTAT S S P K S T C I Q C E K S T N K V N S S C I G T L S R E F G G S F L R M G N V L	1080

1090

с	1	ATGTTGATTACTTANTGAGGTTGTTAGATTCAAGAACATCTAGATGGGGCCAACCCACGCCTCTCAGATCATTCAGGCGATTATATTGGGGGCTGAATTCTAATTTCAGAAAATGG M F D Y L M R L L D S R T S R L G P T H A L R S I H A D Y I G G M N S N F R K W	40
	121	TATTTIGCAGCCCAGTIGGATATCGAIGATITIGGITITIGGATATGGCAAAATGGGAAAATCAAGGGGTCAAATGACCCAGTIGCAACATIGGAGCAAGCIGAACTGCAAATCAAGGGCTCAAATGACCCAGTIGCAACATGGCAAATGGCAAATGGCAAATGGCAAATGGCAAATGGCAAATGACGCCAAGTGCAACTGCAA	80
	241	TCTACAAATATGCTAGCTTTATCTCCTACTGATTCAGGTTTAAGCCATATATCTTTTGATTTGGGGTGAAGCCAACAATATCAGATTTATGCCTGAGTGTATTGTTTTATCTTC S T N M L A L S P T D S V I Q L A I Y L L I W G E A N N I R F M P E C I C F I F	120
	361	AAATGCTGTAATGATTTTTATTTTTCCATCGATGCTGCACACCAGTACAACTGTTACAACTGTTAGACGAGATAATCACTCCCCCCTCTACAACTTTTACGAGACCAATGGTAC K C C N D F Y F S I D P D T P V T T V T P S F L D H I I T P L Y N F Y R D Q S Y	160
	481	ATTCTTGTTGATGGGAAATACCGTCGTCGGCGATAAAGATCACGAGTCTGGATGGGTATGGTATGAACCAATTATTCTGGTACAGCAAAGGTTTGGAAAGACTTGTTTGGCAGAC I L V D G K Y R R R D K D H E S V I G Y D D M N Q L F W Y S K G L E R L V L A D	200
	601	AAGAAATCTAGATGATGAGGAGTCTCCACCAGGAGAAAGGTATGAGGAATTAAATCAAGTATTATGGAATCGTGTCTTTTATAAAACTTTTAAAGAAAACAGAGGCTGGTCTCATGTATTG K K S R L M S L P P G E R Y E E L N Q V L W N R V F Y K T F K E N R G W S H V L	240
	721	GTAAACTITICACAGAGTITIGGATAATTCACAGTGCTGTGTTITIGGTACTACAGGGCTTITTAACTCGCCAACATTATACACAAGAATTATCAACCGGCGTTAGACAACCAAC	280
	841	CAAGCAAGATTGTCAGTGTTGGCGTTTGGAGGTGFGGTGTGCGATTGTTATTGATATTATCAGTTTATTGTCGAGTTAGGGTTACGGGTTATTCCAAGAAAAGGACTGAAGCTGAAGCTGAACCTGTAGT Q A R L S V L A F G G V V A I V I D I I S L L F E L R F I P R K W T G A Q P V S	320
	961	AAAAGATTAGCTTTGTGATTTTGACGTGATTTTGAACGTGGGCCCAAGTGCTACTGTTTATGTTTATGCCATTAAATGTTCAGAACAAGTGGGCTAGTTATTTCGCTTTCGCTTTCGG K R L A L L I L A L I L N V G P S V Y L F M F I P L N V Q N T V G L V I S A F Q	360
	1081	TITICATITICIGTCATATIGGTTTTATATITIGTCTACAGIACCATTGGGGAGGGTTTTTTCCAAAAAACCTAAAGGCAACGATAGAAGGTTTTGCCCCAGCGTCTGTTGGACCAAT F S F S V I M V L Y L S T V P L G R L F S K K P K A N D R R F L P Q R S F V T N	400
	1201	TTCTATTCATTAGCCGAAGGTGATAGAGTTGCATCTTATGGTTTAGGTTTAGGTTTGCTATATTTGTATCATTGAGTCATATTTCTTTTGACTCTATCATTGAGAGACCCGGTTCGT F Y S L A E G D R V A S Y G L W F A I F V S K F I E S Y F F L T L S L R D P V R	440
	1321	GAATTGAGTATAATGAAAATGAGAAGATGGCCGGGGGGGG	480
	1441	ATATTAGACACCTACTTGTGGTACATTGTATGGAATACTGTGTTTTCAGTTTGTCGCTCATTTTACATTGGCGTCCAATTTGGACTCCATGGCGAAATATATTTTCAAGGTTACCCAAA I L D T Y L W Y I V W N T V F S V C R S F Y I G V S I W T P W R N I F S R L P K	520
	1,561	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	560
	1681	GAACATGTTCAAAAACTTATCTATCAAAAACGATAAACCGATAACCCCGGAGTTGAAGGGGGTTCTGTTTTGAAAGAAA	600
	1801	TTTCAAGATCAAGCAGAAGCACAGCGAAGGATTACTTTTTTTT	640
	1921	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	680
	2041	AAAGATACAAAGTTATTAGCGGAAGAGTTTGAAACTGATTGGTTCGTCTTGGTGGAAATTAAAAGAGAAAACTGATGATGATTACCGTACGTA	720
	2161	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	760
	2281	CTGACTAAATTTGGCACGGAGAACGAACGAAAGTTAGAACAAGCTGCAATAATGGCCCATCGTAAATTCAGAATAATCACGTCGATGCAAAGAATAAAGTATTTACTCCCGAGGAAAAAGAA S T K F G T E N D K L E Q A A I M A H R K F R I I T S M Q R L K Y F T P E E K E	800
	2401	AACACAGAATTCTTGTTACGAGCTTATCCTGAACTACCAAATATGTTACCTTGATGAAGAAGATGACGAGGCTTCTGGTGAAATTGTCTACTATTCAGCGTTAGTAGATGGAAGTTGTGCC N T E F L L R A Y P E L Q I C Y L D E E V D E A S G E I V Y Y S A L V D G S C A	840
	2521	ATTATGGAAAATGGTGAAAGAGCCAAAGTACCGTATTAGATTATCTGGGAACCCAATTCTTGGTGATGGGAAATCTGATAACCAAAACCATTCATGATATTTTGCAGAGGAGAATAT I M E N G E R E P K Y R I R L S G N P I L G D G K S D N Q N H S L I F C R G E Y	880
	2641	ATTCAACTAGTTGATGCTAACCAAGATAATTATTTGGAAGAATGCCTTAAAATTAGAAGCATTTTGGCGGAAATTTGAGGAAGCAACTTTCCCCCTTGGATCCTACTCGACTATTGGAA I Q L V D A N Q D N Y L E E C L K I R S I L A E F E E A T F P L D P Y S T D L E	920
	2761	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	960
	2881	GCCAGAACTITGGCCCATATIGGAGGTAAATGGCATTATGGCATCCAGATTTTTTAAATGGAATCTTTATGACAACTAGAGGGGGGGG	1000
	3001	GAAGATATTTATGCCGGTATGAATGTTGTTTTGAGAGGCGGCCGTATCAAACATTGCGAATACATGCGAATGGGAAAGGGAATGGGAATGGGATTAGGATTTGGCTCAATATTGAATTTCACTACA E D I Y A G M N V V L R G G R I K H C E Y M Q C G K G R D L G F G S I L N F T T	1040
	3121	AMGATAGGAGGGGGGGGGGGGGGGGGGGGGGGGAGGAAATGCTTCAAGAGAGAG	1080
	3241	ANTITETTATTATECTITETATITEATITTECTETEGTEGTEGTEGTEGTEGTEGTEGTEGAGETTATACTAGCEGAAGCACCACTATEGEAATATEGCEGATATEGAGACCCATAACTEGACCCAAA N L F I M L S I H L F L L V G A N L A A L T S E S T I C E Y D R F R P I T D P K	1120
	3361	CGGCCACATGGATGTTACAATTGATCCTGTTGCCGTGGCTGGC	1160
	3481	ACAGGATTITATANAGCTATCACAAGGTIGGGTAAACAGTITGCATGTTTGCATGTTTTGAAGTGTTTGTTGTAAAATCTATGCCCATGGTGTGCGAGTAATTTGAATGGT R G F Y K A I T R L G K Q F A S F S P L F E V F V C K I Y A H S L S S D I S I G	1200
	3601	GEAGCCAGGTATCTTGCCACAGGTAGGGGGTTTGCAACTATAAGGTCCCATTGCGCACATGTATTGCGGGTGAGAGTTTGTATTATGGATCCATTTGCGGATTATTGATA G A R Y L A T G R G F A T I R V P F A T L Y S R F A V E S L Y Y G S I C G L L I	1240
	3721	TTTRACTGTTCCCTATCAATGTGGAAGTTGGAATTGTATTGT	1280
	3841	CTCGATTACAAGGAATGTATCCAGTGTTTTACCGGGTAAAGGCAAACGAAGGCTTTCTTCGTGGATAAATTTACCAGGCTCAAAGGAGTCGGGTGTCGGGGTCAAAGGAAAGA L D Y K B C I Q W F Y R G N S K P R L S & W I N F T R L K R S R I V G V K S K R	1320
	3961	TACAGCATAATGAAGAATTAAAGTGGTTAGTGAAGTGAA	1360
	4081	TTTACCAACTCCCAGAACGGATCTAGAGGAACCTATCCAGTCAACAGTATCTTGCAATTCTTATAGCTTGTTCCTATTGGAGTAAACCTCGTAATATTGATGCTTGCT	1400
	4201	GIGICIAIAICAATIGGICCATTITICAATIATTIGTAAGAAATITCCTCATTIGTAGCAGCACTGCAACCAGTGCCAATCAGTATITTTTTTTTTT	1440
	4321	L F Q N W N F S V T V L G F A L S A L I Q C W F L Q M M T I L L V S R E F R H D	1480
	4561	R S N R S W W S G K W A T A G L G W Y I I T Q P M RE A V C K L S E M S Y F A G	1520
	4601	D L V A T H I L F A Q I P I L L I P Y A D K W H T L M L F W L K P G N Q I R P	1560
	4801	ANGTATTTTGATATCGATTGTTGTGATATTGTGTGATGTGTGATGGTGATGGGATGAT	1600
	4021	TAG	1640



FIG. 3. Hydropathy profiles of *C. albicans* Gsc1p (CaGsc1p), Gsl1p (CaGsl1p), and Gsl2p (CaGsl2p), calculated as described by Kyte and Doolittle (19). The number of amino acids is plotted on the *x* axis, and hydropathy is plotted on the *y* axis.

possessed about 16 potential transmembrane helices but harbored no obvious signal sequence at the N terminus, suggesting that *C. albicans* Gsc1p is an integral membrane protein as are other Gsc/Fks proteins (Fig. 3).

S. cerevisiae GSC1/FKS1 and GSC2/FKS2 have been considered genes for the putative catalytic subunits of  $\beta$ -1,3-glucan synthase. Thus, it is possible that in addition to GSC1, other glucan synthase genes are present in the *C. albicans* genome. During the course of this study, we found the partial sequence of *C. albicans FKS2* in a genome database. To determine whether *C. albicans FKS2* is an isogene of *C. albicans GSC1*, a *C. albicans FKS2* is an isogene of *C. albicans GSC1*, a *C. albicans FKS2* sequence as a probe. The gene cloned by *C. albicans FKS2* homology was designated *C. albicans GSL1* (glucan synthase-like gene 1) (Fig. 1). It contained an ORF of 3,273 bp that could encode an 130-kDa integral membrane protein with potentially 10 transmembrane domains (Fig. 2B and 3). Although the size of the *GSL1* ORF was about 57% of that of *C. albicans GSC1*, Gsl1p had a high degree of overall sequence identities with central regions of other Gsc/Fks proteins (54% identity with *C. albicans* Gsc1p, 50.5% with *S. cerevisiae* Gsc1p, 50.7% with *S. cerevisiae* Gsc2p, and 50.3% with FksAp).

To further explore the GSC1-related genes in C. albicans, we also carried out Southern analysis using the SalI-EcoRI fragment of C. albicans GSC1 as a probe (indicated as probe 2 of C. albicans GSC1 in Fig. 1). This SalI-EcoRI fragment encompasses the region that is quite conserved among all glucan synthase genes. At low stringency, this DNA fragment hybridized with a C. albicans genomic DNA sequence that was distinct from GSC1 or GSL1 (data not shown). Therefore, we screened a C. albicans genomic library by using the same hybridization conditions and isolated a third gene, which was termed C. albicans GSL2 (glucan synthase-like gene 2) (Fig. 1). The C. albicans GSL2 ORF consisted of 4,923 bp which could specify an 189-kDa integral membrane protein harboring potentially 16 transmembrane domains (Fig. 2C and 3). C. albicans Gsl2p was also highly related to other Gsc/Fks proteins over the entire sequence (52.9% identity with C. albicans Gsc1p, 47.4% with C. albicans Gsl1p, 52.5% with S. cerevisiae Gsc1p, 53.0% with S. cerevisiae Gsc2p, and 54.3% with FksAp).

Northern blotting revealed that *C. albicans GSC1* mRNA of approximately 6 kb and *GSL1* mRNA of approximately 3.5 kb were expressed in yeast cells, while *GSL2* mRNA was barely detectable (Fig. 4A). The sizes of *GSC1* and *GSL1* mRNAs coincided with the lengths of their ORFs. We also addressed whether morphogenetic transition from yeast to hyphae affected the expression of the *C. albicans GSC* and *GSL* genes. When yeast cells were transferred to RPMI 1640 medium to induce hyphal growth, both *GSC1* and *GSL1* mRNA levels declined at 22 h, while *C. albicans SKN1* mRNA expression was strongly induced upon hyphal induction as reported earlier (Fig. 4B) (24). Finally, *GSL2* mRNA, if any, remained at very low levels in both yeast and mycelial phases (Fig. 4B).



FIG. 4. Northern blotting of *C. albicans GSC1*, *GSL1*, and *GSL2* mRNAs. Poly(A)<sup>+</sup> RNA was extracted from cells in yeast phase (A) and hyphal phase (B) and hybridized with *C. albicans GSC1* probe 1 (*CaGSC1*) and *GSL1* (*CaGSL1*), *GSL2* (*CaGSL2*), *SKN1* (*CaSKN1*), and *ACT1* (actin) probes. Regions of the DNA fragments used for *GSC1*, *GSL1*, and *GSL2* probes are indicated in Fig. 1. Entire coding regions of *C. albicans SKN1* and exon 2 of *C. albicans ACT1* were used to detect the *SKN1* and *ACT1* mRNAs. Positions of the 25S and 18S rRNA markers are indicated.



FIG. 6.  $\beta$ -Glucan and *C. albicans GSC1* mRNA levels in wild-type, GSC1(+/ +/-), and GSC1(+/-/-) strains. Cell wall  $\beta$ -glucan was extracted from CAI4 (column 1), GSC1(+/+/-) (column 2), and GSC1(+/-/-) (column 3) strains, and the levels of total, alkali-soluble, and alkali- and acid-insoluble  $\beta$ -glucan were determined.

selection (Fig. 5B). Thus, two of the three *GSC1* alleles were disrupted by *hisG* sequences in this strain. Further analysis by restriction enzyme mapping revealed that there was a restriction enzyme polymorphism within *GSC1* loci and that the polymorphism was due to the absence of a *Bst*PI site in one of the three *GSC1* alleles. Disruption of any two of the three *GSC1* alleles was possible, but we have never achieved the null mutation of *GSC1* irrespective of extensive transfection and screening, suggesting that *GSC1* is an essential gene in *C. albicans*. In this study, *C. albicans* strains that harbored one and two disrupted *GSC1* alleles were designated GSC1(+/+/-) and GSC1(+/-/-), respectively.

GSC1(+/+/-) and GSC1(+/-/-) strains grew normally and did not exhibit any morphological change as judged by light microscopy. However, the disruption of one of the three GSC1 alleles decreased cell wall  $\beta$ -glucan by 10 to 20% (Fig. 6). The effect of the disruption of GSC1 alleles on  $\beta$ -glucan was more significant when two GSC1 alleles were disrupted;  $\beta$ -glucan content in the GSC1(+/-/-) strain was about half of that in parental CAI4 cells (Fig. 6). Furthermore, there was a correlation between the amounts of  $\beta$ -glucan and GSC1 mRNA. The amount of GSC1 mRNA in GSC1(+/-/-) was about 50% of the wild-type level, whereas the level in GSC1(+/+/-) was only slightly lower than that in the parental strain CAI4 (Fig. 7). These results demonstrate that GSC1 is involved in  $\beta$ -glucan synthesis in *C. albicans*. It should be also noted that there



# FIG. 7. *C. albicans GSC1* mRNA levels in wild-type, GSC1(+/+/-), and GSC1(+/-/-) strains. Poly(A)<sup>+</sup> RNAs extracted from CAI4, GSC1(+/+/-), and GSC1(+/-/-) strains were hybridized with probe 1 of *C. albicans GSC1* (*CaGSC1*) or an *ACT1* probe (actin). The region of DNA used for the *GSC1* probe is indicated in Fig. 1.

### A) CaGSC1 disruption



*albicans GSC1* (*CaGSC1*) and the region of the DNA used for the disruption of *C. albicans GSC1* (*CaGSC1*) and the region of the DNA used for the probe. Restriction endonuclease sites together with nucleotide positions numbered from the *Eco*RI site present in the 5' noncoding region of *GSC1* are indicated. 5-FOA, 5-fluoroorotic acid. (B) Integration of the *hisG* sequences into *GSC1* alleles was confirmed by Southern blotting. Twenty-five micrograms of genomic DNA that was digested with *Eco*RI, *Sal*I, and *Bst*PI was fractionated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized with a 1.0-kb *Bal*I-*Eco*RI fragment of *C. albicans GSC1* as a probe. Bands derived from the *GSC1* allele with a *Bst*PI site, the *GSC1* allele without a *Bst*PI site, the *gsc1*\(\alpha::hisG-URA3-hisG allele, and the *gsc1*\(\alpha::hisG allele are indicated by a, b, c, and d, respectively. Lane 1, CAI4; lane 2, GSC(+/+/-) with *URA3*; lane 3, GSC(+/+/-) without *URA3*; lanes 4 and 5, GSC(+/-/-) with *URA3*; lanes 6 and 7, GSC(+/-/-) without *URA3*.

Involvement of C. albicans GSC1 in β-glucan synthesis. Among C. albicans GSC1, GSL1, and GSL2, GSC1 seems to be the gene that is most closely related to S. cerevisiae GSC1/FKS1 because of its expression level and the size of the ORF. To gain more insight into the physiological roles of C. albicans GSC1, we disrupted the GSC1 gene by using the ura blaster protocol (Fig. 5A). Southern blotting detected the 1.0- and 2.0-kb bands when C. albicans CAI4 genomic DNA was digested with EcoRI, SalI, and BstPI and hybridized with the 1.0-kb BalI-EcoRI fragment of GSC1 as a probe. Unexpectedly, three bands appeared when one of the GSC1 alleles was disrupted by the hisG-URA3-hisG module (Fig. 5B). Because all three bands derived from C. albicans GSC1, strain CAI4 would contain three GSC1 alleles. Further introduction of the hisG-URA3-hisG-disrupted allele subsequently converted either the 1.0- or 2.0-kb band into a 2.8-kb band. This also became a 4.7-kb band after 5-fluoroorotic acid



FIG. 8. Calcofluor White sensitivity of strains CAI4, GSC1(+/+/-), and GSC1(+/-/-). Ten thousands cells of the indicated strains were seeded on YPD agar plates in the presence (+) or absence (-) of Calcofluor White (1 mg/ml) and incubated for 24 h at 30°C. Results of two independent colonies from GSC1(+/+/-) and GSC1(+/-/-) are shown.

was no allele-specific effect by *GSC1* disruption on  $\beta$ -glucan amount and *GSC1* mRNA level (data not shown).

The fact that some of the mutants which were hypersensitive to Calcofluor White were also defective in  $\beta$ -1,3-glucan synthesis (29) prompted us to examine whether the disruption of *C. albicans GSC1* alleles affects the sensitivity to Calcofluor White. As shown in Fig. 8, GSC1(+/-/-) cells were more susceptible to Calcofluor White than parental CAI4 and GSC(+/+/-) cells. On the other hand, echinocandins inhibit the growth of fungal cells through the inhibition of  $\beta$ -1,3glucan synthase. It has been demonstrated in *S. cerevisiae* that *FKS1* is necessary for susceptibility to the echinocandin class of antifungal drugs (6, 9). We therefore examined whether the disruption of one or two *C. albicans GSC1* alleles confers partial resistance to echinocandins. However, the sensitivities of GSC1(+/+/-) and GSC1(+/-/-) to echinocandin B were essentially the same as that of CAI4 (data not shown).

Although the physiological functions of *C. albicans GSL1* and *GSL2* remain to be established, all of these results demonstrate that *GSC1* is required for  $\beta$ -glucan synthesis in *C. albicans.* 

**Purification of \beta-glucan synthase.** Since *C. albicans GSC1* is highly homologous to S. cerevisiae GSC/FKS and is required for  $\beta$ -glucan synthesis in vivo, it is likely that *GSC1* is the gene for  $\beta$ -1,3-glucan synthase of *C. albicans*. We have addressed this possibility by purifying and determining the amino acid sequences of the enzyme.  $\beta$ -1,3-Glucan synthase was extracted from C. albicans membranes with 0.1% CHAPS and 0.02% cholesteryl hemisuccinate and purified by product entrapment (12). By repeating the product entrapment procedure, the enzyme was purified by several hundred-fold, and a 210-kDa protein that was termed p210 was sequentially enriched. Next, we generated monoclonal antibodies by using the partially purified  $\beta$ -1,3-glucan synthase fraction as an antigen. One of them, CF2A4, immunoprecipitated  $\beta$ -1,3-glucan synthase activity, and it specifically reacted with p210 by Western blotting (Fig. 9), demonstrating that p210 is a subunit of  $\beta$ -1,3-glucan synthase.

To confirm that *C. albicans GSC1* encodes p210, the protein band was excised from the gel and partially digested with lysyl endopeptidase. Microsequencing five peptides that were produced by lysyl endopeptidase digestion revealed that all of the sequences determined in the peptides derived from p210 were found in the deduced amino acid sequence of *C. albicans* Gsc1p (Fig. 2A). Given these results, we concluded that *C. albicans GSC1* encodes a subunit of  $\beta$ -1,3-glucan synthase.

### DISCUSSION

We have cloned C. albicans GSC1, the C. albicans homolog of GSC1/FKS1. Unexpectedly, C. albicans CAI4 cells possessed three copies of GSC1 in the diploid genome, and in Southern blotting, one of the GSC1 alleles displayed a pattern different from those of other two GSC1 alleles when the genomic DNA was digested with BstPI. Since no other genes have been reported to be present in more than two copies in CAI4, trisomy may be specific to the GSC1 locus in this strain. Disruption of two GSC1 alleles decreased both GSC1 mRNA and cell wall  $\beta$ -glucan levels by about 50%, and all amino acid sequences of the peptides derived from p210 that was copurified with  $\beta$ -1,3glucan synthase activity were found in the deduced amino acid sequence of Gsc1p. Thus, it appears that GSC1 encodes a subunit of  $\beta$ -1,3-glucan synthase in *C. albicans* and that  $\beta$ -1,3glucan synthase is highly conserved at least in S. cerevisiae, C. albicans, and A. nidulans.

We have shown that the *C. albicans GSC1* mRNA is preferentially expressed in yeast cells. In some experiments, we observed the shorter mRNA of approximately 3 kb with a *C. albicans GSC1* probe. Since 3-kb mRNA was detected only when the 3' region of the ORF was used as a probe, it seems that the shorter *GSC1* mRNA is missing the 5' region of the ORF, and therefore it would encode an N-terminally truncated protein. However, we cannot rule out the possibility that the shorter *GSC1* mRNA was a degraded product of intact mRNA.

In addition to *GSC1*, two *GSC1*-related genes, *GSL1* and *GSL2*, are present in the *C. albicans* genome. Although *GSL1* was expressed as a 3.5-kb mRNA, *GSL2* mRNA was undetectable in either yeast or hyphal cells. This finding implies that the *GSL2* gene is silent and redundant. Disruption of the *GSL* genes is under way in an effort to understand the physiological roles of these genes. Preliminary experiments revealed that the *GSL2* gene is not essential for vegetative growth.

The expected molecular weight of *C. albicans* Gsl1p is less than those of other Gsc/Fks proteins, but the protein encompasses the central regions of Gsc/Fks proteins where the putative long cytoplasmic domain exists. Furthermore, the amino acid sequence of the putative long cytoplasmic domain is highly conserved among all Gsc/Fks and Gsl proteins (Fig. 10). Although the physiological functions of *C. albicans* Gsl proteins



FIG. 9. SDS-polyacrylamide gel electrophoresis of *C. albicans* glucan synthase purified by product entrapment. Lane 1, protein size marker (Rainbow markers, PRN 756; Amersham); lane 2, partially purified  $\beta$ -1,3-glucan synthase after two cycles of product entrapment, separated by SDS-7% polyacrylamide gel electrophoresis and stained by Coomassie brilliant blue R-250; lane 3, Western blotting of the preparation in lane 2. Proteins were transferred to a polyvinylidene difluoride membrane and probed with monoclonal antibody CF2A5.

Gsclp Gsc2p CaGsclp CaGsllp	1:MNTDQQPYQQQ-TDYTQGPG-NGQ-SQBQ-DYDQYGQPLYPSQ-ADGYYDPNVAAGTEADMYGQ-QPPNESYDQDY-TNGEYYGQPPN-MAAQDGENFSDFS-SY-GPPGTPGYDSY-GGQYTASQMSYGEPN-SSG 1:MSYNDPNINQYYSNGDGTG-DGNYFTYQVTQDQSAYDEYGQPIY-TQNQLDDGYYDPN-EQYVDGTQFPQGQDPSQD-QGPYNNDASYYNQPPNM/NPSSQDGENFSDFS-SY-GPPSG-TY-PNDQYTPSQHSYPDQDGSSG 1:MSYNDNNNYYDPNQ-GG-G-MPPHQ-G-GEG-YY-QQQYDDMGQQPHQQDYDPNAQYQ-QQP-YD-MDGYQDQANYGGPNN-AQGYNADEAFSDFSYGQTPGTPGYDQY-GTQYTPSQMSYGDGPNSGG 1:	r 126 r 137 m 123
CaGsl2p FksAp	1:MI-MRI-DSRI-DSRI-DSRI-DSRI-DSRI-DS	- 14 ¥ 150
Gsclp Gsc2p CaGsclp CaGsl1p CaGsl2p	127: STP IYGNYDPNA IAMALPNEPY PAWTAD SQSP VS IEQIED IF IDLTNRLGFORDSMRNMFDHFMVLLDSRSS RMSP DQALLS LHADY IGGDTAN YKKWYF AAQLDMDDE IGFRNMS LGKLSRKARKAKKKNKKAME BANP 138: STP-YGNGVVNGNGQYYDPNA IEMALPNDPYPAWTAD PQSP LP IEQIED IF IDLTNKFGFQRDSMRNMFDHFMTLLDSRSS RMSP BQALLS LHADY IGGDTAN YKKWYF AAQLDMDDE IGFRNMBLGKLSRKARKAKKNKKAME BANP 124: STP IYG-GQGQGYDPTQFNMS-SNLPYPAWSAD PQAP IK IEHIED IF IDLTNKFGFQRDSMRNMFDHFMTLLDSRSS RMSP BQALLS LHADY IGGDTAN YKKWYF SSQDLDDSLGFANMTLGKIGRKARKASKKSKKARKAAE BI 1:	5 267 5 286 5 267 - - 69
FksAp	151: GGNRSSGASTPVYGMDYGNGLPAGQRSREPYPAWASDGQVPVSKEEIED IFIDLVNKFGFQRDSMRNMYDHIMTQLDSRASRMTPNQALLSLHADYIGGDNANYRWYFAAHLDLDDAVGFANMKLGKADRKTRKARKAAKK-KAQENPI	299
Gsclp Gsc2p CaGsclp CaGsl1p CaGsl2p FksAp	206 UTE-5TL-NI EGONSLEAADFRWKAMMOLSPLEXVRHIALYLLCKGEANQVRFTRECLCFIYKCASDYLD95LCQQRQEPHEGDFLNRVIFFIXHFIXNQVFIVDGRFVKERDHHKIVGTDDNQLFWFEGI-AKIVHEGTRI 287 UTE-5TL-NQIEGONSLEAADFRWKAMMOLSPLEXVRQIALFLLCKGEANQVRFTPECLCFIYKCASDYLDFEGOTLNNVIFFIXHFIXNQVFIVDGRFVKERDHHKVIGVDDVQLFWFEGI-AKIVHEGTRI 268 : GQVDALANELEGDYSLEAASIRKKAMNSLTFEERVRQIALFLLCKGEANQVRFTPECLCFIYKCASDYLDSQLQQRPPHEGDTLNNVIFFIXHFIXNQVFIVDGRFVKERDHHKVIGVDDVQLFWFEGI-SRIFFED 1:	414 433 416 - 205
Gsclp Gsc2p CaGsclp CaGsl1p CaGsl2p FksAp	415 : IELPLEERYLRLGDVWDDVFFKTYKETRTWLELVTNFNRIWVMI SI IFWYFAYNSFTFY HENNOOLUDNOPLAAYKWASCALGDTVASLDOIVATLCEWSFVPRWAADOLLSRWFWFLCI IFGINLGP I IFVFAYDKOTYJSTAAB 434 : IDLPAEERYLRLGSI PHDDVFFKTYKETRSWLELVTNFNRIWIHI IS VYWYCAYNAFTFY HENNOOLUDNOPLAAYKWASCALGDTVASLDOIVATLCEWSFVPRWAADOLLSRWFWFLCU HIGINLGP I IFVFAYDKOTYJSTAAB 417 : VDI PQEERFLKLGEVEWKNVFFKTYKE IRTWLHFVTNFNRIWI HIGT IYWYTAYNSFTLY THENNOOLUDNOPLAAYKWATAALGDTVASLDOIVATLCEWSFVPRWAADOLLSRWFWFLCU HIGINLGP I IFVFAYDKOTYJSTAAB 417 : VDI PQEERFLKLGEVEWKNVFFKTYKE IRTWLHFVTNFNRIWI HIGT IYWYTAYNSFTLY THENNOOLUDNOPLAASKWAATAALGDTVASLDOILATLFEW IFVPRWAADOLLSRWFWFLCU HIGINLGP I IFVFAYDKOTYJSTAAB 417 : VDI PQEERFLKLGEVEWKNVFFKTYKE IRTWLHFVTNFNRIWI HIGT IYWYTAYNSFTLY THENNOOLUNNGPLASSRWAACAIGDULASFDOILATLFEW IFVPRWAADOLLSRWFU TVLILIUTV-ANSOSI VTNLGLLKRWFT 1 :	7 564 7 583 4 566 3 87 7 355 7 595
Gsclp Gsc2p CaGsclp CaGsl1p CaGsl2p FksAp	565:VAAVMFFVAVATIIFFSIMPLOOLITSYMKKSTRRYVASOTTAAFAPLIEGLORMSYLVVTVFAAFYSESYFLIVLGLADIRILS-TTAMRTGEYMMGAVLKVPRIVLGVIATDFILFFLITHLATIVVTVFSVCKFYLE 564:VGAVMFFVAVATUFFSVMFLOOLITSYMKKSTRRYVASOTTAAFAPLIEGLORMSYLVVTVFAAFYSESYFLIVLGLADIRILS-TTAMRTGEYMMGAVLKVPRIVLGMINTSTLFFLITHLATIVVTVFSVCKFYLE 567:VSTVFTAVATUFFSVMFLOOLITSYMKKSTRRYVASOTTAAFAPLIEGLORMSYLVVTVFAAFYSESTFLILGLADIRILS-TTAMRTGEYMMGAVLKVPRIVLGMINTSILFFLITHLATIVVTVFSVCKFYLE 567:VSTVFTAVATUFFSVMFLOOLITSYMKKSTRRYVASOTTAAFAPLIEGLORMSYLVVTVFULAVUSSTFLILGLADIRILS-TTAMRTGEYMMGAVLKVPRIVLGMINTSILFFLITHLATIVVTVFSVCKFYLE 567:VSTVFTAVATUFFSVMFLOOLITSYMKKSTRRYVASOTTAAFAPLIEGLORMSTLVMTVFSVCKFYLE 88:TVVAAISHOLSV-LITHFLAINPPGSFXTVFSLASOTTAAFANTSESTFLILGLADIRILSLADIRILS-TTEMRTVOEVINGUSTUNTVINLAUVUSST 356:TSAFOFSVINULISTYFLORLFKRKKANARFLORSSLASOTSGLADAFSCHAATYSESTFLILGLADIRILSLADIRILS-TTEMRTOEVINGUS 556:TSGTVEFFINALTFFFFSINFLOOLFOSSVINFSLASOTTAAFS	713 732 715 220 505 745
Gsclp Gsc2p CaGsclp CaGsl1p CaGsl2p FksAp	714:5 ILTP WANTFILERKEITSKILATTOMEIKYK RVLIGOVINALIIISMYREHLLAIDEBOKILERU-PSEIBGKRTLRAFTFYSODINNETEFFPROSHAFRAGISTFIFPELFONNETETUTE INAFERILLSIDE ID 733:5 ILTP WANTFILERUTSKILATTOMEIKYK RVLIGOVINALIIISMYREHLLAIDEBOKILMUV-PSEIBGKRTLRAFTFYSODINNETEFFPROSHAFRISFFAQSISTFIFPELFONNETETUTE INAFERILLSIDE ID 716:5 ILTP WANTFILERUTSKILATTOMEIKYK RVLIGOVINALIIISMYREHLLAIDEBOKILMUV-PSEIBGKRTLRAFTFYSODINNETEFFPROSHAFRISFFAQSISTFIFPELFONNETTUTE INAFERILLSIDE ID 716:5 ILTP WANTFILERUTSKILATTOMEIKYK RVLIGOVINALIIISMYREHLAIDEBOKILMUV-PSEIBGKRTLRAFTFYSODINNETEFFPROSHAFRIISFFAQSISTFIFPELFONNETTUTE INAFERIL 716:5 ILTP WANTFILERUTSKILATTOMEIKK RVLIGOVINALIIISMYREHLAIDEBOKILMUV-PSEIBGKRTLRAFTFYSODINNETEFFPROSHAFRAISFFAQSISTFIPEPTU 716:5 ILTP WANTFILERUTSKILSOODIN 716:5 ILTP WANTFILTUTSKILSOODIN 716:5 ILTP WANTFILERUTSKILSOODIN 716:5 ILTP WANTFILERUTSKILSOODIN 716:5 ILTP WANTFILERUTSKILSOODIN 716:5 ILTP WANTFILERUTSKILSOODIN 716:5 ILTP WANTFILERUTSKILSOODIN 716:5 IL	2 862 1 881 1 864 2 360 2 654 2 894
Gsclp Gsc2p CaGsclp CaGsl1p CaGsl2p FksAp	863 EDDOF RAVILLEFLING BY VENECFURTH I LABETAAYE-GNENBAEKEDALKSQI DDJEPHCI EFKSAAFEYTLRTHAALKSDTLYR I IAGYENYSKI I KLIYXWEPE I VOHGGNAEGLERENARRIKEFLVSKIRLANF 863 EDDOF RAVILLEFLINGLBY VENCFURTH I LABETAAYE-GNENBEPEKEDALKSQI DDJEPHCI EFKSAAFEYTLRTHAALKSDTLYR I IAGUNSKI I KLIYXWEPE I VOHGGNAEGLERENARRIKEFLVSKIRLANF 865 EDDOF RAVILLEFLINGLBY VENCFURTH I LABETAAYE-NNEDEPEKEDALKSQI DDJEPHCI EFKSAAFEYTLRTHAALKSDTLYR I VASIGEN VARH I KLIYXWEPE I VOHGGNAEGLERENARRIKEFLVSKIRLANF 361 II-QSTSKILLI LEFLINGLBY VENCFURTH I LABETAAYE-GNENBEPEKEDALKSQI DJEPHCI EFKSAAFEYTLRTHAALKSDTLYR I VASIGEN VARH I KLIYXWEPE I VOHGGNAEGLERENARRIKEFLVSKIR 361 II-QSTSKILLI LEFLINGLBY VENCFURTH I LABETAAYE-GNENBEPEKEDALKSGI EFKSARFEYTLRTHAALKSDTLYR I VANGENN VATILLIY VAN EFKJOR I ALEMAARRIKEFL I SKILL 365 IEDOSTSKILLI LEFLINGLBY VENCFURTH I LABETAAYE-GNENBEPEKEDALKSGI I VANGENN VARH I LIYXVINELU VANGEN VARH 361 II-QSTSKILLI LEFLINGLBY VENCFURTH I LABETAAYE-GNENBEPEKEDAKSGI I VANGENN VARH I LIYXVINELU VANGEN VARH 365 IEDOSTSKILLI LEFLINGU VENCFURTH I LABETAAYE FARHARRIKSTI VANGENN VARH I LIYXVINELU VANGEN VARH I LABETARRIKEFU VANGENN 365 IEDOSTSKILLI LIYLINGLBE VENCFURTH I LABETAAFEN I DEDITING VENCFURTH VAN KENTI VANGENN VARH I LIYXVINELU VANGEN VARH I LIYXVINELU VANGEN VARH I LIYXVINELU VANGEN VARH I LIYXVINELU VANGEN VARH VANGEN VARH I LIYXVINEL VANGEN VARH I LIYXVINELU VANGEN VARH I LIYXVINEL VARH VARH I LIYXVINEL VARH VARH I LIYXVINEL VARH VARH VARH I LIYXVINEL VARH VARH VARH VARH VARH VARH I LIYXVINEL VARH VARH VARH VARH VARH VARH VARH VARH	(1011) (1030) (1014) (495) (795) (1042)
Gsclp Gsc2p CaGsclp CaGsllp CaGsl2p FksAp	1012: PHELENAEFIL MYFDLQIAYIDEEPPLTEGEEPRIKSAIIIGHCEILDNGRRAPH WOLSGNPILGOGKSDNQHHALIFYKGEYIGLIBANGDNYLEECLKIRKVIAFFELLUVGUNPHAPGLRYEEQTT-NHPVAIVARTYIFS 1031: PHELENAEFILMYTDLQIAYIDEEPPLNSGEEPRIKSAIIIGHCEILDNGRRAPH WOLSGNPILGOGKSDNQHHALIFYKGEYIGLIANGDNYLEECLKIRKVIAFFELLIGICIBPTGLKYEDGST-NHPVAIVARTYIFS 1015: DDEMENAEFILMYTDLQIAYIDEEPALNBOEEPRIKSAIIIGHCEILDNGRRAPH WOLSGNPILGOGKSDNQHHALIFYKGEYIGVIDAGDNYLEECLKIRKVIAFFELLIGICIBPTGLKYEDGST-NHPVAIVARTYIFS 496: PDERTADASIFYKFFWLWAXILESD	, 1160 ; 1179 ; 1164 ; 632 ; 940 ; 1188
Gsclp Gsc2p CaGsclp CaGsl1p CaGs12p FksAp	1161: RSCM.GDMARKEQTFGTLFARTLSQTGKLHYGHDFINATFHTRGQFKAQKGLHLNEDIYAGNIAMLRGARIKHCHYDCGKGRDLGHCTILNFTHKIGAHSGCMLSRETHNLTGALGHLFFTHNULFFTHFTHRQFKAQKGLHLNEDIYAGNIAMLRGARIKHCHYDCGKGRDLGHCTILNFTHKIGAHSGCMLSRETHNLTGALGHLFTHFTHNULFFTHFTHLNLTGALGH 1161: NSCM.GTMARKEGTFGTLFARTLAGIGKLHYGHPTINATFHTRGQFKAQKGLHLNEDIYAGNIAMLRGARIKHCHYDCGKGRDLGHCTILNFTHKIGAHSGCM, SRETHTLTGALFIDHFTHNULFFGLFKA 1165: NSCM.GTMARKEGTFGTLFARTLAGIGKLHYGHPTINATFHTRGQFKAQKGLHLNEDIYAGNIAMLGARIKHCHYDCGKGRDLGHCTILNFTHKIGAHSGCM, SRETHTLTGALFIDHFTHNULFFGLFKAG 1155: NSCM.GTMARKEGTFGTLFARTLAGIGKLHYGHPTINATFHTRGQFKAQKGLHLNEDIYAGNIAMLGGRIKHCHYDCGKGRDLGHCSTILNFTHKIGAHSGCM, SRETHTLTGALFIDHFTHNULFFGLFKAG 633: NITILGIINAAKEGTFGTLFARTLAGIGKLHYGHPTINGTFKAQKKAGKLILNEDIYAGNIAKGGRIKHCHYDCGKGRDLGHCSTUNFTKKIGGHSGCMSEFTHANLTHFT 941: NITILGIINAAKEGTFGTLFARTLAGIGKLHYGHPTINGTFKAGTKAGKKAGKLILNEDIYAGNIAMVRGGRIKHCHYDCGKGRDLGFGSTUNFTKKIGGHSGCMSEFTHANLTHFT 1189: NITILGIINAAKEGTFGTLFARTLAGIGKLHYGHPTINGTFKAGTKKAGKLILNEDIYAGNIAKSGTKSCHYGGKGRUGGSTUNFTKKIGGHSGCMSEFTHANLTHFT 1189: NITILGYDAGGTFGTLFARTLAGULGKLHYGHPTINGTHAGTKAGKKAGKLILNEDIYAGNIAGRIKHCHYDGKGGRUGGKGRUGGSTUNFTKKIGGHSGGALSFTHTUNGTHAFTHT 1189: NITILGYDAGGTFGTLFARTLAGULGKLHYGHPTINGGTHKGTFKGTFKGTFFKARGTFKGTFFFHNILTHFT 1189: NITILGYDAGGTFGTLFARTLAGULGKLHYGHPTINGTFKGTFKGTFKGTFFFHNILTHFT 1189: NITILGYDGGAGGTFGTLFARTLAGULGKLHYGHTGGTFKGTFFFHNILTHT 1189: NITILGYDGGAGGTFGTLFARTLAGULGKLHYGTFFHTNGTFKGTFKGTFFFHNILTHFTH 1189: NITILGYDGGAGGTFGTLFARTLAGULGKLHYGTFFHTNGGTFKGTFFFHNILTHFTH 1189: NITILGYDAGGTFGTFFFHT	1310 1329 1314 782 1090 1338
Gsclp Gsc2p CaGsclp CaGsl1p CaGsl2p FksAp	1311: FNLTLVNËSLAHES INT YDRNRRKEDVLVE ICTVNFGRAVGAVRETI LITI IVMENAVET VVDLI BRELMKATORFFCHLELER FEVERAGO INSSALLSDLA I GORMI SIGRGFFATSRI PRS I LYSRFGSA I MEDASMIMU 1330: FNLTLVNERALAHES ILLVYDROM I DVLYH GTVNFHR I DAVARTTI HI IVMENAVET VVDLI BRELMKATORFFR I ISI IPMEVERAGO INSSALLSDLA I GORMI SIGRGFFATSRI PRS I LYSRFGSA I MEDASMIMU 1330: FNLTLVNERALAHES ILLVYDROM I DVLYH GTVNFHR I DAVARTTI HI IVMENAVET VVDLI BRELMKATORFFR I ISI IPMEVERAGO INSSALLSDLA I GORMI SIGRGFFATSRI PRS I LYSRFGSA I MEDASMIMU 1335: FNLTLVNERALAHES YNROM UVLYH GTVNFHR I DAVARTTI HI IVMENAVET VVDLI BRELMKATORFFR I ISI IPMEVERAGO INSSALLSDLA I GORMI SIGRGFFATSRI PRS I LYSRFGSA I MEDASMIMU 1335: FNLTLMSLGANNOTAAL - TEONH I GTHLWFUNA I DORVLEVI VCTI ISI ILV UVDLI BRELMKATORFFR I ISI IPPEVIVAQI ISSSVETTU COMSALABORFI SIGRGFFATSRI SIGRAFFATSRI SI ISI GORMAN 1335: FNLTLMSLGANNOTAAL - TEONH I GTHLWFUNA I DORVLEVI VCTI ISI ILV UDEL BRELIXA I ILI IVI. BFFEVIVAQI ISSSVETADI. SIGRAFFATSRI SI SI SIGRAFFATSRI SI SI SI GORMAN, SI GROFFATSRI SI SI SI GORMAN, SI GROFFATSRI SI SI SI GORMAN, SI G	1460 1479 1464 924 1240 1488
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Gsclp Gsc2p CaGsc1p CaGs11p CaGs12p	1601 FILICILAP IAVNLGVLFFCMGMSCCSGP LFGMCCKKTGSVMAGIABGVAV IVH IAFF IVMWVLESFNFVRML IGVVTC IQCQ-RLIFHCMTAIMLTREFKNDHANTAFWTGKWYGKGMGYMAWTQP SRELTAKV IELSEFAADFVLGHV 1620 FILICILAP IVID IGVLFFCMGUSCCSGP LLGMCCKKTGSVMAGIARGIAVVWH IVFF IVMWVLEGFSFVRML IGVVTC IQCQ-RLIFHCMTVULLTREFKNDHANTAFWTGKWYSTGLGYHAWTQP TRELTAKV IELSEFAADFVLGHV 1613 FILI ICALAPVV IDMGCLGVCLAMACCAGPHLGLCCKKTGSVMAGIARGIAVVWH IVFF IVMWVLEGFSFVRML IGVVTC IQCQ-RLIFHCMTVULLTREFKNDHANTAFWTGKWYSTGLGYHAWTQP TRELTAKV IELSEFAADFVLGHV 1613 FILI ICALAPVV IDMGCLGVCLAMACCAGPHLGLCCKKTGSVMAGIANGIAVHH IVFF IVMWVLEGFSFVRML IGVVTC IQCQ-RLIFHCMTVULLTREFKNDHANTAFWTGKWYSTGLGYHAWTQP TRELTAKV IELSEFAADFVLGHV 1613 FILI ICALAPVVINGCAGNNU	1749 1768 1761 1090 1527
Gsclp Gsc2p CaGsclp CaGsl1p CaGs12p	1952: BALTAR OF IGING VAAVFFORGENCERFORVLAALAHAIAHAIVILLUVIFEVMFFLEHKSKPRCVMGMIAMAAIQ-RFVYKLIIALALTREFKHDOSNIAMMTGKMYNMGWESLSOPGREFLCKITELGYFSADFVIGHL 1750: ILICOLPLIIIPKIDKFHSIMLFWLKPSRQIREPIYSLKOTELEKKRVKKYGSLYFLVLAIFAGCIIGFAVASAKIHKHIGDSLDG-VVHNLFOPINTTNNDTGSOMS-TYGSHYY-TH-TFSLKTWSTIK 1769: ILICOLPLIIPKIDKFHSIMLFWLKPSRQIREPIYSLKOTELEKKRVKKYGSLYFLVLAIFAGCIIGFAVASAKIHKHIGDSLDG-VVHNLFOPINTTNNDTGSOMS-TYGSHYY-TH-TFSLKTWSTIK 1769: ILICOLPLIIPKIDKFHSIMLFWLKPSRQIREPIYSLKOTELEKKRVKKYGSLYFLVLUFUVUGFAVASAKIHKHIGDSLDG-TFENLVOPENVSNNDTGSOMS-TYGSHYT-TH-TFSLKTWSTIK 1769: ILICOLPLIFFINDRNHSMLFWLKPSRJEFSLIREPIYSLKQARLKKRVKKYCSLYFLVLUFUVUGFAVASAKIHKHIGGSSIAGCIAVOFAN 1762: ILFOLPLILFFINDRNHSMLFWLKPSRJEFSLIREPIYSLKQARLKKRVKKYCSLYFLVLUFUVUGFAVASAKIHKHIGGSSIAGCIAVOFAN 1951:	1778 1876 1895 1897
FksAp	1779: LLF IMLPALCVPYIDKF BSVILIWLRPS RGIRPIYSLKQSKLRKRVVRPAILYFAMILIELVLIAPIIELARNOULIKANGILLEMOPLO-SINDEITHSTUTSCVPKCMPEIASPSSVILINUN	1905

FIG. 10. Amino acid sequences of Gsc/Fks and Gsl proteins, compared by using the Genetyx program (version 8.0). Amino acids that are conserved among six proteins are boxed. The region corresponding to the putative long cytoplasmic domain is underlined. Dashes indicate gaps introduced to optimize alignment. Gsc1p, *S. cerevisiae* Gsc1p; Gsc2p, *S. cerevisiae* Gsc2p; CaGsc1p, *C. albicans* Gsc1p; CaGsl1p, *C. albicans* Gsl2p; FksAp, *A. nidulans* FksAp.

remain to be established, it is speculated that the highly conserved cytoplasmic domain present in the middle of Gsc/Fks proteins may be important for the catalytic activity.

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