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Cloning of the *Candida albicans* Homolog of *Saccharomyces cerevisiae GSC1/FKS1* and Its Involvement in β-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 β-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 yeast-phase 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 β-1,3-glucan synthase specifically reacted with the 210-kDa protein and could immunoprecipitate β-1,3-glucan synthase activity. These results demonstrate that *C. albicans GSC1* is the gene for a subunit of β-1,3-glucan synthase.**

β-1,3-Glucan synthase (UDP-glucose; 1,3-β-D-glucan 3-β-D-glucosyltransferase; EC 2.4.1.34) catalyzes the formation of a β-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 β-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 β-1,3-glucan synthase activity was present in *gsc1*Δ/*fks1*Δ 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 β-1,3-glucan synthase activity, demonstrating that Rho1p is a regulatory subunit of the enzyme (8, 28).

There is a linkage between β-1,3-glucan and another cell wall component. In *S. cerevisiae*, the nonreducing end of a β-1,3-glucan chain binds to the terminal reducing residue of a chitin chain by a β-1,4-linkage (18). *CHS3*, one of the three chitin synthase genes, is required for the formation of the linkage between β-1,3-glucan and chitin (18). This β-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 β-1,3-glucan synthase activity is present in the membrane fraction of *C. albicans* (27) and that *C. albicans* β-1,3-glucan synthase activity is sensitive to echinocandin B (32) strongly suggest that *S. cerevisiae* and *C. albicans* have similar mechanisms of β-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 *Sau*3AI, 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*

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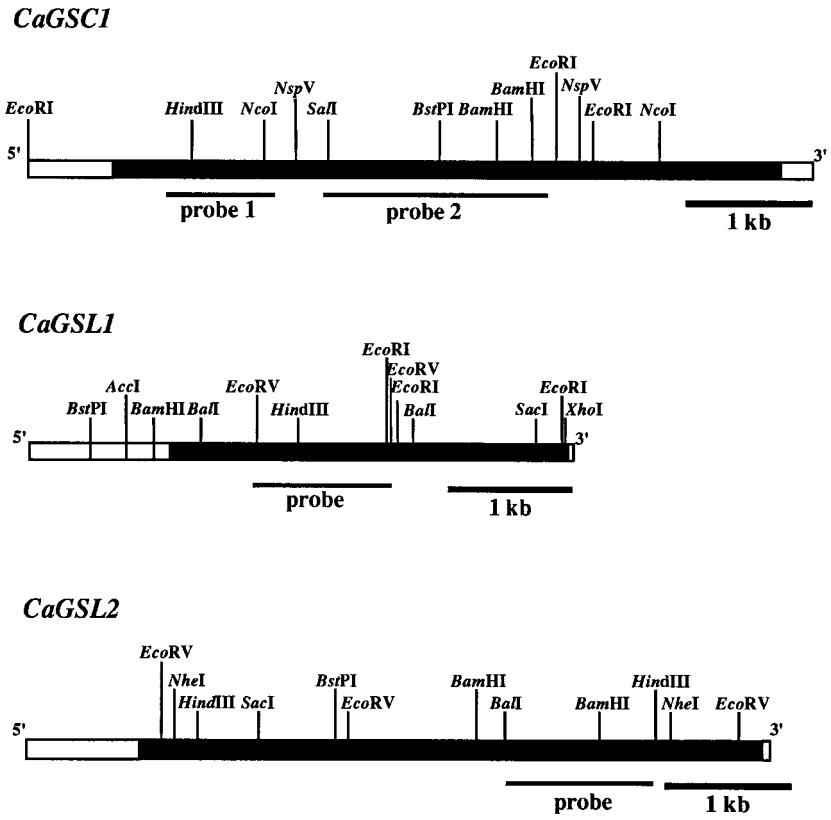

FIG. 1. Restriction maps of *C. albicans GSC1* (*CaGSC1*), *GSL1* (*CaGSL1*), and *GSL2* (*CaGSL2*). Regions of probes used for Southern and Northern blottings are also indicated. Black bars represent ORFs of *C. albicans GSC1*, *GSL1*, and *GSL2.*

homolog was carried out under low-stringency conditions in a buffer containing 0.25 M sodium phosphate (pH 7.2), 2× SSC (1× 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-*Bgl*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 pCA1U with *Acc*I, 100 μg of the DNA was transfected into *C. albicans* CAI4 (*ura3Δ::imm434*/*ura3Δ::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 CAI4 cells were cultured in YPD medium (1% peptone, 2% yeast extract, 2% dextrose) at 30°C.

Determination of cell wall β-glucan. *C. albicans* cells that were grown to mid-logarithmic phase were harvested, washed with H_2O, and used for extracting cell wall polysaccharides. The amount of cell wall β-glucan was determined by fractionating the cell wall polysaccharides as described previously (16).

Purification of β-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.). β-1,3-Glucan synthase was extracted from membranes with 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS)–cholesteryl hemisuccinate detergent mixture, purified by product entrapment, 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 β-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* (β-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

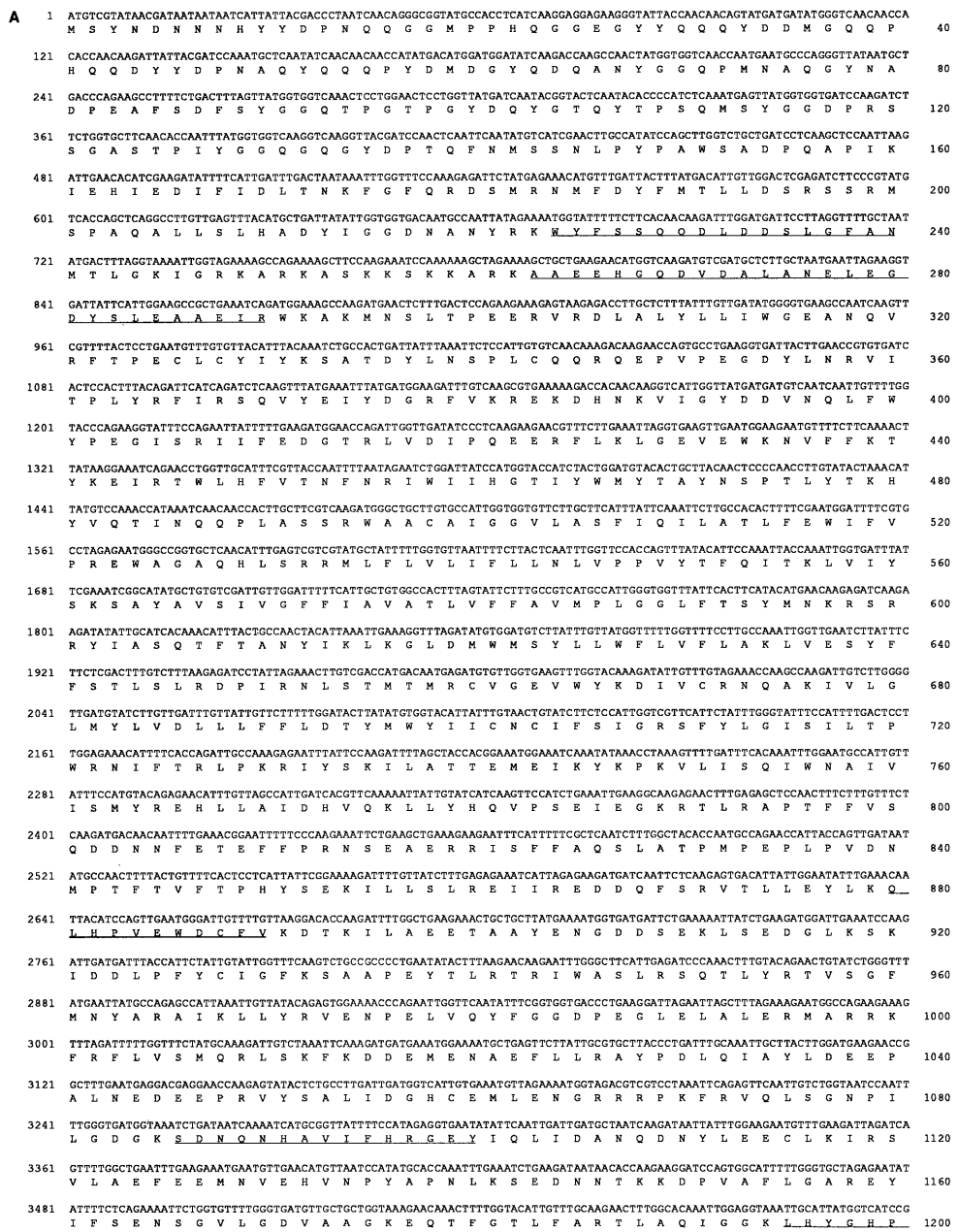

FIG. 2. Nucleotide and predicted amino acid sequences of *C. albicans GSC1* (A), *GSL1* (B), and *GSL2* (C). Underlines represent the corresponding peptide sequences which have been determined with the purified 210-kDa β-1,3-glucan synthase. According to the report by Manuel et al. (21), the CTG codon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

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4321  CCATTTTCAATCTTGTATTCACGTTTTGCTGATTCATCCATTTATATGGGAGCAAGATTGATGTTGATTTTATTATTTGGTACAGTTTCTCATTGGCAAGCACCATTATTATGGTTCTGG
      P  F  S  I  L  Y  S  R  F  A  D  S  S  I  Y  M  G  A  R  L  M  L  I  L  L  F  G  T  V  S  H  W  Q  A  P  L  L  W  F  W   1480

4441  GCTTCATTATCGGCTTTAATGTTCTCCCCATTCATTTTCAATCCTCATCAATTTGCTTGGGAAGACTTTTTCCTTGATTACAGAGATTTCATTAGATGGTTATCTAGAGGTAACACTAAA
      A  S  L  S  A  L  M  F  S  P  F  I  F  N  P  H  Q  F  A  W  E  D  F  F  L  D  Y  R  D  F  I  R  W  L  S  R  G  N  T  K   1520

4561  TGGCACAGAAACTCATGGATTGGTTATGTTAGACTTTCTAGATCACGTATCACTGGTTTCAAACGTAAGTTGACTGGTGATGTTTCTGAAAAAGCTGCTGGTGATGCTTCAAGAGCTCAT
      W  H  R  N  S  W  I  G  Y  V  R  L  S  R  S  R  I  T  G  F  K  R  K  L  T  G  D  V  S  E  K  A  A  G  D  A  S  R  A  H   1560

4681  AGATCCAATGTTTTGTTTGCTGATTTCTTACCAACATTGATTTATACTGCTGGTCTTTATGTTGCTTATACTTTTATTAATGCTCAAACTGGGGTTACTAGTTATCCATATGAAATCAAT
      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  GGATCTACTGATCCACAACCAGTTAATTCTACTTTGAGACTTATTATTTGTGCTTTAGCTCCAGTTGTTATTGATATGGGATGTTTAGGTGTTTGTCTTGCCATGGCATGTTGTGCTGGT
      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

4921  CCAATGTTAGGATTATGTTGTAAAAAGACTGGTGCTGTTATTGCTGGTGTTGCCCATGGTGTTGCCGTCATTGTTCATATTATTTTCTTTATTGTTATGTGGGTCACTGAAGGTTTCAAT
      P  M  L  G  L  C  C  K  K  T  G  A  V  I  A  G  V  A  H  G  V  A  V  I  V  H  I  I  F  F  I  V  M  W  V  T  E  G  F  N   1680

5041  TTTGCCAGATTAATGTTGGGTATTGCCACCATGATTTATGTTCAAAGATTATTATTCAAGTTTTTGACATTATGTTTCTTGACTAGAGAATTTAAGAATGATAAAGCCAATACTGCTTTC
      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  TGGACTGGTAAATGGTATAATACTGGTATGGGATGGATGGCTTTTACTCAACCATCTCGTGAATTTGTTGCTAAAATCATTGAAATGTCGGAATTTGCTGGTGATTTCGTTTTGGCACAT
      W  T  G  K  W  Y  N  T  G  M  G  W  M  A  F  T  Q  P  S  R  E  F  V  A  K  I  I  E  M  S  E  F  A  G  D  F  V  L  A  H   1760

5281  ATTATATTATTCTGTCAATTACCATTATTGTTTATTCCATTAGTTGATAGATGGCATTCAATGATGTTATTCTGGTTGAAACCATCAAGATTGATTAGACCACCAATTTATTCTTTGAAA
      I  I  L  F  C  Q  L  P  L  L  F  I  P  L  V  D  R  W  H  S  M  M  L  F  W  L  K  P  S  R  L  I  R  P  P  I  Y  S  L  K   1800

5401  CAAGCCAGATTAAGAAAGAGAATGGTGAGAAAATATTGTGTTTATATTTTGCCGTGTTGATATTATTTATTGTCATTATTGTTGCACCAGCAGTTGCTTCGGGACAAATTGCTGTTGAT
      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  CAATTTGCCAATATTGGTGGATCTGGTTCTATTGCTGATGGATTATTCCAACCAAGAAATGTCAGTAATAATGATACTGGTAATCATAGACCAAAAACCTACACTTGGAGTTATTTGAGT
      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  ACTCGTTTTACTGGAAGTACCACCCCTTATTCTACAAATCCATTCAGAGTTTAA
      T  R  F  T  G  S  T  T  P  Y  S  T  N  P  F  R  V  *                                                                    1897

B     1  ATGTCATTCAACTCGCCTTCTTTGTACACACCAAATTATACCCCCAATAAGTCACCGCAAGTGCACATAAGACTAGCCATTGTATCCATAGGTGGAATCATTGCCGTCTTAATATCGTTG
         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   GGTGCCGCAATATCTGATTTTTTCTTTGTGTCAGGAAGTGTCCGAAACATTGTTTTGTTATTGATATTGACAGTTGCAAACTCGGGATCTATAGTTTACAATCTAGGGCTTTTGAAATGG
      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   GACAAGTATTCTAAAAATGGGACAGTGGTTGCAGCTATTCTGATGTGCTTATCGGTTCTCACATTTTGTTCTTGGCCATCAATCCACCAGGAAGTTTCAAAACCGTGTTTTCCAACAAT
      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   TTCCCAAAATTAAAGTTAAGAAGCCGATTATTCTCCATATCCTTATGGATTGGTGTATTTGCAGCCAAGTATTCAGAGTCATACTTTTTCTTAATATTGTCGTTGAAAGACCCCATTCAA
      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  F  L  I  L  S  L  K  D  P  I  Q   160

481   ATTCTATCTACCATTGAATTAAACTGTGATAACGGTCATTTTCTATGCCGGTTTCAACCAAAGATTACATTGATATTGTTTTATCTCACTGATTTGATATTATTCTTTTTGGACACTTAT
      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   TTGTGGTATGTGATTTGCAATTGTTTATTTTCAGTGGGACTATCGTTTTCATTAGGTGTGTCTATTTTTACCCCTTGGAAAAATATTTTTTCTCGATTACCAGATCGGATTTTAACCAAG
      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   ATATATTATGGCGATTCCACAGAGTTGATATTGGTGATATCACAAATATGGAACAGCATTATCATTTCCATGTACAGGGAGCATGTTCTCTCGGTTGAACAAGTTTGCAAGTTGATTTAT
      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   CAACGAGGAGCTGATGAAAACACTATACGACCACCACTATTTTTTGTTTACGAAGATGATAACAAATTTTATGATTTTATTAAAATCGAAAAGGAATGGGAAAGAAGGATCACATTTTTT
      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   GCTCAATCGTTATCAAGCCCTTTACCAGAACCATTTCCAGTAGTTTCTACACCAACATTTACCGTTTTGATTCCTCATTACTCAGAAAAAATACTATTAAGTTTACAAGATTTAATTAAA
      A  Q  S  L  S  S  P  L  P  E  P  F  P  V  V  S  T  P  T  F  T  V  L  I  P  H  Y  S  E  K  I  L  L  S  L  Q  D  L  I  K   360

1081  GAACAAAGCTTTTCAAAACTAACGTTGCTAGATTATTTGAAACAACTTCATTCGAAAGAATGGGATTCATTTGTTCAAGATAGTAAGATGATCCAAACTATAAAGGAAATGGATGAAGAC
      E  Q  S  F  S  K  L  T  L  L  D  Y  L  K  Q  L  H  S  K  E  W  D  S  F  V  Q  D  S  K  M  I  Q  T  I  K  E  M  D  E  D   400

1201  AAGTTTGTACGCGAAAATATGGATGATTTGCCGTACTACTGTATCGGGTTCAAAGATTCTTCACCAGAAAATGTTTTACGAACAAGAATTTGGGCTGCATTAAGATGTCAGACATTGTAT
      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  CGTACAGTTTCAGGGTTTATGAATTATGTGACTGCTTTAAAGTTGCTTTACCGAACGGAAGTAATCGGTTTTGAACAAAACGAATTTCCCGAAGAAGAACTTGAAGAATTTGTAAGCAGA
      R  T  V  S  G  F  M  N  Y  V  T  A  L  K  L  L  Y  R  T  E  V  I  G  F  E  Q  N  E  F  P  E  E  E  L  E  E  F  V  S  R   480

1441  AAATTCAATTTACTAATTGCTATGCAGAATTTCCAGAATTTTGCCCCAGACATGAGGACCGATGCCGATTCTTTATTTAAGGCTTTCCCCAACGTCAAGGTTGCCATTTTAGAATCTGAT
      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  AACGATCAAGACTATTATTCAACACTATTAGATGTTTCAAAACGAGATGACAAAAATCAGTATGTTAAAAAATACCGAATCAAGCTATCAGGAAATCCTATTTTAGGTGATGGCAAATCT
      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  GATAATCAAAATAGTGCATTAATATTTTATCGTGGGGAATATATCCAGGTCATAGATTCCAACCAGGATAATTATATCGAGGAATGTCTCAAAATCAAATCTTTGCTAAATGAATTCGAG
      D  N  Q  N  S  A  L  I  F  Y  R  G  E  Y  I  Q  V  I  D  S  N  Q  D  N  Y  I  E  E  C  L  K  I  K  S  L  L  N  E  F  E   600

1801  GAGATGAACTTGGACGTAAGTTTTGGATATCAGACGGAGCACCCAGAAACTAGTTCTGTGGCAATTGTGGGGGCAAGAGAATTCATATTTTCACAGAACATAGGAATTTTAGGAGACATA
      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  GCTGCTGCCAAAGAACAGACTTTTGGAACGTTATTTGCAAGAACAATGGGAGAAATCGGATCTAAACTTCATTATGGCCACCCTGATTTGCTCAATGGAATATTTATGACGACAAGAGGT
      A  A  A  K  E  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  GGAATTTCAAAAGCCCAGAGAGGCTTACATTTGAATGAAGACATTTATGCAGGGATCACTGCAACTTGTCGTGGTGGAAGGATAAAACACTCAGATTACTATCAATGTGGCAAGGGAAGG
      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  GATCTTGGGTTCCAATCAATTGTGAATTTTACAAAGAAAATTGGATCTGGAATGGGCGAACAGCTTTGTCAAGAGAGTATTACTATTTGGGGAGCATGTTACCAATAGATAAATTCTTG
      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  TCATTTTACTATGCTCACGCTGGATTCCATATCAACAACTTGTCTATAATGTTGTCTGTAAAAGCCTTTATGTTTTTGTTGATGAGCCTTGGTGCATTAAACAACGGCACAGCCGCGTGC
      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  ACGGAAGATAACCCAACACCTGGCTGCCATAATTTAGTCCCCGTTCTAAACTGGATTGACCGTTTTGTGCTTTCAGTATTTGTTTGCTTCTTCATTTCATTTTGCCTTTGATAATTCAA
      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  GAGTTTATCGAAAAGGGATTACTCAAAGCAATTTTGCGAATATTATTACACATTGTTTCGTTATCGCCATTTTTTGAAGTTTTTGTGTGTCAAGTATATTCAAGAGCGTTAAGGGACAAT
      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  TTTATATTTGGAGAAGCTAAATATATTGCTACAGGACGAGGATTTGCTATCTCCAGAGTATCATTTGCCACATTGTACTCCCGATATGCCAGTTTATCCATTTACTATGGTGGTGAAATA
      F  I  F  G  E  A  K  Y  I  A  T  G  R  G  F  A  I  S  R  V  S  F  A  T  L  Y  S  R  Y  A  S  L  S  I  Y  Y  G  G  E  I   920

2761  TTTTTGGTGATTTTGTTTGCCTCAATAACTATCTGGAGAAAATCTTTACTATGGTTTGTAATCACTATTATATCCTTATGCTTGGCACCATTCATTTTCAATCCTCATCAGTTTAATTTT
      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  GTTGATTTCTTTGTCGATTATAGGGACTATGTCAGATGGTTGACAAGAGGAAACTCTTCTCTCAAAGAACTGTCCTGGACTCATTATACCAAAGTTAGGAGAGCTCGGTTAACTGGAGAA
      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  AAGTTTGATGGCGGGTATGTTCTGGGTAGAAATACTGCTACATTCAATTTGTTATTGGGGGAGGTAGCGTCACCGTCGATCAATTTGATATTGTATCTATTACCGTATTTGTTTCTTCAT
      K  F  D  G  G  Y  V  S  G  R  N  T  A  T  F  N  L  L  L  G  E  V  A  S  P  S  I  N  L  I  L  Y  L  L  P  Y  L  F  L  H   1040

3121  TCAAGTCCCAAATCTACTTGCATTCAGTGTGAAAAATCCACTAATAAAGTTAACAGCAGTTGTATTGGCACCTTATCTCGAGAATTCGGCGGTTCTTTTCTTCGTATGGGTAATGTCCTT
      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

3241  AACTCTTGCGCCATTGGTTGGAATATGGTTTAA
      N  S  C  A  I  G  W  N  M  V  *                                                                                          1090
```

FIG. 2—*Continued.*

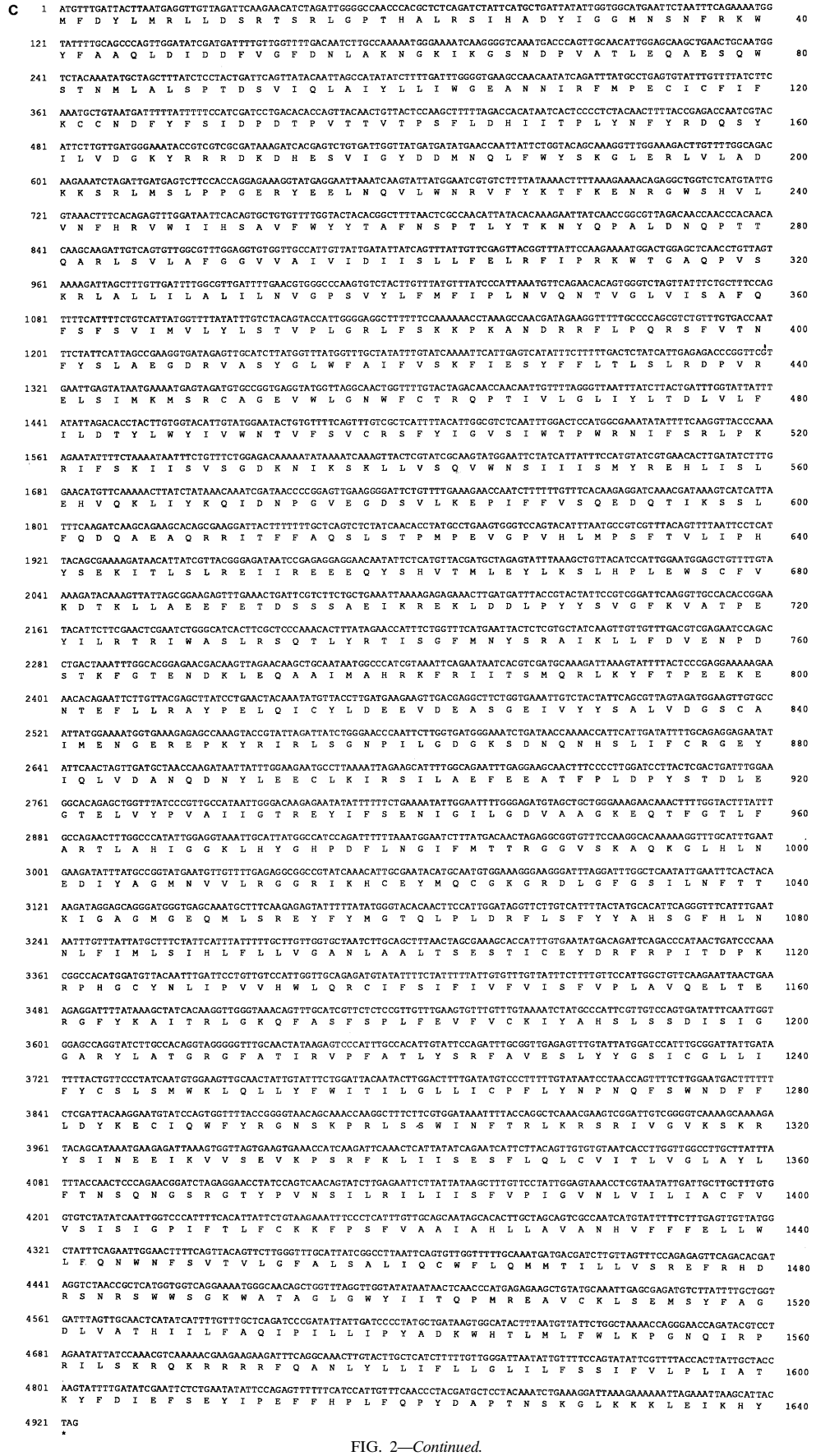


FIG. 2—*Continued.*

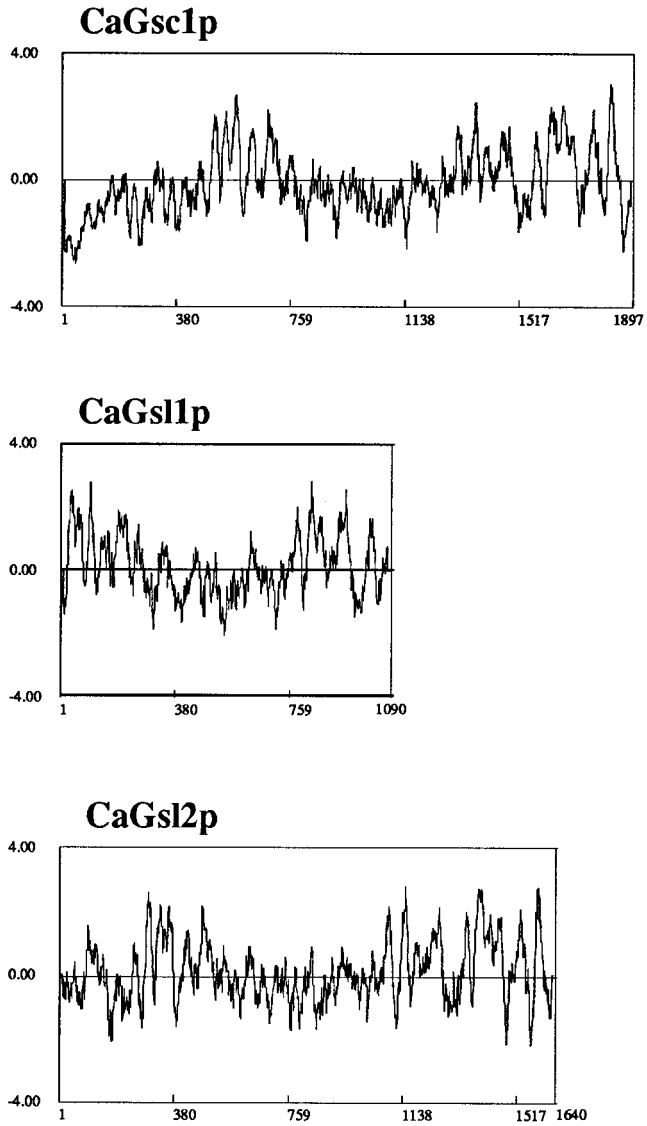


FIG. 3. Hydropathy profiles of *C. albicans* Gsc1p (CaGsc1p), Gsl1p (CaGsl1p), and Gsl2p (CaGsl2p), calculated as described by Kyte and Doolittle (19). The number of amino acids is plotted on the *x* axis, and hydropathy is plotted on the *y* axis.

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 β-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* genomic DNA library was again screened by using the *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 *Sal*I-*Eco*RI fragment of *C. albicans GSC1* as a probe (indicated as probe 2 of *C. albicans GSC1* in Fig. 1). This *Sal*I-*Eco*RI 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).

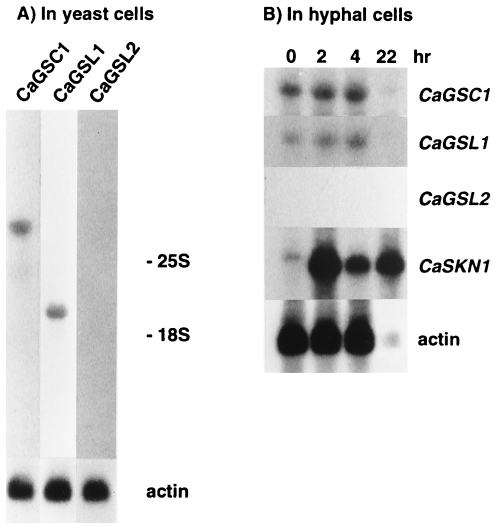
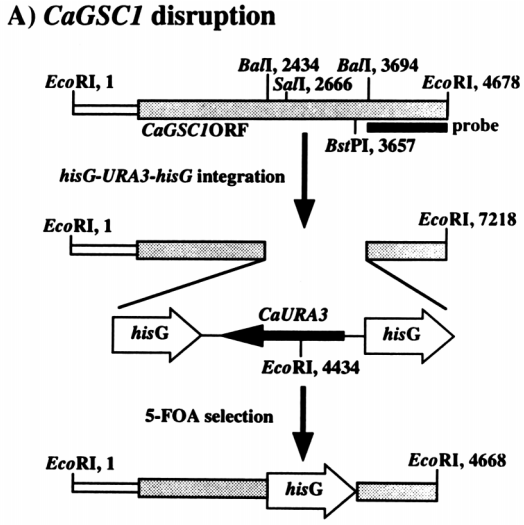


FIG. 4. Northern blotting of *C. albicans GSC1*, *GSL1*, and *GSL2* mRNAs. Poly(A)$^+$ RNA was extracted from cells in yeast phase (A) and hyphal phase (B) and hybridized with *C. albicans GSC1* probe 1 (*CaGSC1*) and *GSL1* (*CaGSL1*), *GSL2* (*CaGSL2*), *SKN1* (*CaSKN1*), and *ACT1* (actin) probes. 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.



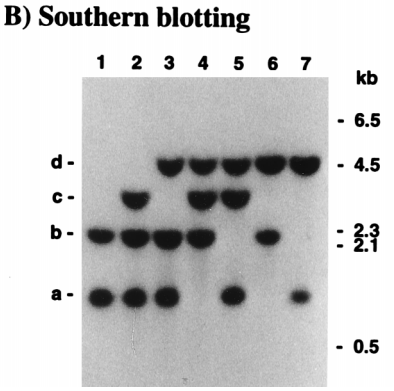


FIG. 5. Disruption of *C. albicans GSC1*. (A) Strategy for the disruption of *C. albicans GSC1* (*CaGSC1*) and the region of the DNA used for the probe. Restriction endonuclease sites together with nucleotide positions numbered from the *Eco*RI site present in the 5′ noncoding region of *GSC1* are indicated. 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*Δ::*hisG-URA3-hisG* allele, and the *gscl*Δ::*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 *Eco*RI, *Sal*I, and *Bst*PI and hybridized with the 1.0-kb *Bal*I-*Eco*RI 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 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.

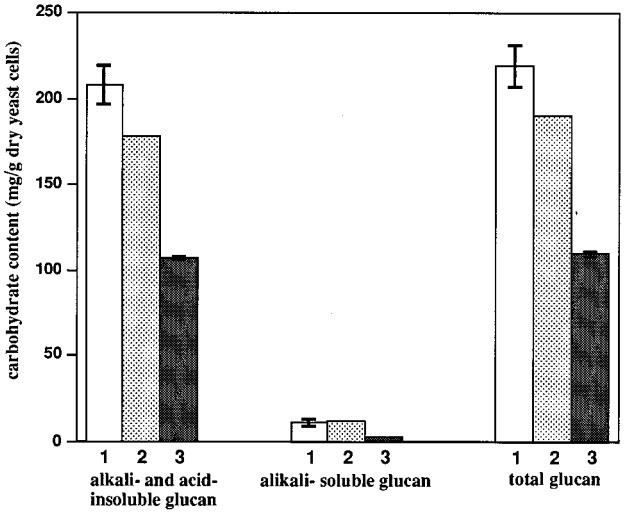


FIG. 6. β-Glucan and *C. albicans GSC1* mRNA levels in wild-type, GSC1(+/+/−), and GSC1(+/−/−) strains. Cell wall β-glucan was extracted from CAI4 (column 1), GSC1(+/+/−) (column 2), and GSC1(+/−/−) (column 3) strains, and the levels of total, alkali-soluble, and alkali- and acid-insoluble β-glucan were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

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 β-glucan by 10 to 20% (Fig. 6). The effect of the disruption of *GSC1* alleles on β-glucan was more significant when two *GSC1* alleles were disrupted; β-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 β-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 β-glucan synthesis in *C. albicans*. It should be also noted that there

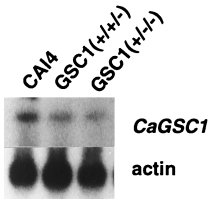


FIG. 7. *C. albicans GSC1* mRNA levels in wild-type, GSC1(+/+/−), and GSC1(+/−/−) strains. Poly(A)$^+$ RNAs extracted from CAI4, GSC1(+/+/−), and GSC1(+/−/−) strains were hybridized with probe 1 of *C. albicans GSC1* (*CaGSC1*) or an *ACT1* probe (actin). The region of DNA used for the *GSC1* probe is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

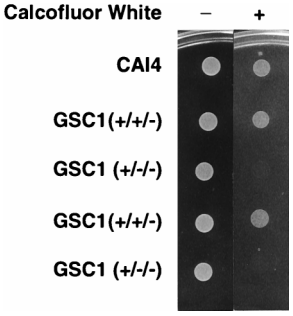


FIG. 8. Calcofluor White sensitivity of strains CAI4, GSC1(+/+/−), and GSC1(+/−/−). Ten thousands cells of the indicated strains were seeded on YPD agar plates in the presence (+) or absence (−) of Calcofluor White (1 mg/ml) and incubated for 24 h at 30°C. Results of two independent colonies from GSC1(+/+/−)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

was no allele-specific effect by *GSC1* disruption on β-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 β-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 β-1,3-glucan 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 β-glucan synthesis in *C. albicans.*

Purification of β-glucan synthase. Since *C. albicans GSC1* is highly homologous to *S. cerevisiae GSC/FKS* and is required for β-glucan synthesis in vivo, it is likely that *GSC1* is the gene for β-1,3-glucan synthase of *C. albicans*. We have addressed this possibility by purifying and determining the amino acid sequences of the enzyme. β-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 β-1,3-glucan synthase fraction as an antigen. One of them, CF2A4, immunoprecipitated β-1,3-glucan synthase activity, and it specifically reacted with p210 by Western blotting (Fig. 9), demonstrating that p210 is a subunit of β-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 β-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 *Bst*PI. 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 β-glucan levels by about 50%, and all amino acid sequences of the peptides derived from p210 that was copurified with β-1,3-glucan synthase activity were found in the deduced amino acid sequence of Gsc1p. Thus, it appears that *GSC1* encodes a subunit of β-1,3-glucan synthase in *C. albicans* and that β-1,3-glucan 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

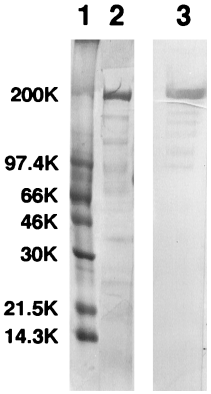


FIG. 9. SDS-polyacrylamide gel electrophoresis of *C. albicans* glucan synthase purified by product entrapment. Lane 1, protein size marker (Rainbow markers, PRN 756; Amersham); lane 2, partially purified β-1,3-glucan synthase after two cycles of product entrapment, separated by SDS–7% polyacrylamide 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.

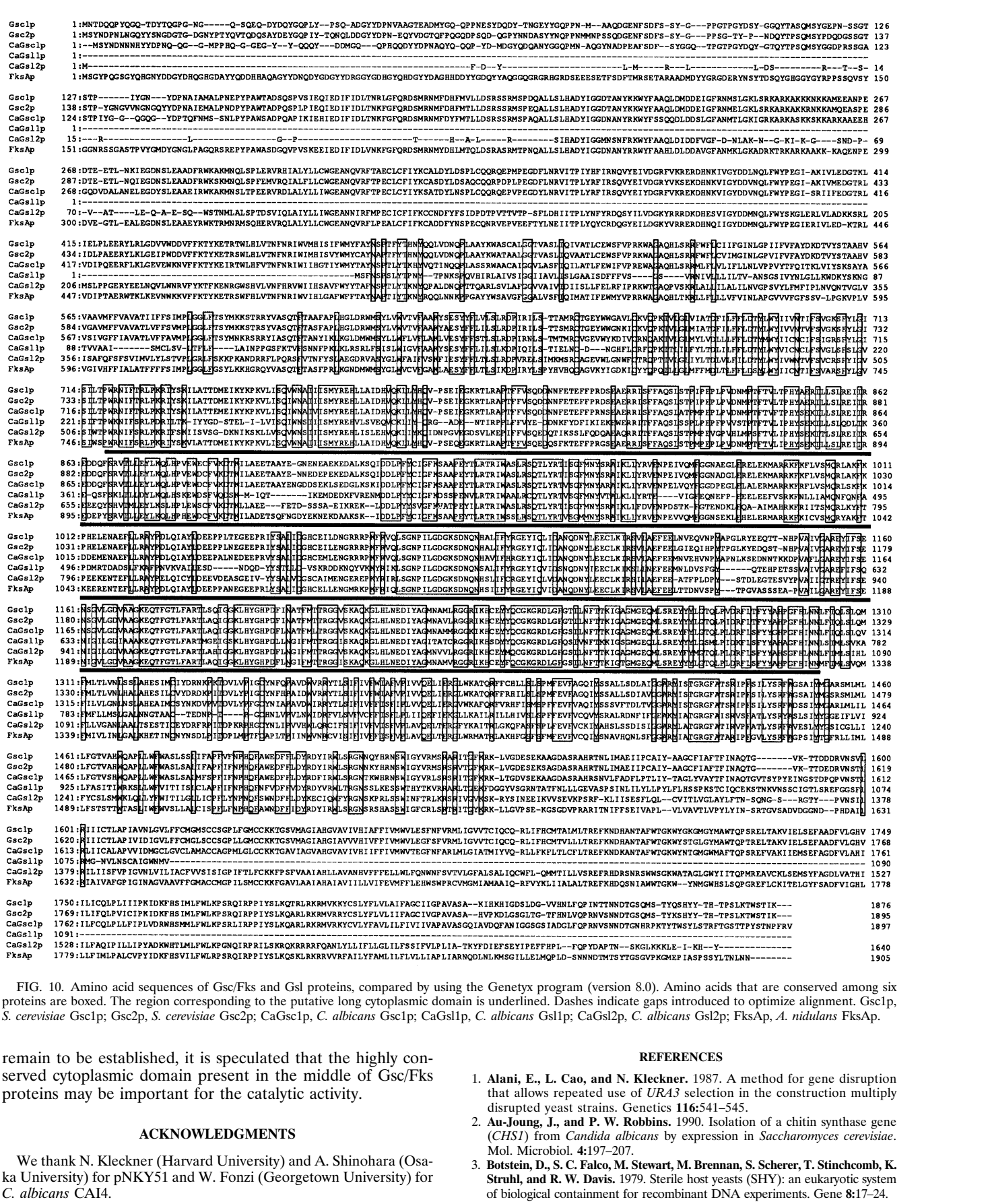


FIG. 10. Amino acid sequences of Gsc/Fks and Gsl proteins, compared by using the Genetyx program (version 8.0). Amino acids that are conserved among six proteins are boxed. The region corresponding to the putative long cytoplasmic domain is underlined. Dashes indicate gaps introduced to optimize alignment. Gsc1p, *S. cerevisiae* Gsc1p; Gsc2p, *S. cerevisiae* Gsc2p; CaGsc1p, *C. albicans* Gsc1p; CaGsl1p, *C. albicans* Gsl1p; CaGsl2p, *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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