The *Candida albicans KRE9* gene is required for cell wall β-1,6-glucan synthesis and is essential for growth on glucose

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ABSTRACT   We have isolated *CaKRE9*, a gene from *Candida albicans*, that is a functional homologue of the *Saccharomyces cerevisiae KRE9* gene involved in β-1,6-glucan synthesis. Disruption of the *CaKRE9* gene in *C. albicans* shows that CaKre9p is required for the synthesis or assembly of this fungal polymer. Homozygous null disruptants of *CaKRE9* grow poorly on galactose and fail to form hyphae in serum, and, in growth medium containing glucose, the gene is essential. Thus, the *CaKRE9* gene product is a potentially useful candidate as a target for fungal-specific drugs.

Opportunistic fungal infections have increased dramatically in the last two decades and have become a significant cause of morbidity and mortality. Yeasts in the genus *Candida* are the major group causing mycoses in humans ranging from mucosal infections to systemic candidiasis (1–4). Much of the structure, physiology, and metabolism of fungal and animal cells is highly conserved (5). This conservation in cellular function has made it difficult to find agents that selectively discriminate between pathogenic fungi and their human hosts. Moreover, resistance to existing antifungal drugs is becoming a problem of increasing concern (6, 7). Thus, there is an urgent need for specific new targets as a starting point to obtain new antifungal drugs. One focus of attention for such new targets is the fungal extracellular matrix, where the cell wall constitutes an essential, fungal-specific organelle that is absent from human cells. The cell wall of fungi is essential not only in maintaining the osmotic integrity of the fungal cell but also in cell growth, division, and morphogenesis (8, 9). The yeast cell wall is composed of highly mannosylated glycoproteins, chitin, and two homopolymers of glucose, β-1,3-glucan, and β-1,6-glucan (8, 10). The β-1,6-glucan polymer exists as a posttranslational modification of cell wall glycoproteins and is essential for fungal cell viability (8–10). It acts as an adhesive, covalently linking glucosylated glycoproteins with the cell wall β-1,3-glucan and chitin polymers in a crosslinked extracellular matrix (11). Cell wall β-1,6-glucan is widespread among fungi occurring in both Ascomycete and Basidiomycete species (12–14). In the dimorphic yeast *Candida albicans*, β-1,6-glucan is particularly abundant, comprising approximately half of the alkali-insoluble glucan (15, 16) compared with a level of ≈20% found in the yeast *Saccharomyces cerevisiae*, which has served as the principal organism for studies of this polymer (8–10).

Although the biochemistry of β-1,6-glucosylation is limited, genetic analysis of β-1,6-glucan synthesis has identified many genes required for the process in *S. cerevisiae*. These genes encode products acting in the endoplasmic reticulum, in the Golgi complex, and at the cell surface (see ref. 10 for a recent review). In the course of this work, the *KRE9* gene was identified, and its product was shown to be required for

β-1,6-glucan synthesis. *KRE9* appears to be a fungal specific gene and encodes a 55-kDa O-glycosylated protein that is found in the extracellular medium when overproduced and is thought to be localized normally to the cell surface, where it participates in glucan synthesis or assembly (17). Disruption of *KRE9* in *S. cerevisiae* leads to serious growth impairment and an altered cell wall containing <20% of the wild-type amount of β-1,6-glucan. The *S. cerevisiae* genome contains a second gene product, Knh1p, highly similar to Kre9p. Knh1p shares 46% identity with Kre9p and appears to be a functional homolog of Kre9p, as overexpression of *KNH1* partially suppressed the severe growth defect of a *kre9* null mutant and the level of alkali-insoluble cell wall β-1,6-glucan (18). When overproduced, Knh1p, like Kre9p, can be found in the extracellular culture medium as an O-glycoprotein and is likely also a cell surface protein under conditions of normal expression. The disruption of both the *KNH1* and *KRE9* genes results in lethality in *S. cerevisiae*. Transcription of *KNH1* is carbon-source and *KRE9* dependent. These results suggest that the *KRE9* and *KNH1* genes are specialized *in vivo* to function under different environmental conditions (18). The essential nature of the *KRE9/KNH1* gene pair and the putative extracellular location of their gene products make these proteins attractive fungal specific targets, a possibility that we have explored further by searching for *KRE9* homologue(s) in *C. albicans*.

MATERIALS AND METHODS

Yeast Strains, Culture Conditions, and Procedures. The different *S. cerevisiae* (SEY6210 background) and *C. albicans* (CAI4 background) strains were grown under standard conditions (yeast extract/peptone/dextrose and yeast nitrogen base/casamino acids). All Ura⁻ *C. albicans* strains were cultured in presence of uridine (50 μg/ml). Glucose growth studies were performed in yeast nitrogen base medium supplemented with glucose at concentrations of 1%, 0.75%, 0.5%, 0.25%, 0.2%, 0.15%, 0.1%, or 0.05%. The ability of *C. albicans* strains to grow on different carbon sources was assessed on solid yeast extract/peptone media supplemented with galactose, sucrose, mannose, maltose, fructose, sorbitol, or glycerol at a final concentration of 2%. When cells were scored for growth in the presence of glucose plus another carbon source, the final glucose concentration was 1%. Hyphal growth of different *C. albicans* strains was induced in presence of galactose and 20% fetal bovine serum at 37°C for up to 18 hr as described (27).

Isolation of the CaKRE9 Gene. A *S. cerevisiae kre9::HIS3* haploid strain containing a wild-type copy of the *S. cerevisiae KRE9* gene (17) on a centromeric *LYS2*-based pRS317 vector was transformed with a *C. albicans* genomic library. This library was contained within the multicopy YEp352-plasmid

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with the *URA3* gene as a selectable marker (19). To screen for plasmids that could restore growth to a *kre9*::*HIS3* mutant, ≈20,000 His⁺ Lys²⁺ Ura³⁺ cells were replica-plated on minimal medium containing α-aminoadipate as a primary nitrogen source to select for growing cells that had lost the *LYS2* plasmid-based copy of *KRE9* and that might possess a copy of the complementing *CaKRE9* gene. These cells were tested further for loss of the pRS317-*KRE9* plasmid by their failure to grow on medium lacking lysine. YEp352-based *C. albicans* genomic DNA was recovered from cells that required lysine for growth. On retransformation in yeast, a specific genomic insert of 8 kb was able to restore partially growth of the *kre9*::*HIS3* haploid strain. By using an Applied Biosystems 373A DNA sequencer, sequencing of this insert was initiated from both ends and was pursued until the *KRE9* ORF was encountered, some 1,436 bp from the 5′ end of the complementing insert. The *KRE9* region then was sequenced on both strands. A 1.6-kb *Dra*I DNA fragment containing the complete *CaKRE9* gene was subcloned from the original insert into the *Sma*I site and the filled *Xba*I site (treated with the Klenow fragment of DNA polymerase I) of YEp352 (see Fig. 3*a*) and was used in complementation studies (see Fig. 2).

Disruption of the CaKRE9 Gene. Gene disruption was performed by the *URA* blaster protocol by using the *hisG-CaURA3-hisG* module (20, 21). The *CaKRE9* gene was disrupted by deleting a 485-bp *Bst*XI-*Bam*HI fragment of the ORF and replacing it with a 4.0-kb *Bgl*II/*Bam*HI fragment carrying the *hisG-URA3-hisG* module from plasmid pCUB-6 (see Fig. 3*a*) (21). The sticky ends were treated with T4 DNA polymerase to accommodate the ligation. This disruption plasmid was digested by *Hin*dIII and *Kpn*I, precipitated with ethanol and sodium acetate, and 100 μg of the 5.2-kb disruption fragment was transformed into CAI4 *C. albicans* cells (21) by the lithium acetate method (22, 23). Putative heterozygous disruptants were selected on minimal medium lacking uracil and containing either glucose or galactose as a carbon source. In preparation for a second round of gene disruption, the *CaURA* gene was excised by using a fluoroorotic acid selection (24). The second round of transformation was performed in the same way as the primary one. The accurate integration of the *hisG-URA3-hisG* cassette into the *CaKRE9* gene and its excision from genomic DNA was verified by Southern hybridization by using three different probes: (*i*) a 405-bp fragment from *C. albicans* genomic DNA containing coding and 3′-flanking sequences of *CaKRE9*; (*ii*) a 783-bp DNA fragment obtained by PCR and covering the entire *CaURA3* coding region; and (*iii*) a 898-bp fragment amplified by PCR that encompasses the whole of the *Salmonella typhimurium hisG* gene (see Fig. 3). All genomic DNAs were digested with the *Bam*HI and *Sal*I restriction enzymes.

β-1,6-Glucan Analysis of *C. albicans* CaKRE9 Mutants. A specific rabbit anti-β-1,6-glucan antiserum was raised against BSA-coupled pustulan (25) and was used to detect antigen–antibody complexes by Western blotting of total cell protein extracts of different yeast strains as described below. Before use, the antiserum was affinity purified via chromatography by using pustulan-Sepharose 6B (Pharmacia), prepared as follows: pustulan was hydrolyzed (17 mg/ml in 0.1M NaOH, 1 h, 100°C) and subsequently was coupled to epoxy-activated Sepharose 6B as per the manufacturer's instructions. For immunodetection of β-1,6-glucan, the following procedure was used. Exponentially growing cells were lysed by using glass beads and were standardized for total cellular protein. Subsequently, total cell lysate material representing equivalent amounts of protein were alkali extracted (0.75M NaOH, 1 h, 75°C). The alkali-soluble fractions then were spotted onto nitrocellulose, and immunoblots were treated in TBST buffer (10 mM Tris, pH 8.0/150 mM NaCl/0.05% Tween 20) containing 5% nonfat dried milk powder and subsequently were incubated with affinity purified rabbit anti-β-1,6-glucan anti-

bodies in the same buffer. After antibody binding, membranes were washed in TBST, and a second antibody directed against rabbit Igs and conjugated with horseradish peroxidase was added. The blots again were washed, and whole cell alkali-soluble β-1,6-glucan was detected by using an enhanced chemiluminescence procedure. For cell wall analysis, the levels of cell wall alkali-insoluble β-1,3- and β-1,6-glucans were determined directly as described (18).

RESULTS

Isolation and Characterization of the CaKRE9 Gene. Using complementation of the *S. cerevisiae kre9* mutant growth phenotype, we isolated a *C. albicans* gene that encodes a protein highly similar to the *S. cerevisiae KRE9* gene product. Partial sequencing of the 8-kb fragment containing the *CaKRE9* gene revealed an ORF of 813 bp (for details, see *Materials and Methods*) encoding a predicted 29-kDa secretory protein of 271 amino acid residues. As with Kre9p and Knh1p (17, 18), the hydrophobic N-terminal region of CaKre9p is a eukaryotic signal sequence, with the most likely cleavage site occurring at the peptide bond between amino acid residues 21 and 22. CaKre9p shares 43% overall amino acid identity with Kre9p and 32% with Knh1p (see Fig. 1). The most conserved region between the three proteins encompasses a large part of the central region and most of the C-terminal portion, with the N-terminal portion being largely unique to each protein. Kre9p, Knh1p, and CaKre9p share a high proportion of serine and threonine residues (26%), potential sites for O-glycosylation, a modification known to occur on Kre9p and Knh1p and characteristic of many yeast cell surface proteins. In addition, all three proteins have lysine and arginine rich C termini and lack potential *N*-linked glycosylation sites. The hydropathy profiles of all three proteins are similar (data not shown), which implies that they possess a similar secondary structure and which is consistent with their sharing related functions in complementing *S. cerevisiae kre9* mutants (ref. 18 and see below).

The ability of CaKre9p to function in *S. cerevisiae* was assessed by measuring its ability to restore the growth and killer toxin sensitivity of a *kre9* null mutant (17). The YEp352-based cloned *CaKRE9* gene from *C. albicans* genomic DNA
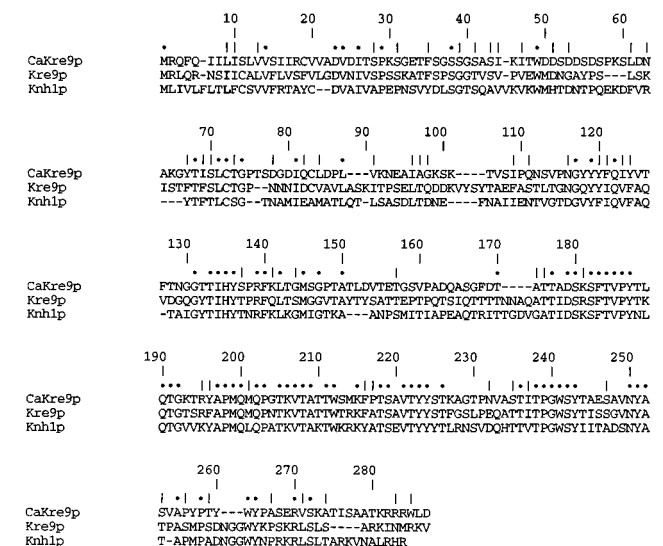

FIG. 1. Comparison of the sequence of Kre9p from *C. albicans* and Kre9p and Knh1p from *S. cerevisiae*. The amino acid residues are shown in single-letter amino acid code. Sequences were aligned with gaps to maximize homology. Dots represent a perfect match between all three sequences whereas a vertical slash indicates a conservative substitution.

was transformed into a diploid strain of *S. cerevisiae* heterozygous for a *kre9::HIS3* deletion. This diploid was sporulated, and a haploid *kre9::HIS3* strain containing a plasmid-based copy of *CaKRE9* was obtained from spore progeny after tetrad dissection. As can be seen in Fig. 2*a*, a strain harboring the *CaKRE9* gene grows at a slower rate than a wild-type strain or the mutant strain with a copy of the *S. cerevisiae KRE9* but significantly faster than the *kre9* null mutant alone, which displays a severe impairment of growth phenotype. The haploid *S. cerevisiae kre9* strain carrying the *CaKRE9* also was tested for killer toxin sensitivity (Fig. 2*b*). K1 killer yeast strains secrete a small pore-forming toxin that requires an intact cell wall receptor for function (26). *ScKRE9* null mutants show an 80% reduction in the level of β-1,6-glucan, rendering the toxin receptor inactive and leading to killer resistance (17). The killer resistance phenotype of the *kre9* mutant allowed a test of suppression by CaKre9p. Overexpression of *CaKRE9* in the *S. cerevisiae* haploid strain carrying a disrupted copy of *ScKRE9* partially suppressed the killer resistance phenotype (Fig. 2*b*). These results imply that ScKre9p and CaKre9p both play related roles in β-1,6-glucan assembly in *S. cerevisiae* and *C. albicans*.

Chromosomal Disruption of the CaKRE9 Gene. In a first round of transformation where transformants were selected on glucose containing plates, Southern blotting results from transformant's DNA revealed that the *hisG-URA3-hisG* module (see *Materials and Methods*) correctly integrated into the *C. albicans KRE9* gene (Fig. 3). When genomic DNA of putative heterozygous *CaKRE9* disruptions was digested with the *Sal*I and *Bam*HI restriction enzymes and was probed with the *CaKRE9* 405-bp *Sal*I–*Bst*XI DNA fragment along with the *hisG* and the *CaURA3* probes, two expected bands could be detected (see Fig. 3*b*, lanes 1 and 2, for representative results):

a 773-bp band corresponding to the wild-type gene that could only be detected by the *CaKRE9* probe and a 4,318-bp diagnostic band, revealed by all three probes, indicating successful disruption of one copy of the *CaKRE9* gene. After removal of the *CaURA3* by using fluoroorotic acid, the 773-bp wild-type band could still be visualized, but the disrupted band from which the *CaURA3* was excised shifted to an anticipated 1,428-bp when probed with the *CaKRE9* and *hisG* probes (see Fig. 3*b*, lane 3).

To test whether the *CaKRE9* gene is essential in *C. albicans*, a second round of disruption was undertaken in the heterozygous strain where one copy of *CaKRE9* was disrupted and the *CaURA3* gene was eliminated. In *S. cerevisiae*, *kre9* mutants grow very poorly on glucose and considerably faster on galactose. This is partly because, in this organism, *KRE9* has a functional homologue, *KNH1*. Transcription of the *KNH1* gene is normally low in wild-type cells grown on glucose but increases ≈5-fold in galactose grown cells, where it partially compensates for the loss of Kre9p and allows partial suppression of the growth defect (18).

To avoid such a possible growth problem in a *C. albicans kre9* disruptant when grown on glucose, the second round transformants were obtained on both galactose and glucose. The population of transformants obtained on galactose was heterogeneous with large and small sized colonies occurring (data not shown). To test for a possible carbon source dependence, 26 of these different sized colonies were plated from galactose to glucose. Among the smaller colonies, eight did not grow on glucose, suggesting they might be homozygous disruptants. Southern blot hybridizations were performed on all eight transformants, and they were shown to be homozygous disruptants for the *CaKRE9* locus: One copy corresponded to the disrupted gene in which the *C. albicans URA3* gene has been
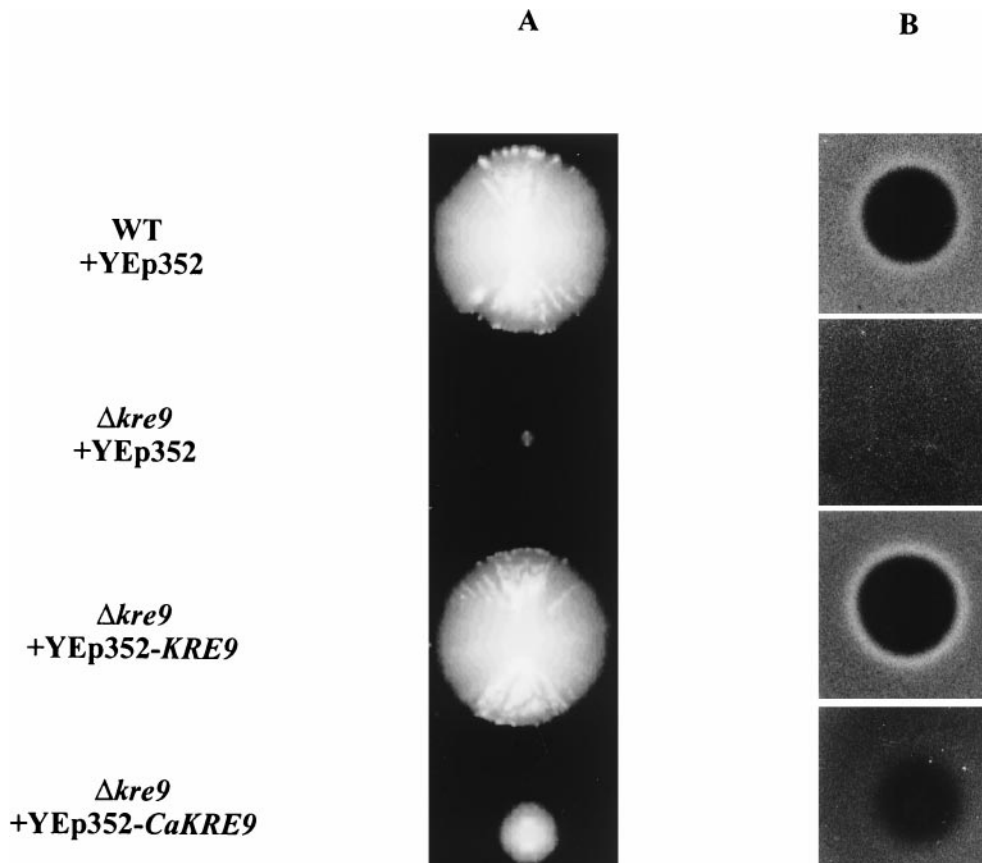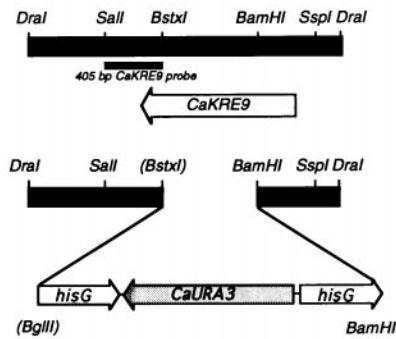
A   **B**

FIG. 2.   Complementation of the growth (*A*) and killer resistance (*B*) phenotypes of the *S. cerevisiae kre9*Δ null mutant by the *C. albicans KRE9* gene. Wild-type cells, a *kre9*Δ mutant, or the same mutant harboring *KRE9* or *CaKRE9* on multicopy vector YEp352 were scored for growth and for killer resistance in a seeded plate assay.

A) Disruption of the *CaKRE9* gene :
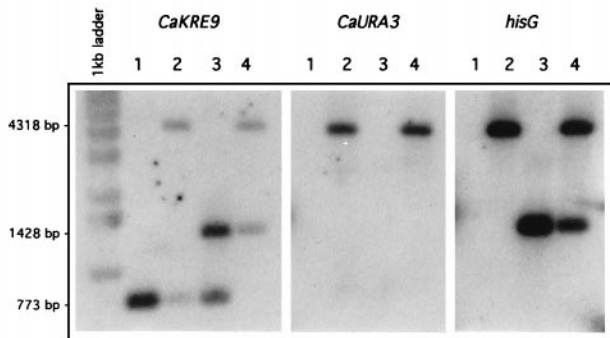

B) Southern results :

FIG. 3. Disruption of *Candida albicans KRE9* gene. (*A*) Schematic representation of the disruption strategy (see *Materials and Methods*). (*B*) Results of the Southern blot verifications of the correct integration of the *hisG-URA3-hisG* disruption module into the *CaKRE9* gene and proper *CaURA3* excision after fluoroorotic acid treatment. Extracted genomic DNAs are from CAI4 wild-type cells (lane 1), *CaKRE9/cakre9∆::hisG-URA3-hisG* heterozygous mutant (lane 2), *CaKRE9/cakre9∆::hisG* heterozygous mutant obtained after fluoroorotic acid treatment (lane 3), and a *cakre9∆::hisG/cakre9∆:hisG-URA3-hisG* homozygous null mutant that was grown on galactose (lane 4).

removed (1,428 bp), and the second represented the inactivation of the remaining wild-type copy by the *hisG-URA3-hisG* module (4,318 bp; see Fig. 3*b*, lane 4 for representative results). Thirty-two Ura$^+$ colonies that were able to grow on glucose also were analyzed by Southern blot hybridization by using the three different probes, and only yeast cells heterozygous at the *CaKRE9* locus could be found (data not shown). Thus, a homozygous disruption of *kre9* in *C. albicans* is lethal when grown on glucose but can grow slowly when galactose is the carbon source.

The ability of the homozygous *cakre9* disruptant to grow on carbon sources other than galactose was assessed. The homozygous *cakre9∆::hisG/cakre9∆::hisG-URA3-hisG* disruptant strain grew at or below the rate of growth on galactose on sucrose, mannose, maltose, fructose, sorbitol, and glycerol as carbon sources (data not shown). To test whether the lethality of the homozygous disruptant on glucose was caused by its inability to sense or use glucose, homozygous disruptant cells were scored for growth in the presence of glucose plus another of the above carbon sources (data not shown). Under such conditions, the homozygous cells could not grow, indicating that the lethality occurs when glucose is present. To extend these results, we examined the response of homozygous *cakre9* disruptants over a range of glucose concentrations.

Compared with wild-type cells, the homozygous mutant failed to grow at glucose concentrations from 1.0% to 0.1%–0.05%, with these lower concentrations approximating the glucose level in human serum (data not shown).

β-1,6-Glucan Analysis of *C. albicans* kre9 Mutants. Whole cell alkali-soluble β-1,6-glucan levels in wild-type and *cakre9* mutants were measured by using an affinity purified anti-β-1,6-glucan antibody (see *Materials and Methods*). Wild-type yeast grown on glucose and galactose yielded a strong β-1,6-glucan signal on either carbon source (see Fig. 4 for results on galactose). Quantitation of alkali-soluble β-1,6-glucan levels revealed that ≈20% of the amount observed in wild-type cells was present in the heterozygous *CaKRE9/cakre9∆* whereas no β-1,6-glucan could be detected in extracts from a *C. albicans* homozygous *cakre9∆* disruptant grown on galactose (Fig. 4). To extend these immunological results, determinations of the levels of cell wall alkali-insoluble glucans were made on the different strains. As seen in Table 1, a *cakre9∆* heterozygous disruptant appears to possess an ≈28% decrease in the level of alkali-insoluble β-1,6-glucan compared with a wild type whereas in homozygous disruptant cells, no β-1,6-glucan could be measured, demonstrating that CaKre9p is required for cell wall β-1,6-glucan biosynthesis.

Hyphal Growth of cakre9∆ Mutants. In *S. cerevisiae*, *kre9* mutants have morphological defects, bud randomly, and fail to form mating projections with α-mating pheromone (17), so the possible role of *CaKRE9* in hyphal formation was investigated. Germ tube formation was induced and hyphae formed when wild-type and heterozygous *cakre9∆* disruptant cells were grown in presence of galactose and fetal bovine serum (27). However, no germ tube formation was observed in the *cakre9∆* homozygous disruptant under the same conditions (Fig. 5).

DISCUSSION

CaKre9p is similar to Kre9p and Knh1p because it is predicted to possess an amino-terminal signal sequence and is rich in serine and threonine residues that could be O-modified (17, 18). Even though the proteins are very similar, the complementation of the growth and toxin sensitivity phenotypes of a
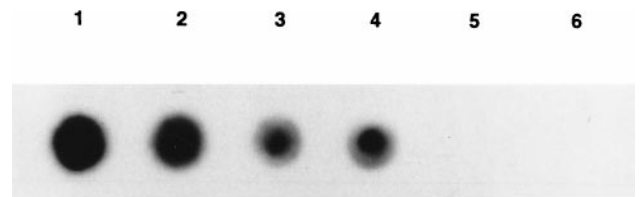
FIG. 4. Detection of cell wall β-1,6-glucan levels in wild-type and *cakre9∆* mutants grown by using galactose as the sole carbon source. A specific anti-β-1,6-glucan antiserum was used to detect whole cell alkali-soluble β-1,6-glucan of different *C. albicans* strains by Western blotting followed by an enhanced chemiluminescence procedure (see *Materials and Methods*).

Table 1. Cell wall polymer levels measured* in different *C. albicans* strains

Genotype	β-1,6-Glucan[†]	β-1,3-Glucan + β-1,6-Glucan[†]
Wild-type	235 ± 15	339 ± 17
Heterozygous *cakre9*Δ	169 ± 19	328 ± 17
Homozygous *cakre9*Δ[‡]	0[§]	377 ± 4
Homozygous *cakre9*Δ[‡]	0[§]	375 ± 21

*Measured as micrograms of alkali-insoluble glucan per milligram dry weight cell wall, as described in *Materials and Methods*.
[†]Values depict mean ±1 SD ($n = 3$).
[‡]These constitute two independent homozygous disruptants.
[§]Values were found to be <1 μg.

S. cerevisiae KRE9 mutant by *CaKRE9* in high copy number was partial. This incomplete complementation may be because the *C. albicans* promoter of *CaKRE9* may not function correctly in *S. cerevisiae*. The isolation in *C. albicans* of a partially functional homologue of the *S. cerevisiae KRE9/KNH1* pair strongly suggests that CaKre9p and Kre9p/Knh1p play similar roles in β-1,6-glucan synthesis or assembly in both species. Comparative studies with *C. albicans* have so far identified and characterized four genes, *KRE1*, *SKN1*, *KRE6*, and *KRE9*, involved in β-1,6-glucosylation based on their relatedness to those in *S. cerevisiae*, indicating that synthesis of this polymer is conserved functionally and is essential for the growth of *C. albicans* (refs. 16 and 19 and this work), indicating that both yeast species likely share similar sets of genes for the production of this glucan.

Results presented here establish that CaKre9p is required for synthesis of β-1,6-glucan in *C. albicans*. When one chromosomal copy is deleted, as found in the heterozygote, the levels of β-1,6-glucan are reduced. The anti-β-1,6-glucan antibody detected ≈20% of the wild-type levels of the alkali-soluble polymer in the heterozygous *cakre9*Δ. An enzymatic digestion procedure that measures β-1,3-glucanase-resistant, alkali-insoluble glucan detected a value of β-1,6-glucan of 70% of the wild-type level in such a heterozygous *cakre9*Δ strain. These differences in the levels of the alkali-soluble and alkali-insoluble fractions of the β-1,6-glucans suggest that the majority of the residual β-1,6-glucans in this heterozygous mutant

is crosslinked to other polymers and is alkali-insoluble. Both evaluation methods, however, indicate that the homozygous disruptant lacks any detectable β-1,6-glucan polymer.

One explanation for the observation that a homozygous disruptant of *CaKRE9* can grow when a carbon source other than glucose is used is that a second functionally homologous gene may be present in the *C. albicans* genome. However, in contrast to the situation observed in *S. cerevisiae*, a homozygous *cakre9*Δ null mutant has no detectable β-1,6-glucan whereas the *kre9*Δ null mutant retains 20% of the wild-type levels of this polymer, an amount thought to be contributed by the homologous *KNH1* gene (18). Thus, if there were another *KRE9/KNH1* homologue in *C. albicans*, we would expect to find a certain amount of cell wall β-1,6-glucan when the homozygous *cakre9*Δ was grown on galactose, yet we do not. Consistent with this, efforts to find a second *C. albicans* gene have so far been unsuccessful. Low stringency Southern blots, PCR approaches using degenerate primers, and a functional complementation approach with galactose as a carbon source have not yielded a second gene. It remains to be determined whether a duplicated copy of *CaKRE9* is present in *C. albicans*, but the complete absence of β-1,6-glucan in the homozygous *cakre9*Δ deletion suggests that this is not likely to be the case.

We offer two kinds of possible explanation for the galactose-specific survival of homozygous *cakre9* null mutants. The first is that some compensatory mechanism exists in galactose-grown cells that does not occur when glucose is present, perhaps through glucose repression. For example, in the absence of glucose, a lack of β-1,6-glucan might be compensated for by an increase in levels of β-1,3-glucan, chitin, or mannoproteins or in altered crosslinking of these polymers. In a contrasting model, glucose-grown cells respond to a wall defect by attempting to compensate in a way that has lethal consequences in a *cakre9* mutant. For example, cells may overproduce chitin or crosslink it to other polymers to such an extent that it prevents growth of the cell wall. Such a lethal compensatory pathway would be glucose-specific and would not occur when cells are grown on galactose.

A number of attributes suggest that the *KRE9* gene of *C. albicans* is a potentially useful antifungal target. *CaKRE9* is involved in a fungal specific process, cell wall β-1,6-glucan synthesis. The gene is not found in the sequenced bacterial (28,
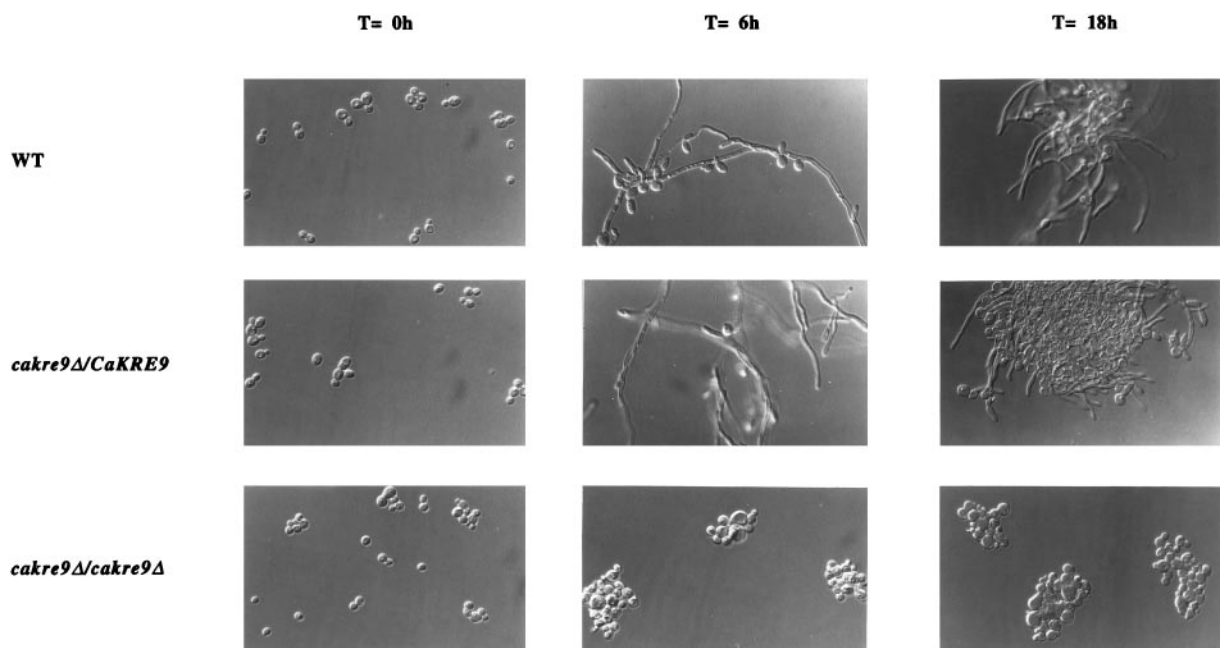

Fig. 5. Induction of hyphal formation of different *C. albicans* strains. CAI4 wild-type, heterozygous mutant, and homozygous mutant cells were induced to form hyphae in presence of galactose and 20% fetal bovine serum at 37°C for up to 18 h. Cells were visualized by Nomarski optics.

29) and archaebacterial genomes (30) and appears to be absent from metazoans, with no known homologues present in the *C. elegans*, mouse, and *Homo sapiens* databases. *CaKRE9* is an essential gene when grown on glucose or when glucose is present at levels comparable to those found in human serum. Thus, a *C. albicans* strain in which CaKre9p would be inactivated/inhibited by a drug should be unable to grow in the presence of glucose. In the event that, in a human host, an alternative carbon source would be present in the absence of glucose, a CaKre9p inhibited *C. albicans* would, based on our laboratory studies, be expected to grow poorly and be unable to form hyphae. This inability of *cakre9* mutants to form germ tubes or hyphae extends the morphological budding and mating projection defects seen in *S. cerevisiae kre9* mutants (17); thus, Kre9p and CaKre9p or the presence of β-1,6-glucan may be necessary for implementing normal, polarized cell wall growth in buds and hyphae.

The exact secretory pathway location of the *KRE9* gene product is not known, but the available data suggest that it functions extracellularly at the cell surface (17, 18). If this is the case, an inhibitor of CaKre9p need not enter the fungal cell to be effective, thus reducing the risk of resistance occurring through reduced uptake or increased efflux of the drug by membrane pumps.

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