Isolation of *canCHS1A*, a variant gene of *Candida albicans* chitin synthase

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A *canCHS1A* gene encoding the chitin synthase of *Candida albicans* was cloned. DNA sequencing and comparison with another *canCHS1* gene described elsewhere indicated that the *canCHS1A* gene encoded a polypeptide with 775 amino acid residues, a protein with one less amino acid than that encoded by the *canCHS1* gene. A six-base alteration was observed between the two genes, suggesting that the *canCHS1A* gene is a variant gene of the *canCHS1*. The pH profile/activity relationship of *canChs1A* in permeabilized cells was identical to that of *canChs1*. The *canChs1A* enzyme was competitively inhibited by polyoxin D (5.2 µM) and nikkomycin Z (12 µM). When the cloned gene was expressed in a *Saccharomyces cerevisiae chs2* mutant that exhibited aberrant morphology, the normal structure was restored. We conclude that the function of the *canCHS1A* gene is similar to that of *sacCHS2* in *S. cerevisiae*.

Keywords: chitin synthase, *Candida albicans*, morphology

INTRODUCTION

Candida albicans is a dimorphic fungus and an opportunistic pathogen that can cause serious infections in humans, particularly in immunocompromised patients. There is a high degree of correlation between its virulence and ability to undergo morphogenesis from yeast to hyphal cell type (Soll, 1992). The hyphal form plays a major role in pathogenicity and is the form involved in tissue invasion. Among the changes observed in the yeast–hyphal transition in *Candida* is a three- to fivefold increase in chitin levels in the cell wall of hyphae (Chattaway *et al.*, 1968; Sullivan *et al.*, 1983). Therefore, it is important to understand the mechanism of the primary septum formation and yeast–hyphal transition in this pathogen.

In the yeast *Saccharomyces cerevisiae*, there are three kinds of chitin synthase (UDP-*N*-acetyl-D-glucosamine:chitin 4-β-*N*-acetylglucosaminyltransferase, EC 2.4.1.16). Each enzyme has a specific function: chitin synthase 1 (*sacChs1*) is a repair enzyme (Cabib *et al.*, 1989), counterbalancing

the activity of the chitinase; chitin synthase 2 (*sacChs2*) participates in the formation of the chitin ring that completes the primary septum at cytokinesis (Bulawa & Osmond, 1990); chitin synthase 3 (*sacChs3*) is responsible for chitin in the ring at bud emergence and in the cell wall (Shaw *et al.*, 1991). Of these, *sacChs2* appears to be the most important chitin synthase because disruption of the yeast *CHS2* gene resulted in the non-viability of the yeast, though this depended on the genetic background of the strain (Bulawa & Osmond, 1990). In aberrant microcolonies, there seems to be no completed cell divisions (Silverman *et al.*, 1988).

The counterparts of these three chitin synthases in *S. cerevisiae* have been cloned from *C. albicans* (*canChs1*, *canChs2* and *canChs3*) (Au-Young & Robbins, 1990; Chen-Wu *et al.*, 1992; Sudoh *et al.*, 1993). Based on their amino acid sequence homologies, *sacChs1* (Bulawa *et al.*, 1986) and *sacChs2* (Silverman, 1989) in *S. cerevisiae* correspond to *canChs2* (Chen-Wu *et al.*, 1992) and *canChs1* (Au-Young & Robbins, 1990) in *C. albicans*, respectively, and *sacChs3*, which is encoded by the gene designated as *CAL1* (Valdivieso *et al.*, 1991) or *CSD2* (Bulawa, 1992), corresponds to *canChs3* (Sudoh *et al.*, 1993). However, there is no evidence that they functionally complement each other. The previously cloned *canCHS1* gene has been isolated as a chitin synthase gene from *C. albicans* by complementation of the *chs1* mutant in *S. cerevisiae* (Au-Young & Robbins, 1990). In this study,

Abbreviations: *canChs1*–3, *Candida albicans* *Chs1*–3; *canCHS1*–3, chitin synthase gene 1–3 of *Candida albicans*; *Chs1*–3, chitin synthase 1–3; *CHS1*–3, chitin synthase gene 1–3; *sacChs1*–3, *Saccharomyces cerevisiae* *Chs1*–3; *sacCHS1*–2, chitin synthase gene 1–2 of *Saccharomyces cerevisiae*; UDP-GlcNAc, UDP-*N*-acetylglucosamine.

The DDBJ accession number for the nucleotide sequence data reported in this paper is D43627.

we isolated the *sacChs2* counterpart from *C. albicans* (*canChs1A*) using the Southern hybridization method under low stringency conditions. We report here the cloning of the *Candida canCHS1A*, which revealed a variant of the *canCHS1* (Au-Young & Robbins, 1990), and show that some enzymic properties of *canChs1A* are similar to those of *sacChs2*. As expected, the expression of the *canCHS1A* gene complements the chitin synthase 2 mutation of *S. cerevisiae*, *chs2*.

METHODS

Strains and media. The *Saccharomyces cerevisiae* strains used in this study were D3C (*MATa ura3*) transformed with pMS1 as the source of *Chs1* (Bulawa *et al.*, 1986) and RRA400-1U (*MATa his3 leu2 trp1 ura3 chs1::URA3 chs3::HIS3*) transformed with YpLCS2 for *Chs2*. *S. cerevisiae* RRA400 (*MATa his3 leu2 trp1 ura3 chs3::HIS3*) was obtained from Motoyama *et al.* (1994). RRA400-1U was constructed by transforming strain RRA400 and *Bam*HI- and *Hind*III-digested linear plasmid YIpCS1U, which contains the *URA3* gene in *sacCHS1*. We confirmed by Southern blotting that *S. cerevisiae* 400-1U harboured only the *sacCHS2* gene and that *sacCHS1* was disrupted by the *URA3* gene (data not shown).

Yeast strains were grown in YPD [1%, w/v, Bacto yeast extract (Difco), 2%, w/v, peptone (Difco), 2%, w/v, glucose], YND [2%, w/v, glucose, 0.7% yeast nitrogen base without amino acids (Difco), nutritional requirements of amino acids], YPGal (composition as YPD except that 2%, w/v, galactose replaced glucose) or YNGal (as YND except that 2%, w/v, galactose replaced glucose). Transformation was achieved by the electroporation technique of either Delome (1989) or Becker & Guarente (1990). *C. albicans* IFO1060 was used for the manipulation of the genomic DNA library (Sudoh *et al.*, 1993). *Escherichia coli* JM109 was used for the propagation of plasmids. *E. coli* was grown in LB medium, and 50 µg ampicillin ml⁻¹ (Wako) was added where necessary. Solid medium plates contained 2% (w/v) Bacto agar (Difco).

Plasmid constructions. The 3.4 kb *Sma*I fragment of the *CHS1* gene from pMS1 (Bulawa *et al.*, 1986) was blunt-ended and ligated with *Hinc*II-digested pUC119 to yield pUCS1. The 1.0 kb *Hind*III fragment of *URA3* from pMS1 was blunt-ended and ligated with *Nco*I-digested pUCS1 to construct YIpCS1U. A YE-type plasmid, pYPR3831 (Fukuda *et al.*, 1994), which contains the *GAL1* promoter and GAPDH terminator, was digested with *Eco*RI, self-ligated to exclude a coding sequence and digested with *Bam*HI and *Hind*III. The resulting transcription unit was cloned to pUC18 to yield pUCGAL.

A synthetic *Hind*III linker was ligated into the *Sma*I-digested pUCGAL vector to yield pUCGALH. To construct YpLX, we digested pUCGALH with *Hind*III, and a 1 kb fragment was ligated to *Hind*III-digested YEp351'. PCR amplification was performed with the Perkin-Elmer/Cetus DNA thermal cycler and GeneAmp kit (Takara). The 5'GCGGCGTCTAGAATG-CACAACATTAACAATGG3' and 5'GCGGCGTCTAGAC-TAATTTAATGGATTGTG3' primers were used for the amplification of *canCHS1A*; the 5'GACTCTAGAATGACG-AGAAACCCG3' and 5'GATGCGGCATCTAGATTAGCC-CTTTTGTGGAA3' primers were used for the amplification of *sacCHS2*. Thirty-five cycles consisting of a 94 °C 1 min melting step, a 55 °C 1 min annealing step, and a 72 °C 2 min extension were run. These PCR products amplified for *canCHS1A* and *sacCHS2* were digested with *Xba*I, and the *Xba*I site of pYEURA3 (Clontech) was inserted to construct pYEUCA1 and pYEUCS2, respectively. To construct

YpLCA1, the former PCR product was digested with *Xba*I and ligated to the *Xba*I-digested YpLX plasmid.

Southern blot analysis. The total DNA content of *C. albicans* was extracted as described previously by Sudoh *et al.* (1993). DNA was separated by electrophoresis on an agarose gel and transferred onto Hybond-N (Amersham). Hybridization to a ³²P-labelled random-primed probe was done with a rapid hybridization buffer (Amersham) at 56 °C under low stringency conditions or at 65 °C under high stringency conditions.

DNA sequencing. DNA sequencing was done by the method of Sanger *et al.* (1977). The pUC18 or pUC19 derivatives were constructed with a deletion kit (Takara), or by a restriction enzyme manipulation. The United States Biochemical sequencing kit or the AutoCycle sequencing kit in combination with the ALF DNA sequencer was used for the reaction (Pharmacia).

Assay of chitin synthases. When the *GAL1* promoter was to be induced, cells were cultured in 5 ml YND medium for 2 d. Culture (5 ml) was inoculated into 100 ml YND medium and cultured to OD₆₆₀ ~ 1.0. The cells were collected by centrifugation, washed with YNGal medium, resuspended in 100 ml YNGal medium, and cultured overnight. Cells (1 × 10⁸ ml⁻¹) were treated with digitonin solution containing 0.5% digitonin and 20 mM Tris/HCl, pH 7.5, for 30 min at 30 °C. An appropriate trypsin concentration (20 µg ml⁻¹; Sigma T-8253, type III) showing maximal chitin synthase activity was added to the permeabilized cell preparation. The optimal amount of trypsin depended on the manufacturer's lot. After a 15 min digestion at 30 °C, soybean trypsin inhibitor (1.5-fold that of the trypsin concentration) was added to each reaction tube. A 30 µl portion of the cell suspension was added to 20 µl of the assay mixture. Assay conditions were as described by Sburlati & Cabib (1986) for *sacChs1* and *sacChs2* and as described by Au-Young & Robbins (1990) for *canChs1* and *canChs1A*. For determination of the pH profiles, 50 mM Tris-maleate buffer in the range pH 5.5–8.5 was used as reported by Au-Young & Robbins (1990). A portion of 10⁶ permeabilized cells was equivalent to 13 µg protein.

Staining with Calcofluor. Calcofluor white MR2 (fluorescent brightener 28; Sigma F-6259) was used. Cells were mixed with an equal volume of 100 µg Calcofluor ml⁻¹ and held at room temperature for 1 h. Cells were examined with an Olympus microscope equipped with epi-illumination and a 100 W mercury lamp.

Protein determination. Protein was measured using the Bio-Rad DC protein assay kit with BSA as a standard.

RESULTS

Isolation and DNA sequencing of *canCHS1A*

When the probe with the ORF of the *S. cerevisiae CHS* gene was used under low stringency conditions, the Southern blots confirmed that a *S. cerevisiae CHS2* gene (*sacCHS2*) homologue was present in the *C. albicans* genomic DNA. Through the hybridization, 10 kb *Bgl*II, 4 kb *Eco*RI, 23 kb *Sac*I, and 20 kb and 3.5 kb *Xba*I fragments were observed at 56 °C (Fig. 1). None of these signals were observed at 65 °C. For the isolation of the *sacCHS2* counterpart from *C. albicans*, a *Bgl*II-digested *C. albicans* genomic DNA library was constructed and screened with the use of the *sacCHS2* gene under the same conditions used for the Southern hybridization. Four clones, isolated from the library, gave identical restriction

Fig. 1. Southern hybridization analysis of genomic DNA with *sacCHS2* as probe. A Southern blot of total DNA from *C. albicans* IFO1060, digested with *Bgl*II (lane 1), *Eco*RI (lane 2), *Sac*I (lane 3), or *Xba*I (lane 4), was hybridized at 56 °C with the 4 kb *Hind*III-*Xba*I-digested *sacCHS2* probe (Silverman *et al.*, 1988), which encodes the ORF, as described in Methods. The positions and sizes of the *Hind*III-digested lambda DNA markers are shown.

Fig. 2. pH-activity profile of the *C. albicans* chitin synthase activity expressed in *S. cerevisiae*. After trypsin treatment, digitonin-permeabilized cells (10^6 cells, equivalent to 13 µg protein) were assayed at pH values between 5.5–8.5 with 50 mM Tris-maleate buffer (Au-Young & Robbins, 1990) containing 5 mM magnesium acetate or 2.5 mM cobalt acetate. Digitonin-permeabilized cells are from *S. cerevisiae* RRA400-1U transformed with YpLCA1 and grown in galactose medium; 1 nmol UDP-*N*-acetyl[3 H]glucosamine is equal to 1600 d.p.m.

Table 1. Comparison of the ORFs of *canCHS1* and *canCHS1A*

Nucleotides are numbered from the first methionine codon of *canCHS1* (Au-Young & Robbins, 1990) as number 1. The *canCHS1* encodes a polypeptide with 776 amino acid residues.

<i>canCHS1</i> (2328 nt)	<i>canCHS1A</i> (2325 nt)
C at 1324 (Leu ⁴⁴²)	T at 1324 (Leu ⁴⁴²)
GAA from 1333 to 1335 (Glu ⁴⁴⁵)	Deletion
G at 1503 (Leu ⁵⁰¹)	A at 1500 (Leu ⁵⁰⁰)
C at 2304 (Tyr ⁷⁶⁸)	T at 2301 (Tyr ⁷⁶⁷)

enzyme maps. Although their physical maps were nearly similar to the *canCHS1* gene reported earlier (Au-Young & Robbins, 1990), the internal partial sequence of our clone was different from that of *canCHS1* (data not shown). Thus, one of the inserts, pCACS1, was sequenced. A single, continuous ORF of 2325 bases for a predicted protein of 88151 Da was found in the 2740 bp sequence. The nucleotide sequence has no introns in the ORF. A comparison of the nucleotide sequence of the cloned DNA fragment with the *canCHS1* gene revealed that GAA, which would encode glutamic acid at positions 1333–1335 in the *canCHS1* gene, was missing in our clone. Other changes included substitutions of T, A and T in place of C (position 1324), G (1503) and C (2304) of the *canCHS1* gene, respectively, although none of these nucleotide alterations gave rise to any amino acid substitutions in the protein specified by this gene (Table 1). Within the *canCHS1A* DNA sequence confirmed, no alteration was found in 297 bp upstream from the first ATG and 117 bp downstream from the stop codon TAG.

Since the *canCHS1A* encoded a polypeptide of 775 amino acids, the protein sequence identity was 99 %. Therefore, *canCHS1A* is probably a variant gene of *canCHS1*.

To examine whether another *canCHS1* gene is present in this strain, we carried out PCR analysis and focused on the Glu⁴⁴⁵ position. The specific primers needed for amplifying the regions from nucleotides 1089 to 1694 containing the Glu⁴⁴⁵ codon were designed. The sequences of five clones derived from the PCR product were all that of *canCHS1A* (data not shown).

Characterization of canChs1A

We expressed the *canCHS1A* gene in *S. cerevisiae* cells by using the *GAL1* promoter to examine whether or not the protein specified by this gene was chitin synthase. Since chitin is synthesized at a rapid rate in digitonin-permeabilized cells supplied with UDP-*N*-acetylglucosamine (UDP-GlcNAc) (Fernandez *et al.*, 1982), these were used in our assays. Chitin synthase activity of RRA400-1U under these assay conditions was at background level and, therefore, the endogenous Chs2 levels were negligible. When YpLCA1, a plasmid carrying the *canCHS1A* gene whose transcription was under the control of the *GAL1* promoter, was introduced into this strain, a significant level of chitin synthase activity was detected, and this activity was completely dependent on galactose. In contrast, these same cells cultured in medium containing glucose showed no activity, compared with those transfected with the vector control (data not shown). The expressed canChs1A was also zymogenic, as are sacChs1 and sacChs2; trypsin treatment of the protein activated the enzyme activity approximately tenfold. The pH-activity profiles of canChs1A expressed in strain RRA400-1U are shown in Fig. 2. The apparent pH optimum of the *C. albicans* chitin synthase activity was 6.8

Fig. 3. Inhibition of *S. cerevisiae* Chs1 (■), Chs2 (○) and canChs1A (●) by polyoxin D (a) and nikkomycin Z (b); 10^6 cells were used.

when Mg^{2+} was present. On the other hand, with Co^{2+} , the optimal activity shifted to acidic pH 6.5. In this case, the activity was reduced to 37% of the total activity. The activity of canChs1A was greater with the Mg^{2+} cation than with the Co^{2+} cation; for *S. cerevisiae* the activity was reversed. Therefore, the pH profile of canChs1A was almost similar to another canChs1 described by Au-Young & Robbins (1990).

We examined the effects of the chitin synthase inhibitors polyoxin D and nikkomycin Z. As a control, *S. cerevisiae* chitin synthase activity was measured in the presence of the cation that yields the greatest activity: Mg^{2+} for sacChs1 and Co^{2+} for sacChs2 (Sburlati & Cabib, 1986). Under these conditions, both polyoxin D and nikkomycin Z were more inhibitory for sacChs1 than for sacChs2 (Fig. 3). Inhibitory patterns of sacChs1 with Mg^{2+} , canChs1A with Mg^{2+} and sacChs2 with Co^{2+} were compared. The *C. albicans* Chs1A had almost the same inhibitory pattern as that of sacChs2 (Fig. 3). sacChs1 was strongly inhibited by polyoxin D and nikkomycin Z, whereas canChs1A and sacChs2 were somewhat less sensitive to these inhibitors. The difference in the responses of these drugs (between 50–100-fold) demonstrates that the drug sensitivity of

canChs1A is similar to that of sacChs2, even though the activities of these enzymes depended on cation requirements.

Kinetic measurements in which the substrate concentration was varied showed competitive inhibition for canChs1A with polyoxin D and with nikkomycin Z (data not shown). Apparent K_i values of polyoxin D and nikkomycin Z for canChs1A were approximately 5.2 μM and 12 μM , respectively. Dixon plot analysis indicated that both compounds inhibited competitively and that their calculated K_i values were closer to those of sacChs2 than to those of sacChs1 (Cabib, 1991).

Complementation of the *S. cerevisiae* *chs2* mutation

To address the *canCHS1A* functions in *S. cerevisiae* *in vivo*, we used a low-copy vector and galactose inducible expression plasmid carrying a *canCHS1A*. The presumed ORF of *canCHS1A* was amplified by PCR using specific primers and cloned into the *Xba*I site of the expression plasmid pYEura3. The resulting pYEUC1 was transformed into *S. cerevisiae* 4992H and 5002H, which were *chs2*-deficient mutants derived from YPH499 and YPH500 whose mating types are *MATa* and α , respectively. For our control, the *sacCHS2* gene was similarly amplified and cloned into pYEura3 to yield pYEUCS2. The plasmid was stably segregated in these strains even when grown in a complete medium. Transformation of 4992H or 5002H cells with pYEUC1 or pYEUCS2 did not alter the morphology of these cells unless they were cultured in galactose-containing medium (Fig. 4a, e). Buds had formed, and a daughter cell had not separated from the mother cell. Calcofluor, which specifically binds to 1,4- β -linked polysaccharides and stains the boundary between mother and daughter cells, did not distinguish the boundary between the mother and daughter cells of the *chs2* mutant (Fig. 4e). After bud emergence, cells with normal morphology lost the ability to form chitin at the budding site (data not shown). These findings show that the *chs2* mutation of *S. cerevisiae* is lacking in both septum formation and cell division as previously reported by Silverman *et al.* (1988). When 4992H cells harbouring pYEUC1 or pYEUCS2 were cultured in the medium containing galactose, both transformants showed normal morphology and underwent regular budding, which was confirmed by Calcofluor staining of the budding site (Fig. 4f), demonstrating that both *sacCHS2* or *canCHS1A* genes were equally capable of rescuing a *chs2* mutation. Further, the functional complementation of the *sacCHS2* gene by the *canCHS1A* gene was totally independent of the mating type because induction of *canCHS1A* gene in 5002H cells whose mating type was α by galactose also resulted in the normal phenotype and budding (Fig. 4c, d).

DISCUSSION

We succeeded in identifying a homologue of *S. cerevisiae* from *C. albicans* by Southern blot analysis under low

Fig. 4. Complementation of cell morphology in strains which express the *canCHS1A* gene. Cells of the *chs2::HIS3* mutant were transformed with pYEUCS2 or pYEUCA1 and grown in glucose or galactose, and then examined with a phase contrast microscope (a–d) or stained with Calcofluor and observed with a fluorescence microscope (e, f). Strain 4992H transformed with pYEUCS2 was grown in glucose (a) and in galactose (b). Strains 4992H and 5002H were transformed with pYEUCA1 and grown in galactose (c, d). Cells of strain 4992H without the expression of the *canCHS1A* gene in glucose medium are shown in (e) and with the expression in galactose medium in (f). Bar, 10 μ m.

stringency conditions. The deduced protein of the gene cloned in this study was compared with another *canCHS1* (Au-Young & Robbins, 1990). There were several base alterations in those genes but minor base alterations did not affect the amino acid sequence. The *canChs1A* protein is missing one amino acid at the Glu⁴⁴⁵ position of nucleotide 1629, but this is unlikely to be a critical region since it is not located in the consensus region of chitin synthases (Bowen *et al.*, 1992). The differences in these sequences may be the result of differences at the strain level. Therefore, the *canCHS1A* gene seems to be a variant gene of *C. albicans* chitin synthase (Au-Young & Robbins, 1990). The PCR analysis was targeted to screen another chitin synthase gene by amplification of the Glu⁴⁴⁵ region. We failed to find any other sequence besides the *canCHS1A* type, suggesting that the *canCHS1* type sequence is not present in the *C. albicans* IFO1060 strain.

In order to characterize the enzymic properties of *canChs1A*, we analysed a yeast strain expressing the

canCHS1A gene. The optimal pH range of *canChs1A* was neutral when the best activating cation (Mg^{2+}) was used, whereas the optimum shifted slightly to an acidic pH when Co^{2+} was used. It is unclear why significant changes in the optimal pH depend on the use of a particular cation in the assay. The pH profiles were similar to those described for *canChs1* (Au-Young & Robbins, 1990). The K_m value for UDP-GlcNAc was 0.4 mM for *canChs1A* with Mg^{2+} (data not shown), in good agreement with the values reported for other chitin synthases; 0.5 mM for *sacChs1* with Mg^{2+} , 0.65 mM for *sacChs2* with Co^{2+} , and 0.28 mM for *sacChs2* with Mg^{2+} (Cabib, 1991).

The drug sensitivity of *canChs1A* was tested against polyoxin D and nikkomycin Z, both of which are potent competitive chitin synthase inhibitors. *sacChs1* was strongly inhibited by both inhibitors, whereas *canChs1A* and *sacChs2* were similarly inhibited, but were 50–100 times less sensitive to both inhibitors. The sensitivity of *sacChs2* in permeabilized cells corresponded to the results

of experiments by Cabib (1991), in which a mixed membrane fraction of chitin synthase was used. This result shows that canChs1A has the same responses and mode of action to these inhibitors. Although both drugs seem to react differently in the active sites of the two enzymes sacChs1 and sacChs2 (Cabib, 1991), it is supposed that the spatial construction of the active site of canChs1 and sacChs2 is similar. It is difficult to explain the difference in metal ion requirements, but it may depend on the environment of the active site. Based on the amino acid sequence homology, the *CHS1* gene of *C. albicans* shows homology to the *CHS2* gene of *S. cerevisiae* (Au-Young & Robbins, 1990). The homology is emphasized by maximal parsimony similarity analysis using full-length chitin synthase polypeptides (Beth Din & Yarden, 1994).

The *canCHS1A* gene product seems to function in the *S. cerevisiae* *chs2* mutant lacking the ability to form septa and to participate in cell division. This is supported by evidence showing normal budding and cell morphology in mutants that had the *canCHS1A* gene expressed when a controllable promoter such as *GAL1* was used. This was also observed by staining with the fluorescent dye Calcofluor, strongly suggesting that the *canCHS1A* gene complements the *chs2* null mutation of *S. cerevisiae*.

In *S. cerevisiae*, Chs2 is thought to be the most important chitin synthase among the three because disruption of the *sacCHS2* gene results in the most severe defect in cell growth. Since *canCHS1A* in *C. albicans* shares high sequence similarity to *sacCHS2*, it is thought to be the counterpart of *sacCHS2*. Since we could demonstrate that the *canCHS1A* gene functionally complements the *sacCHS2* gene, the above hypothesis is strongly supported. The real function of the *canCHS1* gene in *C. albicans* cells still remains to be clarified; however, Gow (1994) has proposed that the homozygous disruption of the *canCHS1* gene might be lethal. This suggests that *sacCHS2* and *canCHS1* have different functions in each organism because the null mutation of the *sacCHS2* gene in *S. cerevisiae* did not result in the lethal phenotype (Bulawa & Osmond, 1990). However, canChs1A also shared a somewhat higher sequence similarity with canChs2 (43% identical) whereas canChs3 is not quite identical to canChs1A (25%). Thus, it would be of interest to examine whether or not *canCHS2* genes also functionally complement the *sacCHS2* gene. However, attempts to express *canCHS2* in *S. cerevisiae* have been unsuccessful to date (Chen-Wu *et al.*, 1992).

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