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A *Candida albicans RAS*-related gene (*CaRSR1*) is involved in budding, cell morphogenesis and hypha development

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***Candida albicans*, the most important human fungal pathogen, is a dimorphic fungus that can grow either as a yeast or as a hyphal form in response to medium conditions. A *RAS*-related *C. albicans* gene (*CaRSR1*) was isolated as a suppressor of a *cdc24*^{ts} bud-emergence mutation of the baker's yeast, *Saccharomyces cerevisiae*. The deduced protein encoded by *CaRSR1* is 248 amino acids long and 56% identical to that encoded by the *S. cerevisiae RSR1* (*BUD1*) gene. Disruption of *CaRSR1* in *C. albicans* indicated that *CaRSR1* is involved in both yeast and hypha development. In the yeast phase, *CaRSR1* is required for normal (polar) bud site selection and is involved in cell morphogenesis; in the yeast–mycelial transition it is involved in germ tube emergence; and in the development of the hyphae it is involved in cell elongation. The disruption of *CaRSR1* leads to reduced virulence in both heterozygote and homozygote disruptants in a dose-dependent manner. The reduced virulence can be attributed to the reduced germination and shorter hyphae resulting from the disruption of *CaRSR1*.**

Keywords: *C. albicans* morphogenesis, *RSR1*, *BUD1*, GTPase, yeast bud site selection

INTRODUCTION

Candida albicans is the most important fungal pathogen in humans. The organism is diploid and lacks a known sexual cycle. It is dimorphic and grows in a yeast or hyphal form in response to environmental conditions. In response to an external signal, the cells of the yeast phase form a germ tube which subsequently evolves into a hypha. During pathogenesis, this transition may play a role in the progression of the disease state (Cutler, 1991). The pattern of bud formation in the yeast phase is also regulated. The pattern of bud site selection is affected in a distinct way by environmental conditions. As an example, buds emerge primarily at one pole of the mother cell when cells are grown at 23–28 °C and pH 7·4. However, when cells are grown at 37 °C and pH 4·5 the bud sites are not adjacent to previous bud sites and are scattered over the entire cell surface (Chaffin, 1984). Selection of sites for germ tube formation, which seemed random, was suggested to be under different regulation. Cell wall expansion during growth of *C. albicans* is also regulated (Staebell & Soll, 1985). The apical zone accounts for the first two-thirds of surface expansion of bud growth, which is then shut down and followed by a more general pattern of cell wall expansion. During mycelial growth, at least 90% of cell wall expansion is due to growth in the apical zone.

Many of the genes associated with bud site selection and polarized growth in *Saccharomyces cerevisiae* have been identified. They are known to affect specifically either the orientation or the assembly of the polarity axis (for reviews see Mischke & Chant, 1995; Roemer *et al.*, 1996). These include bud site selection genes *RSR1* (also known as *BUD1*) to *BUD9*, and the polarity-establishing genes *CDC24*, *CDC42*, *CDC43* and *BEM1*. The latter group of genes is also required for bud formation and viability. Rsr1 is a Ras-related GTPase required for normal bud site selection in both haploid and diploid *S. cerevisiae* cells. Deletion of *S. cerevisiae RSR1* (*ScRSR1*) causes randomization of bud position (Bender & Pringle, 1989). It has been suggested that in *S. cerevisiae*, the *RSR1* GTPase cycle links spatial bipolar signals to polarity establishment functions (Zheng *et al.*, 1995).

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Abbreviation: 5-FOA, 5-fluoroorotic acid.

The GenBank accession number for the sequence reported in this paper is U46158.

Since the two phases of growth of *C. albicans*, yeast phase and filamentous phase, require polar development, the possibility of a common pathway was intriguing. This study intended to determine whether the yeast and hyphal phases share a common development pathway. The *C. albicans* functional homologues of *S. cerevisiae* polarity establishment genes are the natural candidates for genes involved in polar budding, and therefore are candidates for involvement in filamentous growth. In this study, we intended to isolate either one of the polarity-establishing homologues of *S. cerevisiae* or bud site selection homologues and to test the effect of these genes on yeast and hypha development in *C. albicans*. A *C. albicans* gene was isolated as a suppressor of an *S. cerevisiae* $cdc24^{ts}$ mutation. Based on its nucleotide sequence and motif analysis it was found to be similar to *ScRSR1*. We show that *CaRSR1* is required to restrict the buds of the yeast phase to the cell poles and is involved in cell morphogenesis, germ tube emergence and hypha growth. The results obtained identify *CaRSR1* as a gene involved in both the yeast and hyphal phases. The gene is not essential but disruption of this gene in *C. albicans* affects significantly the virulence of the pathogen.

METHODS

Yeast strains, media and methods. The *S. cerevisiae* strains used were: TD4 (*MAT*a *ura3-52 his4-519 leu2-3,112 trp1 can1*; G. Fink, Whitehead Institute, Cambridge, USA); KGC24-3 (*MATα cdc24-4*^{ts} *ura3-52 his4 leu2-3,112 trp1-284*; Coleman *et al.*, 1986); and DJTD2-16D (*MATα cdc42-1*^{ts} *ura3 his4 leu2 trp1 gal*; Bender & Pringle, 1989). The *C. albicans* strains used are listed in Table 1. YPD, SD and SC media for *S. cerevisiae* (Sherman, 1991) were used for growth of *S. cerevisiae* and *C. albicans*. When including leucine in SD or SC, its concentration was modified to 100 mg l^{-1}. Lee medium (Lee *et al.*, 1975) adjusted to pH 7·2 (1·5 %, w/v, agar for plates) was used for growth and for yeast–hypha transition assays. Serum (foetal calf) Sabouraud medium (Biological Industries) and 3 % Sabouraud medium (Difco; 1:1, v/v) were used for yeast–hypha transition assays (Kwon-Chung & Bennett, 1992). *S. cerevisiae* and *C. albicans* transformations were performed by the LiAc procedure (Ito *et al.*, 1983). Approximately 5 μg DNA was used for each *C. albicans* transformation. SC solid medium devoid of uracil was used for selection of *C. albicans* Ura^+ transformants. Selection of Ura^- *C. albicans* auxotrophs was performed on medium containing 5-fluoroorotic acid (5-FOA) according to Boeke *et al.* (1984), except that uracil was replaced by 25 μg uridine ml^{-1}.

Budding pattern determination. *C. albicans* cells were grown exponentially for at least nine generations in SC-uracil medium with agitation at 30 °C to a final cell density of $1·2–3·7 \times 10^6$ cells ml^{-1}. The cells were washed with deionized water, suspended in 0·1 mg Calcofluor White M2R ml^{-1} (Sigma fluorescent brightener 28) for staining of bud scars (Maeda & Ishida, 1967) and observed by fluorescence microscopy. Cells that had budded at least twice (represented by the total number of bud scars, buds and unseparated daughter cells) were scored for bud site location. Cell poles were defined for this experiment as the two extreme thirds of the cell length. For spherical cells where the cell poles were not easily identified (CAI4-5 strain), the pole was defined as the third of the cell proximal or distal to the mother cell. When a spherical cell was not attached to its mother cell, it was scored only if it had already budded at least three times. Spherical cells that had budded twice and were not attached to a mother cell were not scored. They comprised less than 5 % of the cells that had budded at least twice.

Germ tube formation induction

Lee medium at 37 °C. *C. albicans* cells from SC-uracil plates were spread on Lee medium plates and incubated at 26 °C for 2 d. Cells were suspended in deionized water, and kept on ice while being counted. Lee broth (20 ml) in a 125 ml glass flask was inoculated with cells to an initial density of $2·3–3·8 \times 10^6$ cells ml^{-1}, and agitated in a gyratory water-bath shaker (New Brunswick G76) at 36·5 °C. Each 200 μl sample was supple-

Table 1. *C. albicans* strains

Strain	Parent	Genotype	Source/reference
SC5314			Gillum *et al.* (1984)
CAF2-1	SC5314	*Δura3::imm434/URA3*	Fonzi & Irwin (1993)
CAI4	CAF2-1	*Δura3::imm434/Δura3::imm434*	Fonzi & Irwin (1993)
CAI4-10	CAI4	*RSR1/Δrsr1::hisG–URA3–hisG* *Δura3::imm434/Δura3::imm434*	This work
CAI4-11	CAI4-10	*RSR1/Δrsr1::hisG* *Δura3::imm434/Δura3::imm434*	This work
CAI4-5	CAI4-11	*Δrsr1::hisG/Δrsr1::hisG–URA3–hisG* *Δura3::imm434/Δura3::imm434*	This work
CAI4-5a	CAI4-5	*Δrsr1::hisG/Δrsr1::hisG* *Δura3::imm434/Δura3::imm434*	This work
CALY5a1	CAI4-5a	*Δrsr1::hisG/Δrsr1::hisG* *LEU2::RSR1 URA3 CaARS/LEU2* *Δura3::imm434/Δura3::imm434*	This work
CALY5a20	CAI4-5a	*Δrsr1::hisG/Δrsr1::hisG* *LEU2::URA3 CaARS/LEU2* *Δura3::imm434/Δura3::imm434*	This work

mented with 20 µl SDS/formalin fixative (Odds *et al.*, 1985). Samples were kept for subsequent microscopic observation. At least 200 yeast cells from each sample were counted, monitoring the ratio of yeast cells having germ tube(s)/hyphae.

Serum medium. Cells were grown overnight in YPD at 30 °C, then inoculated into test tubes containing serum and Sabouraud medium (1:1) and incubated without shaking at 37 °C. Cells were sampled for 85 min after inoculation and fixed with SDS/formalin. At least 450 cells of each strain were examined microscopically to detect germ tube formation.

Virulence tests. To test the importance of *CaRSR1* in virulence, immunocompetent and neutropenic 4-week-old male mice (Harlan–Sprague–Dawley) were used. The mice were caged and fed according to National Institutes of Health guidelines for ethical treatment of animals. The *C. albicans* strains tested were grown in YPD medium overnight at 37 °C, washed twice in saline and resuspended to a concentration of 10^7 cells ml^{-1} as determined by cell count. Infection was performed via the lateral tail vein. Immunocompetent mice were infected with 10^6 cells per mouse. Cyclophosphamide (150 mg kg^{-1}) was used to induce neutropenia by an intraperitoneal administration of the drug 1 d prior to the infection and 3 d post-infection. The neutropenic mice were infected with 10^4 cells per mouse.

Nucleic acid methods. Standard recombinant DNA techniques (Sambrook *et al.*, 1989) were followed. Plasmid DNA was extracted using the Qiagen plasmid purification kit. A Sequenase 2.0 kit was purchased from United States Biochemical and used according to the manufacturer's directions for chain-termination DNA sequencing (Sanger *et al.*, 1977). Oligonucleotides were synthesized by BioTechnology General. Analysis of *CaRSR1* DNA and deduced protein sequences was assisted by either DNA Strider (Marck, 1988) or the University of Wisconsin Genetics Computer Group software package (Devereux *et al.*, 1984). For Southern blot analysis, *C. albicans* strains were grown in YPD and genomic DNA was prepared according to Hoffman & Winston (1987). Digested DNA was blotted onto Hybond-N+ membrane (Amersham) and hybridized with a digoxigenin-dUTP (Boehringer Mannheim)-labelled DNA probe and detected by chemiluminescence (CSPD; Boehringer Mannheim). *Escherichia coli* DH5α was used as a host for amplification of plasmids. Strain NS2626 (*dam13*::Tn*9*; Sternberg *et al.*, 1986) was used for amplification of pLY006.

Construction of the disruption vector for *C. albicans*. Plasmid pLY005 (Fig. 5) was used as a template for PCR-derived deletion. The primers used were 5′CAATTAATGATCACCCAAAGTCTAGCTCAGGAAGC, identical to the (+) strand in the region of the *Bcl*I site, and 5′GGCGCTGATCATCGGTGACAGAGTACACCAATAAGAACCC, which introduces a new *Bcl*I site, connected to a sequence identical to the (−) strand, 441 bp upstream of the existing site. PCR amplification therefore yielded a 7·1 kb fragment of pLY005 lacking 441 bp (encoding residues 87–233 of CaRsr1). This fragment was extracted from an agarose gel, digested with *Bcl*I (to remove the ends) and self-ligated to yield pLY006. The disruption vector was constructed by using a cassette (Fonzi & Irwin, 1993) of *Salmonella typhimurium hisG* gene direct repeats flanking a *CaURA3* gene, cloned in plasmid pCUB6. The *Bcl*I-linearized pLY006 was ligated to the 3·7 kb *Bam*HI–*Bgl*II fragment of pCUB6 carrying the disruption cassette. The ligation product pLY007 was used as a source for the 5·4 kb *Spe*I–*Hin*dIII (*Carsr1*Δ::*hisG–URA3–hisG*) disruption fragment.

Construction of a *CaRSR1 C. albicans/E. coli* shuttle plasmid (pLY008). A 3·3 kb *Hin*dIII–*Hin*dIII fragment of pLY001 (*CaRSR1*) was ligated to the *Hin*dIII unique site in the polylinker of pCA-I. pCA-I (from William Fonzi, Georgetown University, USA) is a modified version of pRC2312 [pUC9 sequences (*bla*, *ori* and *lacZ*), and *C. albicans URA3*, *CaARS* and *LEU2*; Cannon *et al.*, 1990] in which a multiple cloning site adaptor has been inserted.

RESULTS

Isolation of a *C. albicans* suppressor of an *S. cerevisiae cdc24* mutation

In a search for genes regulating polarized growth of *C. albicans*, efforts were conducted to isolate the *C. albicans* functional homologue of *S. cerevisiae CDC24*. A *C. albicans* genomic library cloned in a high-copy-number plasmid (Rosenbluh *et al.*, 1985) was used to transform an *S. cerevisiae cdc24*^{ts} mutant (KGC24-3). Cells were plated on SD agar supplemented with uracil, histidine and tryptophan and incubated at 24 °C to select Leu$^+$ transformants. The plates were then replicated onto YPD supplemented with 1 M sorbitol agar and incubated at a non-permissive temperature of 36 °C. This procedure allowed isolation of a functional homologue of *ScCDC24* or multicopy suppressors of this gene. Sorbitol supplementation has been shown to facilitate the isolation of multicopy suppressors of this *Sccdc24*^{ts} mutation (Bender & Pringle, 1989). Several colonies were isolated and the linkage of the suppression of the temperature-sensitive phenotype of *Sccdc24* to the transforming plasmids was confirmed. One of the isolated plasmids, pTM1861, was further studied and subjected to subcloning in the *S. cerevisiae/E. coli* high-copy-number shuttle vector pRS426 (Christianson *et al.*, 1992; Fig 1). To identify the DNA fragment encoding the gene that suppresses *Sccdc24*^{ts}, each subclone was used to transform the *S. cerevisiae* mutant. Transformants were tested for growth at 36 °C by plating drops of $1{\cdot}3 \times 10^3$ cells onto duplicate SC agar plates supplemented with 1 M sorbitol (Bender, 1993). The plates were incubated for 45 h at either 36 °C or 24 °C. As a control, the suppression of *Sccdc24*^{ts} by *S. cerevisiae RSR1* cloned in YEp24 (pPB117; Bender & Pringle, 1989) was tested. No significant difference was noticed in the suppression of *Sccdc24* by either *ScRSR1* or the sequence cloned from *C. albicans* (data not shown).

Effect of the *C. albicans* cloned DNA on the morphology of *S. cerevisiae cdc42* mutant and wild-type strains

S. cerevisiae genes which have been isolated as suppressors of a *cdc24* mutation are involved in cell morphogenesis (Bender & Pringle, 1989; Herskowitz *et al.*, 1995). To determine whether the cloned *C. albicans* sequence that suppresses *Sccdc24* has a discernible effect on the overall morphology of *S. cerevisiae*, it was used to transform some *S. cerevisiae* strains. Wild-type cells (TD4) were transformed with plasmid pLY005 containing the *C. albicans* cloned sequence or the vector

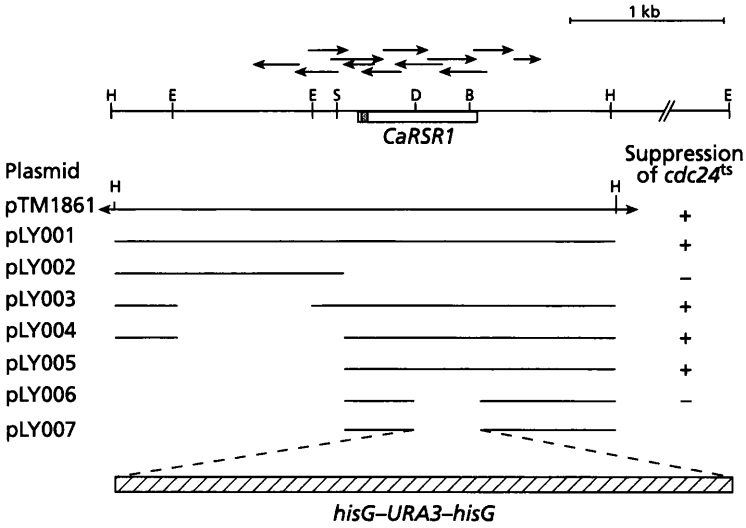

Fig. 1. Genomic site and restriction map of *C. albicans RSR1*. The gene is denoted by an open box, and includes an intron (dark box). *C. albicans* DNA cloned in YEp13 (pTM1861) was subjected to subcloning in pRS426 (pLY001-006). The clones were assayed for suppression of the *S. cerevisiae* $cdc24^{ts}$ mutation (indicated by + or −). The disruption cassette was inserted into pLY006 (Methods). The *SpeI–HindIII* fragment of the resulting pLY007 was used as a disruption fragment of genomic sites. The arrows above the restriction map indicate the sequencing reactions. H, *Hin*dIII; E, *Eco*RI; S, *Spe*I; D, *Dra*III; B, *Bcl*I.

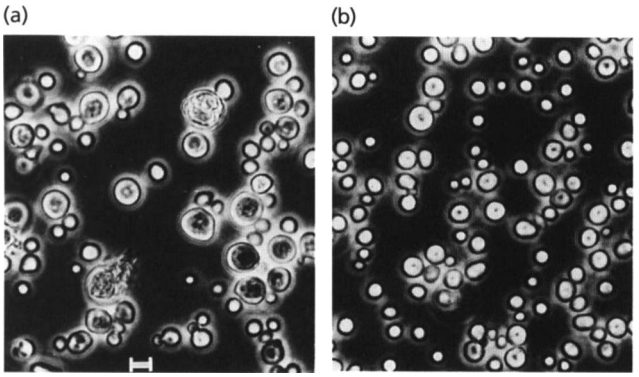

Fig. 2. Effect of *CaRSR1* on cell size of an *S. cerevisiae cdc42* mutant. Cells of *S. cerevisiae* DJTD2-16D transformed with (a) pTM1861 (*CaRSR1*) and (b) YEp13 vector were plated on SC-leucine and incubated at 26 °C for 3 d. Cells were suspended in SC medium and observed by phase contrast microscopy. Bar, 5 μm.

pRS426. Some morphological effect induced by the presence of pLY005 was noticed in wild-type *S. cerevisiae* but the effect was minor. Microscopic examination of the cells did not reveal significant differences in cell size or shape. Very large cells that comprise less than 1% of cells sampled were detected in strains transformed with pLY005 or pRS425. The normal ovoid structure was evident among the cells transformed with the vector, whereas the cells transformed with pLY005 were more spherical.

CDC24 and *CDC42* interact genetically and according to more recent results the proteins they encode interact physically (Bender & Pringle, 1989; Zheng *et al.*, 1994, 1995). Therefore, it was of interest to test the cloned *C. albicans* sequence that suppresses the *Sccdc24* mutation for its effect on an *S. cerevisiae cdc42-1* mutant. The colonies formed by these transformants were very small. The cells were found to vary considerably in size and included large cells, some of which disintegrated during microscopic examination (when resuspended in SC medium; Fig. 2). This instability was not seen in cells transformed with vector sequences only. Thus, the cloned suppressor of *Sccdc24* did not appear to be the *C. albicans* functional homologue *Sccdc42*.

Sequence of the *C. albicans* suppressor of *Sccdc24*

The *C. albicans* gene was sequenced (Fig. 3) and the deduced sequence indicated that the *C. albicans* suppressor of *Sccdc24* encodes a Ras-related protein of 248 amino acids (Fig. 4). The deduced protein is most similar to that encoded by *S. cerevisiae RSR1* (Bender & Pringle, 1989; $P = 3{\cdot}8e{-}89$). The gene, designated *CaRSR1*, is deduced to encode a protein shorter by 24 amino acids than that encoded by *ScRSR1* and the proteins are 56% identical. *CaRSR1* potentially includes a short exon of seven nucleotides followed by an intron of 74 nucleotides and a second exon of 737 nucleotides. The deduced splice junctions are identical to those that occur in *S. cerevisiae* and seen in other *C. albicans* genes (Langford *et al.*, 1984; Smith *et al.*, 1988). The region located between nucleotides −242 and −37 consists of 80% AT, and two clear TATA boxes (at −64 and −56) are evident. It is likely that this region may therefore represent the upstream activating sequence of *CaRSR1*. DNA motifs which serve as 3′-end signals of mRNA in *S. cerevisiae* (Guo & Sherman, 1995; Russo *et al.*, 1991, 1993) can be found downstream of the TGA stop codon of *CaRSR1*. Four suggested efficiency element motifs (TATATATA, TATGTA, TATATA, TATGTA) are located some 36–156 nucleotides downstream of the termination codon. They are followed by a suggested positioning element motif (AATAAA) and a major poly(A) site (CA) typical of 3′-end signals.

The deduced CaRsr1 protein is very similar to Ras superfamily proteins in regions important for GTP binding, effector interaction and membrane attachment (Fig. 4; reviewed by Lowy & Willumsen, 1993; Valencia *et al.*, 1991). Both *S. cerevisiae* and the deduced *C. albicans* Rsr1 proteins are related to the Rap family and share with Rap proteins most of the amino acid residues

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   1  AAG AAA AGT AAA GCA TAT TGA GTA AAT GTT ATC GGT TTA TTT AGT TGA AAG AAA CGC GTT
  61  TAT TTT TAG GTG TGT GTG TGT AAA ACA AAA ATT GAA GAG AAT GTT TAG TTT TTA ATT TTT
 121  CGT CTT CTG TGT TTT CTC GTC AAT GTT AAA CAA GTG TGA AAT TGG GGT TAG ATA GGT GTT
 181  ATT ACT ACT ATC ATT TGC TCG TTA TAC AAA TAA AAT ACA AAT TTC TAT TTG ACC CAC TGT
 241  GTA GTA ATG GCA AAT CAT TAT TGA AAA GAC CTG AGT CGT TTG CTT GTA TTT ACA AAT CGA
 301  TAA CAA AAG ACG AAT TTA TTC TAA TTT TAC TCG TAG TAA AAA TTC CAC ATA AAT TTT AAT
 361  GCC AAC CTC TCT TGG CCC TGA ATT TTG AAT TCA TGC AGT GCT GGT GGT AGT AGT GGA TTC
 421  TCT TTC TGA AAA AAA AAA AAT AAA AAA GTA CGT GTT ACA TTG ATG CTT CTT AAT CGA TTA
 481  ATA CAA TTA AAA TAT TAT TAT TTA TCA CTC CCA CCA AAA CAG CAA AAA AAG AAA AGC AAA
                          SpeI
 541  AAA AAG AAA AGC AAA ACA CTA GTC AAC AGA GAA AAA AAA AAC ACC AGA AAA AAA ATA GTA

 601  ATT CTG TAT ACC TTT ATA CAA ATA AGA TTT TAA AGC CTA TTC ACA AAC ATC AAT CTT CTG
                          .........

 661  TCA GAT TTC AAT GAG AGG TAT GTA CAT TCA ACA AAA GCC CGT TAC ACT TGT ATT TCA ATA
               met arg a
                ..........                ....

 721  ACC TTA TAT ACT AAC TTT TGT TTT GTC ACA GAT TAT AAA GTC GTA GTA TTG GGT GCT GGT
                                          sp tyr lys val val val leu gly ala gly

 781  GGG GTA GGT AAA TCC TCA ATC ACC GTG CAA TTT GTC CAG GGT GTA TAC GTC GAA AGT TAC
      gly val gly lys ser ser ile thr val gln phe val gln gly val tyr val glu ser tyr

 841  GAC CCT ACA ATT GAA GAC TCC TAT AGA AAA CAA ATC GAA GTG GAT GGC AGG GCT TGT GAT
      asp pro thr ile glu asp ser tyr arg lys gln ile glu val asp gly arg ala cys asp

 901  CTA GAG ATT TTA GAT ACA GCA GGG GTG GCA CAA TTC ACA GCC ATG AGA GAA TTG TAC ATT
      leu glu ile leu asp thr ala gly val ala gln phe thr ala met arg glu leu tyr ile

 961  AAA AGT GGT AAA GGG TTC TTA TTG GTG TAC TCT GTC ACC GAT*GAA AAT TCG CTT AAA GAA
      lys ser gly lys gly phe leu leu val tyr ser val thr asp glu asn ser leu lys glu

1021  TTA TTA GCA CTT CGT GAA CAA GTG TTG AGA ATA AAA GAT AGT GAC AAT GTC CCT ATG GTA
      leu leu ala leu arg glu gln val leu arg ile lys asp ser asp asn val pro met val

1081  TTG GTT GGG AAC AAG TGT GAT TTA GAA GAT GAC CGT GTT TTA AGT ATA GAG GAT GGG GTG
      leu val gly asn lys cys asp leu glu asp asp arg val leu ser ile glu asp gly val

1141  AAA GTG AGT CAA GAT TGG GGA TTA GTA CCA TTC TAT GAA ACA AGT GCC ATG TAC AAA ACA
      lys val ser gln asp trp gly leu val pro phe tyr glu thr ser ala met tyr lys thr

1201  AAT GTG GAT GAA GCG TTC ATT GAT GTT GTC AGA CAA ATC ATG AGA AAA GAA GCC GCT ATC
      asn val asp glu ala phe ile asp val val arg gln ile met arg lys glu ala ala ile

1261  AGT GCC GAA AAG AAA CAA CAA AAA GAA TTA CAA AAA CAA CAA CAG CAA CAG CAG CAA GAA
      ser ala glu lys lys gln gln lys glu leu gln lys gln gln gln gln gln gln gln glu

1321  CAA GAT GCT GAA GGA CAA CAA CAA CAA CAG AAA TCA GGA AAA TCC AAA TCG TCT GCA ACA
      gln asp ala glu gly gln gln gln gln gln lys ser gly lys ser lys ser ser ala thr

1381  CAA AAG GAT GCA ACA GCA GAT GGC CAA ACA GAT GTC AAT GCC AGA TTG AAA CAA TCA ATT
      gln lys asp ala thr ala asp gly gln thr asp val asn ala arg leu lys gln ser ile
        BclI
1441  AAT GAT CAC CCA AAG TCT AGC TCA GGA AGC AAG TTC TGC ACA ATT ATT TGA CCC AAT CAA
      asn asp his pro lys ser ser ser gly ser lys phe cys thr ile ile OPA

1501  TCA ATC AAT CAA TCA AAT AAT ATA TCT ATA TAT ATA TGT ATT AGA GTA ATT TTT TTT TCT
1561  TGG AGG GTT AAG ACC CTT TAT ATA ATT TCT AAT TAA TTT AAA GGT TTA AAT GAT TTT TTA
1621  CAA ATA AGG GAA TTG ATC TCT ATG TAA GAA TAA AAT GGA GTT GGT GAA GAG ATT TGA CAT
1681  ACC CAA TGT ACA AAT TCA AGT CAC TTT TCC CCC TGA AAA AAA TAT AGA CCG TGT ATG TTC
1741  CTG AAA AGT TCA AGA TTT TAA TTT GCC ACG GGG AGT AAA GTT CGA AAT AAT GAA AGG ACC
1801  AAG TAA TCT AGT AGA GTT GAC TTT GTG TTA TTG TTA AAC AAT TAC ACT ACT TTA CAA TAG
1861  TAG TAA ACT TTT ATG TCA GTA CGT AAT ATA TAC ATT GCA AGA AAT GGA AAT CGT AAA
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Fig. 3. *CaRSR1* DNA and deduced protein sequences. Two potential TATA sequences are overlined. The intron is underlined. Its splicing conserved motifs are indicated by dots. Putative 3′-end-forming signals of mRNA, i.e. efficiency elements, a positioning element and the major site of polyadenylation, are indicated by a double underline. *Spe*I and *Bcl*I restriction sites are indicated. The location in which the synthetic *Bcl*I site was introduced to yield pLY006 is indicated by an asterisk. For motif details see Results.

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                                                                          70
Rsr1 Ca     MRDYKVVVLGAGGVGKSSITVQFVQGVYVESYDPTIEDSYRKQIEVDGRACDLEILDTAGVAQFTAMREL
Rsr1 Sc     -----L-----------CL---------LDT----------T--I-NKVF---------I---------
Rap1B Hs    --E--L----S------AL-------IF--K-----------V---AQQ-M--------TE------D-
RapA Do     --E--L----S------AL-------IF--K-----------V---CQP-M--------TE------D-
strict cons.    K    G   GK  L        F      T                      DTAG E
sites                PM1              G1    PM2                    PM3

                                                                         140
Rsr1 Ca     YIKSGKGFLLVYSVTDENSLKELLALREQVLRIKDSDNVPMVLVGNKCDLEDDRVLSIEDGVKVSQDWGL
Rsr1 Sc     -----M----------RQ--E--ME----------R-----I---A--INE--I-V-E-IE--SK--R
Rap1B Hs    -M-N-Q--A----I-AQSTFND-QD----I--V--T-D---I----------E--VGK-Q-QNLARQ-NN
RapA Do     -M-N-Q--A----I-AQSTFND-QD----I--V--TED---I----------E--VGK-Q-QNLARQ-NN
strict cons.                                                 G K DL
sites                                                        G2

                                                                         210
Rsr1 Ca     VPFYETSAMYKTNVDEAFIDVVRQIMRKEAAISAEKKQQKELQKQQQQQQQEQDAEGQQQQQKSGKSKSS
Rsr1 Sc     --------LLRS----V-V-L----I-N-MESV-V-DARNQS-QFSKIESPSTRLPSSAK-DTKQSNNKQ
Rap1B Hs    CA-L-S--KS-I--N-I-Y-L----N--TPVPGKAR-KSS
RapA Do     CA-L-S--KS-I--N-I-Y-L----N--APVEKCK--KSQ
strict cons.  F E GA     V   F
sites           G3

                                                                         248
Rsr1 Ca     ATQKDATADGQTDVNARLKQSINDHPKSS                          SGSKFCTII
Rsr1 Sc     SSKGLYNKSS-GQAKVKQSTPV-EKH-P-HAVPKSGSSNRTGISATSQQKKKKKNAST---L
Rap1B Hs                                                           -QLL
RapA Do                                                            --LL
strict cons.                                                       C
sites                                                              term
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Fig. 4. Amino acid sequence alignment of the deduced *C. albicans* Rsr1 (this work), *S. cerevisiae* Rsr1 (Bender & Pringle, 1989), human Rap1B (Matsui *et al.*, 1990) and *Discopyce ommata* RapA (Ngsee *et al.*, 1991). The coordinates for CaRsr1 are indicated. Dashes indicate identity with CaRsr1. Blanks indicate gaps inserted to maximize alignment of the sequences. Amino acids strictly conserved in the Ras superfamily (Valencia *et al.*, 1991) are indicated by bold letters. Phosphate/Mg-binding (PM) and guanine-binding (G) residues as well as the C-terminus ('term') are indicated by a line. The putative effector region is overlined by broken double lines.

that participate in nucleotide binding, with a few exceptions of residues 11 and 28–31. Rap and Ras families share the effector-binding domain (residues 32–40). Rap and Rsr proteins share some distinctive features (non-glutamine at residue 61, uncharged residue at position 63), in a region (residues 59–65) that forms loop L4, important for the interaction with the GTPase-activating protein. Alanine 62 of both Rsr1 proteins seems unique. The deduced C-terminus motif of CaRsr1, CTII, most strongly resembles the CaaL (a = aliphatic residue) motif of Rap and Rho protein families (for a review, see Schafer & Rine 1992), which is modified by a geranylgeranyl moiety required for association with membranes.

The C-terminal extension of the Ras superfamily proteins (residue 167 up to the C-terminus motif) is highly variable. It may be mobile, sticking out of the cytoplasmic globular GTP-binding domain, and may act as a flexible spacer between this domain and the membrane-bound C-terminus (Valencia *et al.*, 1991). ScRsr1 and the deduced CaRsr1 have a longer C-terminal extension than Rap proteins from different origins. The CaRsr1 variable region is glutamine-rich (19 out of 78 residues). A 36-amino-acid inner stretch (175–210) is highly hydrophilic and includes two polyglutamine (seven and five residues) stretches. The low frequency of cytosine among the first nucleotides of codons of *CaRSR1*, except for glutamine codons (CAA, CAG), suggests a conservation of polyglutamine in CaRsr1. Polyglutamine stretches were shown to act as transcriptional activation domains (Courey *et al.*, 1989). The polyglutamine of the deduced CaRsr1 may have a role in protein–protein interactions, as suggested for other proteins (Burke *et al.*, 1996; Stott *et al.*, 1995).

Disruption and reintroduction of the *CaRSR1* gene

In an attempt to elucidate the physiological role of *CaRSR1*, *C. albicans rsr1/rsr1* strains were constructed. This was performed by gene disruption using the method of Alani *et al.* (1987) and adapted for *C. albicans* by Fonzi & Irwin (1993). This method allows the repeated use of *URA3* selection, which is necessary to disrupt the two alleles of a given gene in *C. albicans*. A disruption vector was constructed as described in Methods. The deletion of 59% of the *CaRSR1* ORF (corresponding to amino acid residues 87–233) covers nearly half of the guanine-binding domain and most of the C-terminal extension. The 5·4 kb *Spe*I–*Hin*dIII disruption fragment was used to transform *RSR1/RSR1* strain CAI4 (Table 1). Ura^+ transformants (*RSR1/rsr1*, CAI4-10) were recovered as presumed heterozygotes. To allow the second round of disruption, Ura^- cells were selected on a medium containing 5-FOA. The resulting cells of CAI4-11 were then retransformed with the same disruption fragment to yield *rsr1/rsr1* Ura^+ strains. Two *rsr1/rsr1* transformants (including CAI4-5) were subjected to phenotypic characterization with identical results.

Uracil auxotrophs *rsr1/rsr1* (including CAI4-5a) were obtained by a second round of 5-FOA selection. For reintroduction of the *CaRSR1* gene into these *rsr1/rsr1* disrupted strains, a plasmid containing *CaRSR1* (pLY008) was directed into the *LEU2* locus. This 8·6 kb *Kpn*I fragment was employed to transform the *rsr1/rsr1* strains to Ura^+. All the disruptions and the reconstitution of *CaRSR1* were verified by Southern analysis of *Eco*RI-digested genomic DNA, using a 1·8 kb *Spe*I–*Hin*dIII fragment of pLY005 as a probe (Fig. 5): 6·2 kb bands indicate wild-type alleles (lanes 1–4). The disrupted allele is longer, split into two bands (7·3 and 2·2 kb) as expected from the presence of an *Eco*RI site in the *URA3* gene (lanes 3 and 5). The loop-out allele is expected to be 6·9 kb but it lacks this internal site (lanes 4–7). The reintroduced allele is represented by a new 2·2 kb band as expected from a fragment starting at the *Eco*RI site 5′ to the *Spe*I site, and ending at the *Eco*RI site of the pCA-1 vector (lane 7).

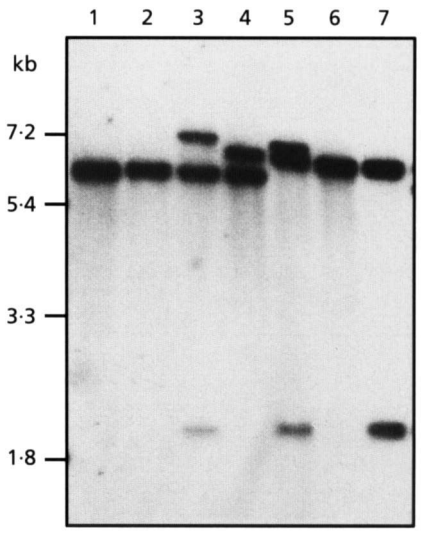

Fig. 5. Disruption and reintroduction of the *CaRSR1* gene. Southern analysis with the CaRsr1 fragment (1·8 kb *Spe*I–*Hin*dIII fragment of pLY005 insert) as the probe. *C. albicans* DNA digested with *Eco*RI was from the following strains (full relevant genotypes). Lanes: 1, SC5314 (*RSR1/RSR1*); 2, CAI4 (*RSR1/RSR1*); 3, CAI4-10 (*RSR1/rsr1*Δ::*hisG–URA3–hisG*); 4, CAI4-11 (*RSR1/rsr1*Δ::*hisG*); 5, CAI4-5 (*rsr1*Δ::*hisG–URA3–hisG/rsr1*Δ::*hisG*); 6, CAI4-5a (*rsr1*Δ::*hisG/rsr1*Δ::*hisG*); 7, CALY5a1 (*rsr1*Δ::*hisG/rsr1*Δ::*hisG LEU2*::*RSR1 URA3 CaARS/LEU2*).

All phenotypic assays were performed with uracil prototrophic strains (*RSR1/RSR1*, *RSR1/rsr1* and *rsr1/rsr1*) and, when required, with CAF2-1 (*RSR1/RSR1*) as a control having one *URA3* allele, and with the *RSR1* reconstituted strains including CALY5a1.

CaRSR1 is involved in yeast cell morphogenesis, is required for polar bud site selection, and affects the maximum cell density

Wild-type and *Carsr1* mutants were grown on solid or in liquid medium, in YPD or in SC (with or without uridine), and at different temperatures (24 or 30 °C).

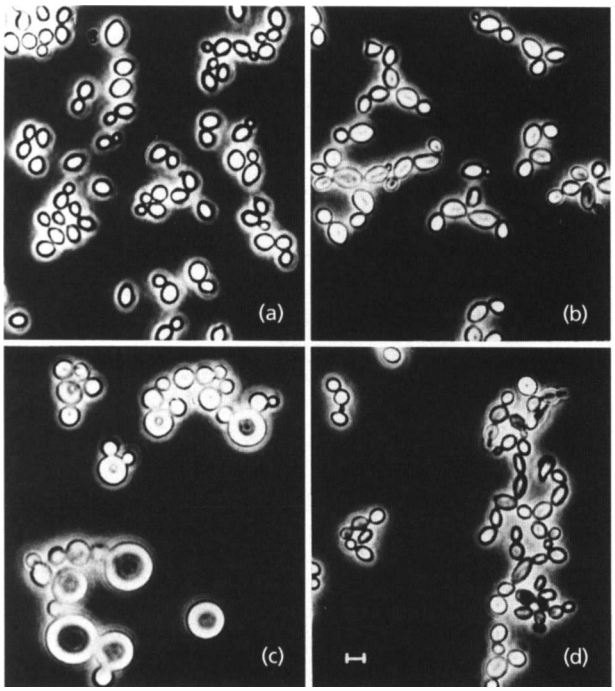

Fig. 6. Morphology of *C. albicans* strains. Cultures were grown in YPD, agitated at 30 °C for 21 h, to stationary phase. Cells were fixed with SDS/formalin and observed by phase contrast microscopy. (a) SC5314 (*RSR1/RSR1*); (b) CAI4-10 (*RSR1/rsr1*); (c) CAI4-5 (*rsr1/rsr1*); (d) CALY5a1 (*rsr1/rsr1 RSR1*). Bar, 5 μm.

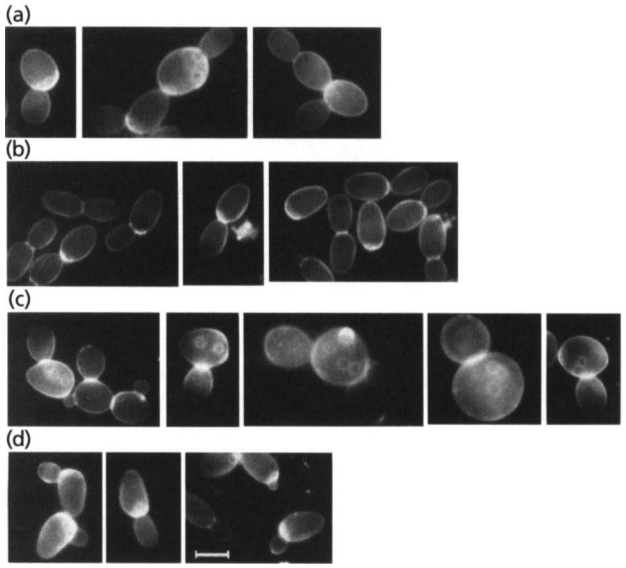

Fig. 7. Budding location of *C. albicans* strains. Calcofluor-stained cells of (a) SC5314 (*RSR1/RSR1*), (b) CAI4-10 (*RSR1/rsr1*), (c) CAI4-5 (*rsr1/rsr1*) and (d) CALY5a1 (*rsr1/rsr1 RSR1*). Bar, 5 μm.

Cell shape was examined microscopically. Under all the different conditions, homozygote cells (*rsr1/rsr1*) were more heterogeneous in size and shape than wild-type, heterozygote (*RSR1/rsr1*) or reconstituted (*rsr1/rsr1*, *RSR1*) strains. The cells of the homozygote disruptant were larger and tended to be spherical while cells of the wild-type and the heterozygotes were ovoid (Fig. 6). The loss of *CaRSR1* leads to a more spherical shape. Therefore, it appears that *CaRSR1* is involved in the polar expansion of the surface of the yeast phase cells of *C. albicans*.

The *S. cerevisiae RSR1* gene is required for normal bud site selection in both haploid and diploid cells (Bender & Pringle, 1989). The effect of *CaRSR1* on the budding pattern of *C. albicans* was examined (Methods). The wild-type strain SC5314 and the heterozygote (*RSR1/rsr1*) budded exclusively at the cell poles (Table 2; Fig. 7). In most of the cells which had a total of at least two daughter cells (identified as buds or bud scars), cell division events occurred in one pole. In cells that budded in both poles, a preference for one pole was noticed since the numbers of bud scars at each pole were not similar (data not shown). In *rsr1/rsr1*, the budding location was scattered over the entire cell surface. Reintroduction of the *CaRSR1* gene in CAI4-5a restored the polar budding pattern. Therefore, *CaRSR1* is required for defining a distinct pattern of budding in *C. albicans*. The normal pattern is a polar one, as seen in the wild-type and all heterozygotes of *CaRSR1*.

When cells were grown in YPD broth at 30 °C, the maximum cell density of the strains was different. The cell density of SC5314 (wild-type) and CAF2-1 (*URA3/*

Table 2. Bud site selection in *C. albicans* strains

Only cells that budded at least twice were scored. See Methods for details.

Strain	Relevant genotype	Budded cells (%)		Cells (total)
		Polar	Non-polar	
SC5314	*RSR1/RSR1*	100	0	208
CAI4-10	*RSR1/rsr1*	99	1	272
CAI4-5	*rsr1/rsr1*	34	66	341
CALY5a1	*rsr1/rsr1 RSR1*	97	3	297
CALY5a20	*rsr1/rsr1*	32	68	320

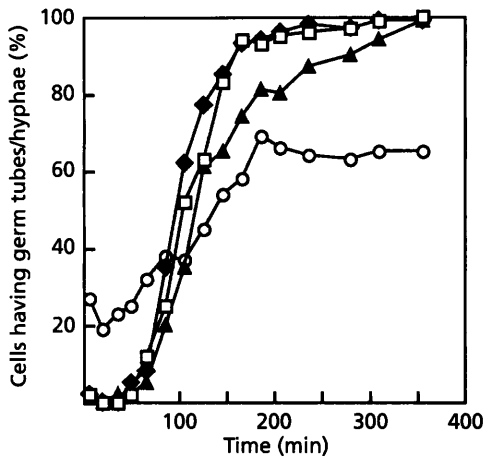

Fig. 8. Germ tube formation by *C. albicans* in Lee medium at 37 °C. The results presented are from one of two consistent repeats of the experiment. For details see Methods. □, SC5314 (*RSR1/RSR1*); ◆, CAF2-1 (*RSR1/RSR1*); ▲, CAI4-10 (*RSR1/rsr1*); ○, CAI4-5 (*rsr1/rsr1*).

*ura3*Δ) was $8{\cdot}4 \times 10^8$ and $7{\cdot}0 \times 10^8$ cells ml^{-1}, respectively. The maximum cell density reached by the heterozygote was $5{\cdot}3 \times 10^8$ cells ml^{-1} but a maximum cell density of only $1{\cdot}5 \times 10^8$ cells ml^{-1} was reached by the homozygote disruptants. Similar differences were observed after growing the strains in SC-uracil broth at 26 °C. Therefore, *CaRSR1* might be involved, in a dose-dependent manner, in determining the maximum cell density that can be reached.

CaRSR1 is involved in germ tube formation and in hyphal elongation

CaRSR1 affects morphogenesis and bud site selection, suggesting that *CaRSR1* is involved in polar growth and polar budding of the yeast phase cells of *C. albicans*. To address the question of whether yeast budding and hypha development are controlled by a common pathway we attempted to determine the effect of disruption of *CaRSR1* on germ tube formation and hypha elongation. Cells were grown under conditions inducing yeast growth (Lee agar at 26 °C) for 45 h. Unlike the SC5314 wild-type, CAF2-1 (*URA3/ura3*) or the heterozygote CAI4-10 (*RSR1/rsr1*), the homozygote disruptant *rsr1/rsr1* developed some hyphae under conditions where the yeast–mycelium transition does not normally occur. To verify that the wild-type and heterozygote strains can undergo this transition, cells of these strains were transferred to medium that induces hypha development (Lee broth at 37 °C; see Methods). The percentage of yeast cells forming germ tubes or developing hyphae was monitored (Figs 8 and 9). All the strains began germ tube formation after a lag of about 50 min. Maximum germination was achieved by 200 min with the exception of the heterozygote, which required 350 min to reach this maximum. However, cells of CAI4-5 (*rsr1/rsr1*) responded slowly and no

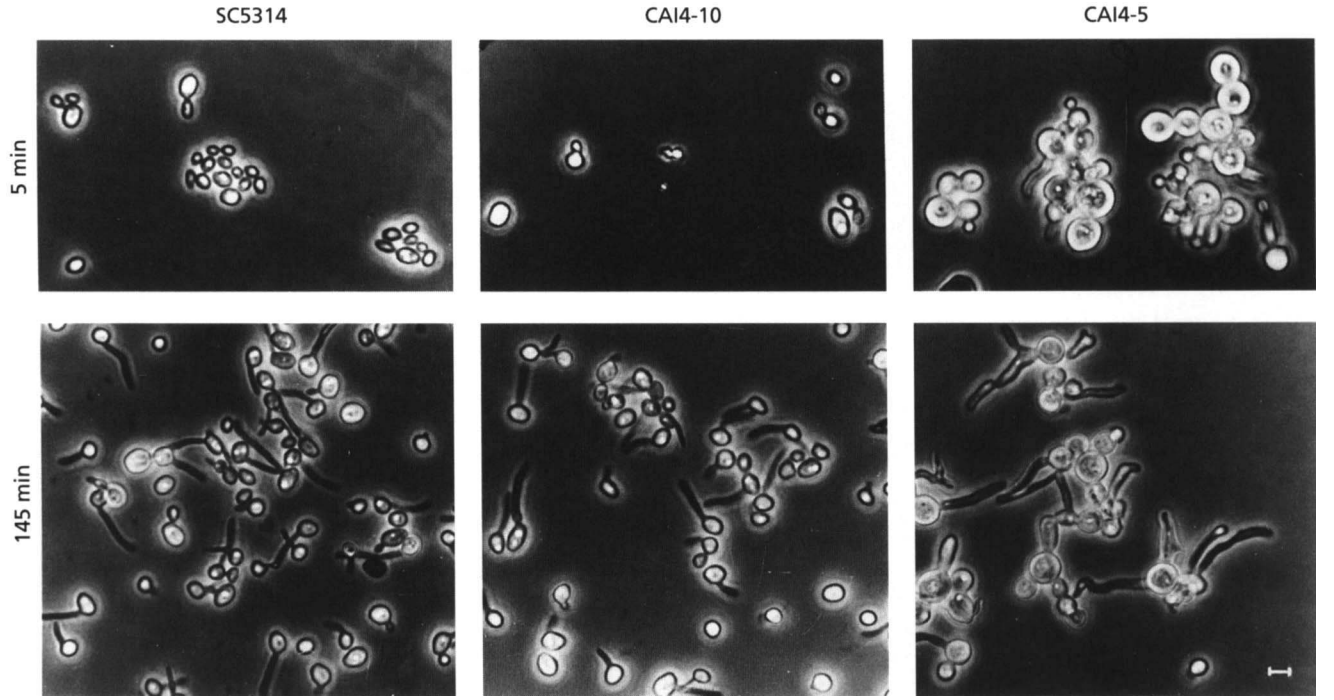

Fig. 9. Germ tube formation. *C. albicans* cells were grown under conditions inducing yeast phase growth and observed 5 and 145 min after being transferred to conditions inducing germ tube formation (Methods). SC5314, *RSR1/RSR1*; CAI4-10, *RSR1/rsr1*; CAI4-5, *rsr1/rsr1*. Bar, 5 μm.

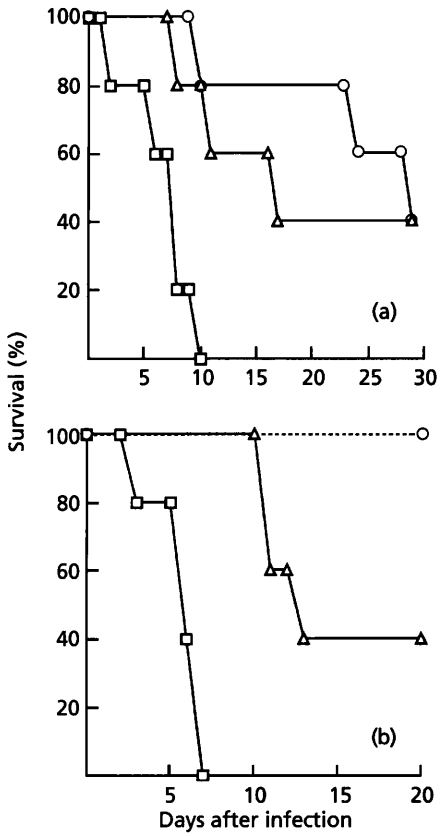

Fig. 10. Survival curves of mice ($n = 5$) infected with *C. albicans* strains (see Methods for details). (a) Immunocompetent mice; (b) neutropenic mice. □, SC5314; △, CAI4-10; ○, CAI4-5.

more than 45% of cells formed germ tubes under the same experimental conditions, while the other strains reached 100% germination.

Hyphal growth was also affected in the homozygote disruptant. The maximum hyphal length after 22 h at 37 °C was 240 μm and 280 μm for the wild-type SC5314 and CAF2-1, respectively. The length of the hyphae of CAI4-10 (*RSR1/rsr1*) during the same time period was 160 μm, and that of the homozygote disruptant CAI4-5 (*rsr1/rsr1*) only 60 μm. These results suggest that *CaRSR1* is involved in both germ tube emergence and in hyphal elongation. The differences cannot be interpreted as a response to the dosage of *URA3* since CAF2-1, like CAI4-10, contains one *URA3* allele. Taken together, our results suggest that the same gene that affects yeast cell shape affects germ tube and hypha development.

When the signal used to induce germination was serum medium, *CaRSR1* was not found to affect significantly the ability to form germ tubes. Some 87% of the cells of the homozygote disruptant (CAI4-5) germinated by 85 min, a time in which 99% of the wild-type (SC5314) and 95% of the heterozygote (CAI4-10) cells had germinated. However, comparison of the same three strains after 25 min in serum medium revealed that the homozygote disruptant had a longer lag in its response to the germinating conditions. Only 15% of cells germinated whereas 30% of cells of the heterozygote and wild-type formed germ tubes during this time. Thus, the effect of *CaRSR1* on germination under these conditions appears to be primarily in response to the signal that initiates germination rather than germ tube emergence.

RSR1 is required for virulence

The importance of the transition from the yeast form to the mycelial form for virulence has been discussed for many years. The application of new molecular biological methods that offer an opportunity to introduce precise mutations in the otherwise isogenic background of the strains used allows more accurate assessment of the importance of specific genes for virulence and the importance of various developmental phases such as germ tube formation and hyphal development for the infection process. Therefore, we examined the effect of *CaRSR1* on virulence. Infection of both immunocompetent and neutropenic mice with the wild-type, heterozygote *CaRSR1/Carsr1* and the homozygote disruptant indicated a marked reduction of virulence in a gene-dose-related manner (Fig. 10). Thus, *CaRSR1* appears to be required in the infection process and can be regarded as a virulence gene of *C. albicans*.

DISCUSSION

The objective of this study was to search for common components of polar processes of *C. albicans* in the yeast form and in the hyphal form. The candidates for the polarity components were genes of *C. albicans* which are functional homologues of genes of *S. cerevisiae* involved in either polarity establishment or bud site selection. A *C. albicans* gene, *CaRSR1*, was isolated as a suppressor of a $cdc24^{ts}$ mutation of *S. cerevisiae*. Cdc24 of *S. cerevisiae* is required for polarity of bud shape, budding location determination (Sloat *et al.*, 1981) and mating (Chenevert *et al.*, 1994). The cloned *C. albicans* gene is most similar to *RSR1* (*BUD1*) of *S. cerevisiae* (Bender & Pringle, 1989).

CaRSR1 is involved in polarity of budding and yeast phase cell morphogenesis

Unlike wild-type *C. albicans* cells which bud at cell poles, *rsr1/rsr1* cells bud randomly over the cell surface. Moreover, the *rsr1/rsr1* *C. albicans* strains display a phenotype of heterogeneous cell size and shape. They tend to be large spherical cells. A phenotype of large spherical cells is known for several budding mutants of *S. cerevisiae*, i.e. *cdc24* (Hartwell *et al.*, 1973; Sloat *et al.*, 1981), *cdc42*, *cdc43* (Adams *et al.*, 1990), *bem1*, *bem2* (Bender & Pringle, 1989) and *cln1cln2* and *bud2* (Benton *et al.*, 1993), and also for *Schizosaccharomyces pombe* homologues of these proteins (Chang *et al.*, 1994; Fukui *et al.*, 1986). It is attributed to a general expansion of the cell cortex. *CaRSR1* is concluded to be required for polar budding and to be involved in ovoid cell mor-

phogenesis. *S. cerevisiae* Rsr1 has been demonstrated to be localized throughout the cortex and suggested to be locally converted to the GTP-bound activated form at the marked bud site (Michelitch & Chant, 1996). It is plausible that a similar mechanism underlies the role of CaRsr1 in controlling cell shape.

The budding pathway of *C. albicans* resembles that of *S. cerevisiae*

The *RSR1* gene of *C. albicans* is similar in four respects to that of *S. cerevisiae*: suppression of an *Sccdc24*^{ts} mutation, sequence similarity, requirement for polar budding and viability of *rsr1/rsr1* deletion mutants. *CaCDC42*, which was isolated as a suppressor of an *Sccdc42*^{ts} mutation (L. Yaar, J. Clifford & Y. Koltin, unpublished), is 87% identical to *ScCDC42* (Johnson & Pringle, 1990). *CaRSR1* was demonstrated to affect *Sccdc42* mutant cells. This might be a consequence of the interaction of CaRsr1 with ScCdc42, ScCdc24 or with another protein that usually interacts with Cdc42 (which is another Ras superfamily member). All the above suggest similarity of the budding pathway in the two organisms. *Schiz. pombe* differs from these two yeasts by having one protein, Ras1p, involved in both the pheromone pathway and morphogenesis.

The size variability and spherical shape characteristic of the *rsr1/rsr1* mutants in *C. albicans* were not reported for a *S. cerevisiae rsr1/rsr1* deletion mutant that appears to grow as wild-type but with some exceptions (Gimeno *et al.*, 1992) buds randomly (Bender & Pringle, 1989). The spherical shape has been reported for a *Schiz. pombe RAS1* mutant (Fukui *et al.*, 1986). *CaRSR1* might therefore play a more dominant role in cell morphogenesis than its *S. cerevisiae* counterpart. This may be attributed to the limited identity of *S. cerevisiae* and *C. albicans* Rsr1 proteins (56% amino acid sequence identity) in comparison with that of Cdc42 (87% amino acid sequence identity) of the two yeasts. The differences between the variable regions of the two Rsr1 proteins (including the polyglutamine of CaRsr1) may cause interactions with different proteins. Other unshared characteristics of the two fungi cannot be ruled out as the cause of the differences.

CaRSR1 is involved in the yeast–hypha transition and in hyphal length determination by a novel pathway

We have demonstrated that *CaRSR1* is required by cells grown in hypha-inducing (37 °C; Lee) medium for full extent and rate of germ tube emergence and for normal extent of hyphal elongation. These conditions have been shown to induce germ tube formation independently of *C. albicans CST20*, *HST7* or *CPH1* genes, which are required for hypha formation under other inducing conditions (Köhler & Fink, 1996; Leberer *et al.*, 1996; Liu *et al.*, 1994). We suggest that the role of *CaRSR1* in the transition of *C. albicans* from yeast to hyphal growth, under these conditions, occurs through a novel pathway of hyphal formation, different to the mycelial formation pathway which is analogous to the pseudohyphae/mating response pathway of *S. cerevisiae*. This pathway seems distinct from the one transducing the serum signal for yeast–hypha transition, which is almost unaffected by the disruption of the *CaRSR1* gene. The presence of hyphae among *C. albicans rsr1/rsr1* disrupted yeast cells, while grown under conditions which induce growth of yeast cells, suggests that *CaRSR1* might be involved in repressing hyphal growth under conditions which do not favour the yeast–hypha transition. Taken together with the reduced final densities of the mutant cells when growing as the yeast form in liquid media (YPD, 30 °C; SC-uracil, 26 °C), a model can be offered in which CaRsr1 is involved in transduction of signals, enhancing the yeast–hypha transition under inducing conditions (Lee broth; 37 °C) and repressing the transition under non-inducing conditions (Lee medium; 26 °C). The suggested role of *CaRSR1* in transducing signals for growth phase transition, combined with its involvement in determining final cell density of cultures, might account for the reduced virulence of the disrupted *C. albicans* mutant. A similar reduction in virulence has been recorded for a number of genes (Bulawa *et al.*, 1995), including *CST20* (Leberer *et al.*, 1996). The effect demonstrated in our study is not as dramatic as one would anticipate based on the fact that *CaRSR1* appears to affect a pathway critical for determination of both yeast cell shape and hypha development. Since *RSR1* disruption does not totally abolish the ovoid shape of cells of the yeast phase, germ tube emergence and hypha elongation, its product is suggested to be redundant or to activate another protein during both phases of growth. Partial penetrance (heterogeneous morphology) of a deletion mutation was previously observed for the *S. cerevisiae* bud emergence mutant *bem1* as well (Chenevert *et al.*, 1992).

Concluding remarks

We demonstrated that in *C. albicans CaRSR1* is required for bud site selection, and is involved in yeast phase cell morphogenesis, germ tube emergence and hypha elongation. We conclude that this gene plays a significant role in facilitating polar bud site selection and a moderate role in yeast cell morphogenesis and transition to and growth in the filamentous phase. We propose that these roles are executed by a common function of focusing cell surface growth to surface sites. The pathways might involve CaCdc42 interaction with Ste20-like protein kinases, such as those involved in polarity/cytoskeletal functions in growth of *S. cerevisiae* and *Schiz. pombe* which are independent of pseudohyphal response and pheromone response (Cla4, Cvrcková *et al.*, 1995; Pak1, Ottilie *et al.*, 1995; Shk1, Marcus *et al.*, 1995).

Many questions concerning the polar processes and the role of *CaRSR1* remain to be answered, such as *RSR1* involvement in the *CST20/HST7/CPH1* pathway of filamentous growth. Also, are there more common components, other than *CaRSR1*, which are shared between the polarity pathways of the yeast and hyphal phase? What are the additional cell components in-

volved in determining ovoid cell shape, germ tube emergence and hyphal length and do they include the gene products of the *C. albicans* homologues of *S. cerevisiae CDC24*, *CDC42* and other polarity establishment genes, or is there a functional redundancy with the unknown *RAS* like that demonstrated for *S. cerevisiae RSR1* (Morishita *et al.*, 1995; Ruggieri *et al.*, 1992)? It is likely that *C. albicans* will display a higher degree of complexity than *S. cerevisiae* and that additional components involved in these morphogenetic processes will be identified.

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