Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 13223–13228, November 1996
Microbiology

Candida albicans strains heterozygous and homozygous for mutations in mitogen-activated protein kinase signaling components have defects in hyphal development

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ABSTRACT **The *Candida albicans* genes, *CST20* and *HST7*, were cloned by their ability to suppress the mating defects of *Saccharomyces cerevisiae* mutants in the *ste20* and *ste7* genes, which code for elements of the mating mitogen-activated protein (MAP) kinase pathway. These *Candida* genes are both structural and functional homologs of the cognate *Saccharomyces* genes. The pattern of suppression in *Saccharomyces* is related to their presumptive position in the MAP kinase cascade. Null alleles of these genes were constructed in *Candida*. The *Candida* homozygous null mutants are defective in hyphal formation on some media, but are still induced to form hyphae by serum, showing that serum induction of hyphae is independent of the MAP kinase cascade. The *Candida* heterozygotes *CST20*/*cst20* and *HST7*/*hst7* are also defective in hyphal formation. This lack of dominance of the wild-type allele suggests that gene dosage is important in *Candida*.**

Candida albicans, the most frequently isolated fungal pathogen in humans, is dimorphic; it switches between growth as a budding yeast form and a filamentous hyphal or pseudohyphal form. This switch is induced by many different environmental cues including high temperature, neutral pH, and serum. Dimorphism is thought to contribute to *Candida's* virulence (1). The direct isolation of mutations that prevent switching is difficult because *C. albicans* is diploid and has no known sexual cycle. To circumvent these problems, several studies have taken the "candidate gene" approach in which a gene is identified that is likely to be involved in dimorphism, and *Candida* strains homozygous for a null allele of that gene are constructed (2, 3). The gene is deemed to be required for dimorphism if the null mutant shows a defect in the induction of hyphae by one of the external cues.

One approach used to identify candidate genes is to clone *Candida* homologs of *Saccharomyces cerevisiae* genes known to regulate filamentous growth. *Saccharomyces* also switches from a yeast form to a filamentous pseudohyphal form, when the yeast cells are starved for nitrogen. This switch requires elements of the mating signal transduction pathway including the protein kinases Ste20, a $p65^{PAK}$ kinase homolog, Ste11 [mitogen-activated protein (MAP) kinase kinase kinase] and Ste7 (MAP kinase kinase), and the transcription factor Ste12 (4). The *C. albicans* homolog of *STE12, CPH1*, was cloned by its ability to enhance filamentous growth of *Saccharomyces* (5). Cph1 shows considerable amino acid identity with Ste12 and suppresses both the filamentation and mating defect of the Saccharomyces *ste12* mutant (6). The *cph1*/*cph1* null mutant is defective in hyphal formation under a number of conditions that induce wild-type *Candida* strains to filament. However, *cph1*/*cph1* strains are still induced to form normal hyphae by serum. This result implicates *CPH1* in *Candida* dimorphism, but suggests that serum induces hyphae by a Cph1-independent pathway.

The finding that the *Candida CPH1* gene complemented both the filamentation and mating defect of the *Saccharomyces ste12* mutant, suggested that other members of a *Candida* MAP kinase (MAPK) cascade could be isolated by suppression of the corresponding *Saccharomyces* mutant. In this report we describe the isolation of *Candida* homologs of *STE20* and *STE7,* two other kinases of the *Saccharomyces* mating MAPK cascade. We constructed strains heterozygous and homozygous for null alleles of these genes in *Candida* and found that both heterozygotes and homozygotes show defects in hyphal formation. However, these mutant strains still form hyphae in response to serum.

MATERIALS AND METHODS

Isolation of *C. albicans* Genes That Suppress the Mating Defects of *ste7* and *ste20*. A *C. albicans* genomic library, prepared from strain 1006 on a *URA3*, 2 micron vector (5), was used to transform *Saccharomyces* strains JKY1(*ste7*) and JKY40(*ste20*) to Ura^+ by a modified lithium acetate method (7). To identify clones with restored mating ability, 10^5 transformants were replica plated to the tester lawn JBY311 on yeast extract/peptone/dextrose (YPD), incubated for 20 h, and replica plated to minimal medium where only diploids could grow. Clones that mated were identified and the suppressing plasmids were isolated.

Plasmids. pJK7 is an isolate from this library containing the *HST7* open reading frame. The insert of pJK7 cloned into Bluescript SK^+ (Stratagene), pJK19, was used for sequencing. The disruption construct, pJK41, was made by inserting a *Bgl*II and an *Sph*I site by site-directed mutagenesis and replacing bases 205-1765 of the *HST7* open reading frame with the *hisG-URA3-hisG* cassette on the *Bgl*II/*Sph*I fragment of pMB7 (8). To complement the homozygous deletion (*hst7*/*hst7*) *Candida* strains with the intact *HST7* gene, the polylinker *Bam*HI and *Xba*I restriction sites were destroyed by blunting and religation, and a *Bam*HI and *Xba*I site was introduced into pJK19 by site-directed mutagenesis between bases 268 and 312 downstream of the *HST7* open reading frame. The *Candida URA3* gene on the *Bam*HI/*Xba*I fragment of pMB7 was cloned into these sites to make pJK58. The insert of pJK58, cut out by the polylinker sites *Kpn*I and *Sac*I, was used to replace one of the two disrupted copies of *HST7* with an intact copy by means of a double crossover event.

Abbreviations: MAP, mitogen-activated protein; MAPK, MAP kinase; YPD, yeast extract/peptone/dextrose.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. U73457).

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The library isolate pJK34 contains the full-length *CST20* open reading frame on a 7.2-kb insert. The isolate pJK25 contains a 295-bp 5′ truncated form of this gene on a 4.8-kb insert. The pJK25 insert, cloned into Bluescript to yield pJK42, was used for sequencing most of the gene. The first 0.3 kb were sequenced from the pJK34 insert cloned into Bluescript, pJK62. The gene disruption construct of *CST20*, pJK51, was made by replacing the *Bgl*II/*Pst*I fragment of pJK42 with the *hisG-URA3-hisG* cassette from pMB7. To reintroduce an intact *CST20* copy into the *cst20/cst20* deletion strains, a complementing integrating construct was made containing *CST20* with the *URA3* selectable marker at its 3′ end, by cloning the *URA3* gene on the *Bam*HI/*Xba*I fragment of pMB7 into the *Hpa*I site of pJK42 to make pJK60, whose insert was excised to induce a double crossover event with the polylinker sites *Kpn*I and *Sac*II. A second construct for this purpose, pJK61, was made by cloning the pJK25 insert into pRC2312, a vector containing the *Candida URA3* and *LEU2* genes, which can be linearized at the unique *Sal*I site in *LEU2* for integration at the chromosomal locus (9).

pHL14 contains the *CPH1* gene (5), a *Candida* homolog of the *S. cerevisiae* gene *STE12*, and was used in the assays for complementation of *Saccharomyces* mating and pseudohyphal growth.

S. cerevisiae* Strains.** For mating assays, all isogenic haploid strains were derived from JBY597 (10) *MATa** *ura3-52 leu2-3 112 trp1*Δ*63 his3*Δ*200 or his3-11,15 ade2 kss1 lys2*::*FUS1*::*LacZ* Gal$^+$. JKY1 (*ste7*::*LEU2*) was constructed from this strain by one-step gene replacement with pDH 90 (from Don Higgins and Kelly Tatchell, North Carolina State University, Raleigh). JKY40 (*ste20*::*TRP1*), whose sterility phenotype is leaky, was constructed from JBY597 with pEL46-2 (11). Gene disruptions were confirmed by Southern analysis. JKY86 was derived from JBY837 (*MAT***a** *ste11*::*URA3*) and JKY87 was constructed from JBY841 (*MAT***a** *ste12*::*URA3*) (10), by disrupting *URA3* with *HIS3* using the plasmid B2388 (from Y. Kassir, Technion, Haifa, Israel). JBY311 (*MAT*α *lys9 ura3-52*) (10) was used as a tester strain for mating. For assays of diploid pseudohyphal growth, we used isogenic strains in the Σ1278b background containing disruptions of *STE20, STE11, STE7*, and *STE12* as described in ref. 4. The *STE/STE* wild-type strain was L5366.

***S. cerevisiae* Mating and Pseudohyphal Growth Assays.** To determine the effect of a cloned *Candida* gene on the behavior of a *Saccharomyces* mutant, we transformed *Saccharomyces* with a plasmid containing the *Candida* gene and assayed the behavior of two transformants for mating or pseudohyphal growth. Mating assays were done using the replica patch test (12), and pseudohyphal growth was assayed by constructing the appropriate diploids and observing colony morphology after 5 days growth on SLAD medium without histidine (13). For controls, the 2 micron *URA3* vector used for the construction of the library, B2205, was used.

***Candida* Growth and Genetic Analysis.** *C. albicans* was routinely grown in YPD medium and at 37°C, unless otherwise noted. Sequential disruption of both alleles of the *Candida* genes was performed using the strategy developed for *S. cerevisiae* by Alani *et al.* (14), as adapted for *Candida* by Fonzi and Irwin (8). This strategy uses the *Candida URA3* gene, flanked by two repeats derived from the *Salmonella typhimurium hisG* gene, as a selectable marker. Segregants that have lost the *URA3* gene by recombination between the repeats can be selected on medium containing 0.1% (wt/vol) 5-fluoroorotic acid and 0.2 mM uridine, and the second allele of the gene can be disrupted reusing *URA3* as the selectable marker.

The strains used for gene disruption were CAI4 (*ura3*::*imm434*/*ura3*::*imm434*) (8) and JKC18, which is derived from CAI4 and is *cph1*/*cph1 ura3*/*ura3* (5). Transformation and selection for Ura$^-$ heterozygotes were performed as described (5). Transformants were screened by PCR for homologous recombination of the transforming construct. Homologous recombination was confirmed by Southern blot analysis. Integration of the intact wild-type gene for rescue of the *hst7*/*hst7* and *cst20*/*cst20* homozygous disruption strains was verified by Southern blot analysis, but the location was not determined. For the *hst7*/*hst7* strains tranformed with an intact copy of *HST7* on pJK58, expression of the gene was confirmed by Northern blot analysis. The wild-type strain used for assay of hyphal growth was a clinical isolate, sc5314 (15), the parent strain of CAI4. To analyze the effect of the introduced mutations on hyphal growth, mutants were assayed before loss of the *URA3* selectable marker, because Ura$^-$ strains are impaired in hyphal formation. Hyphal growth of Ura3$^+$ strains, whose *URA3* gene was at the locus of the disrupted gene in the *hisG-URA3-hisG* cassette, was assayed at 37°C on Spider medium (5), on medium 199 buffered to pH 7 with Hepes (3) and solidified with 2% agar, on Lee's medium (16) solidified with 2% agar, and on solid agar medium containing bovine calf serum (Sigma), made by adding serum at concentrations between 0.5 and 20% to a mixture of water and 2% agar. Hyphal growth of single cells was assayed at 37°C in liquid Lee's medium (16) and in 20% calf serum.

RESULTS

***Candida* Homologs of *S. cerevisiae STE7* and *STE20*.** Functional *Candida* homologs of *S. cerevisiae STE7* and *STE20* were isolated by complementation of the mating defect of *Saccharomyces ste7* and *ste20* mutants. Transformation of a *ste7* mutant with a *Candida* library cloned in a high copy vector yielded *CPH1*, a homolog of *Saccharomyces STE12,* and *HST7*, which has been reported to be the structural and functional homolog of *Saccharomyces* Ste7 (17). Transformation of a *ste20* mutant yielded *HST7* and 10 distinct isolates of a DNA segment, which could encode a protein with homology to *S. cerevisiae* Ste20. The predicted 1230 amino acid protein is a member of the Ste20/p65^{PAK} protein family based on the domains it shares with other members of this family. The carboxyl-terminal kinase domain of this protein is 78% identical (Fig. 1*B*) with the Ste20 kinase domain (11, 18). In addition, Cst20 is 69% identical with Ste20 in a putative Cdc42 binding domain (Fig. 1*A*), which it shares with other members of the Ste20/p65^{PAK} kinase family like the *Schizosaccharomyces pombe* Ste20 homolog Pak1/Shk1, and the rat protein p65^{PAK} (20–22). The amino-terminal portion of the *Candida* protein is longer than that of the *Saccharomyces* Ste20. Because of its functional and structural homology to the *Saccharomyces STE20* gene, this *Candida* gene is called *CST20*.

***Candida* Genes Function in *Saccharomyces*.** HST7 clones restore both mating and pseudohyphal growth (Fig. 2) to *S. cerevisiae* containing a *ste7* disruption, as previously reported (17). Plasmids containing enough of the 5′ portion of the gene to express the putative Cdc42 binding domain of *CST20* (pJK25 and pJK34) suppress both the mating and pseudohyphal defects of a *Saccharomyces ste20* mutant. In contrast, plasmids with a 5′ truncation of *CST20* lacking the presumptive Cdc42 binding domain (pJK21 and pJK29) suppress the mating defect, but not the filamentation defect (data not shown). These data suggest that there may be distinct domains of Ste20 that are devoted to each morphogenetic process. The *Candida* homologs of the *Saccharomyces STE7* and *STE12* genes bypass mutations in genes that act earlier in the MAPK cascade: *CPH1* suppresses the pseudohyphal defect in *ste20*, *ste11*, *ste7*, and *ste12* strains and partially suppresses the mating defect in *ste20, ste11*, and *ste7* mutants. *HST7* suppresses the pseudohyphal and mating defects in *ste20, ste11*, and *ste7*, but not *ste12*. *CST20* fails to suppress the mating or pseudohyphal defect in any sterile mutants tested except for *ste20*.

***Candida* Heterozygotes for Null Alleles of *HST7* and *CST20*.** *C. albicans* heterozygotes made by gene disruption (*HST7*/*hst7* or *CST20*/*cst20*) (Fig. 3) do not have the same hyphal morphology as the parent strain. Three independent single dis-

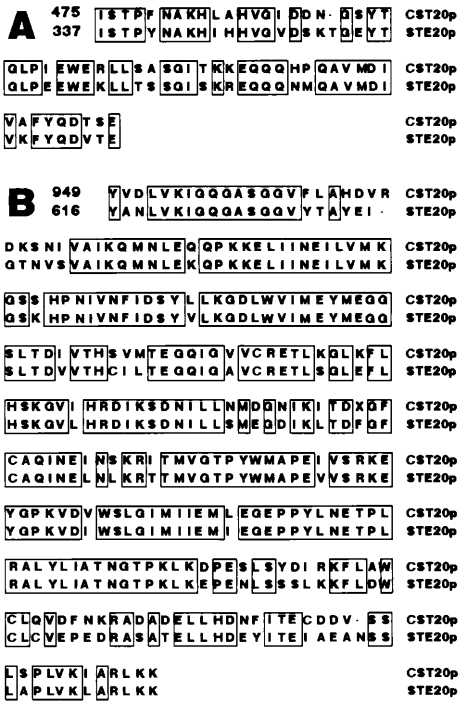

FIG. 1. Cst20 and Ste20 share homologous Cdc42-binding domains and catalytic domains. Translation such that CTG codes for serine (19). (*A*) Cdc42 binding domains. (*B*) Kinase domains.

ruptions of *HST7* were constructed. The three independent Ura$^+$ *HST7*/*hst7* heterozygotes all show reduced hyphal formation on Spider medium as compared with the *HST7*/*HST7* parent (Fig. 4*A*). These heterozygotes show variability in the reduction of hyphal formation between strains and from colony to colony (Fig. 4). Overall, they show more peripheral hyphal growth than their *hst7*/*hst7* derivatives. Two independent *CST20*/*cst20* disruptants were constructed. Both of these heterozygotes also show a drastic reduction of peripheral hyphal growth on Spider plates as compared with the *CST20*/*CST20* parent (Fig. 5*B*). The *CST20*/*cst20* heterozygotes are more homogeneous than the *HST7*/*hst7* heterozygotes and have a greater reduction in peripheral hyphal growth.

***Candida* Homozygotes for Null Alleles of *HST7* and *CST20*.** Deletion of both alleles of the *HST7* gene (*hst7*/*hst7*) by disruption of the remaining copy in any one of the three *HST7*/*hst7* heterozygotes resulted in complete loss of hyphal formation at the periphery of colonies (Fig. 5*F*) on Spider medium, even after 3 weeks of incubation. Sparse short hyphae growing vertically into the agar are formed at the center of the colonies. Two *cst20*/*cst20* disruption strains of *Candida albicans*, each derived from an independently constructed *CST20*/*cst20* heterozygote have similar phenotypes (Fig. 5*C*). They have a reduction of peripheral hyphal growth as compared with both the heterozygote and *CST20*/*CST20* parent; the hyphae are both shorter and more sparse. However, peripheral hyphal growth in the *cst20*/*cst20* strains is not completely abolished on Spider medium, as it is in *hst7*/*hst7* strains. On Lee's medium and on medium 199 (pH 7) solidified with 2% agar, hyphal growth of the strains disrupted for *HST7* and *CST20* is also reduced compared with the wild-type parent, but the difference is less drastic than on Spider medium (data not shown).

To verify that hyphal phenotypes of the *hst7*/*hst7* and *cst20*/*cst20* homozygotes are a consequence of the deletions we constructed, we reintroduced a functional copy of the cognate genes and observed that hyphal formation was restored (Fig. 5 *D* and *G*). In neither case does reintroduction of a functional copy of the gene restore hyphal growth equivalent to that observed in the wild-type parent from which the strains were derived.

Despite the failure of the *cst20*/*cst20* and the *hst7*/*hst7* homozygotes to form significant hyphae on Spider medium, both strains form hyphae on agar plates containing serum. Germ tube formation—i.e., outgrowth of a hypha from a yeast-form cell—is also unaffected in *cst20*/*cst20* and *hst7*/*hst7* strains in 20% liquid serum and in liquid Lee's medium. None of the strains carrying mutations in the known *Candida* homologs of the MAPK cascade (*cst20*/*cst20*, *hst7*/*hst7*, or *cph1*/*cph1*) blocks the serum induction of hyphae. One explanation for the failure of the mutants to block serum induction

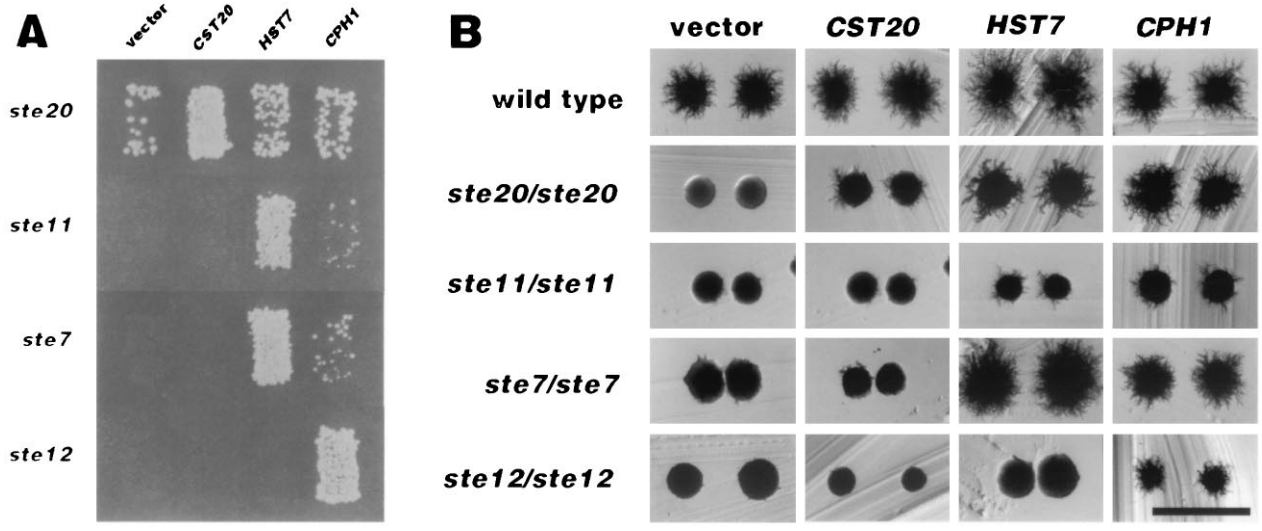

FIG. 2. (*A*) *Candida* genes suppress the mating defects in haploid *S. cerevisiae ste* mutant strains. *Saccharomyces* strains were transformed with the 2-micron vector B2205 (column 1), and with plasmids based on this vector: pJK25 carrying the *CST20* gene (column 2), pJK7 carrying *HST7* (column 3), and pHL14 carrying *CPH1* (column 4). Haploid *Saccharomyces* strains are JKY40 (*ste20*), JKY1 (*ste7*), and JKY 87 (*ste12*). Patches of transformants were grown on selective medium to maintain the plasmid, replica plated to YPD plates covered with lawns of the mating tester strain JBY311, incubated for 4 h, replica plated to minimal medium, and incubated for 2 days. (*B*) *Candida* genes complement pseudohyphal growth defects in diploid *ste*/*ste S. cerevisiae* strains containing mutations in genes encoding components of the mating MAPK cascade. Ste$^+$ (L5366), *ste20*/*ste20* (L5624), *ste11*/*ste11* (L5625), *ste7*/*ste7* (L5626), and *ste12*/*ste12* (L5627) were transformed with *Candida* genes on 2-micron plasmids, as indicated above the columns. The same plasmids were used as in the mating patch assays. Transformants were streaked on SLAD medium and incubated for 5 days. (Bar = 1 mm.)

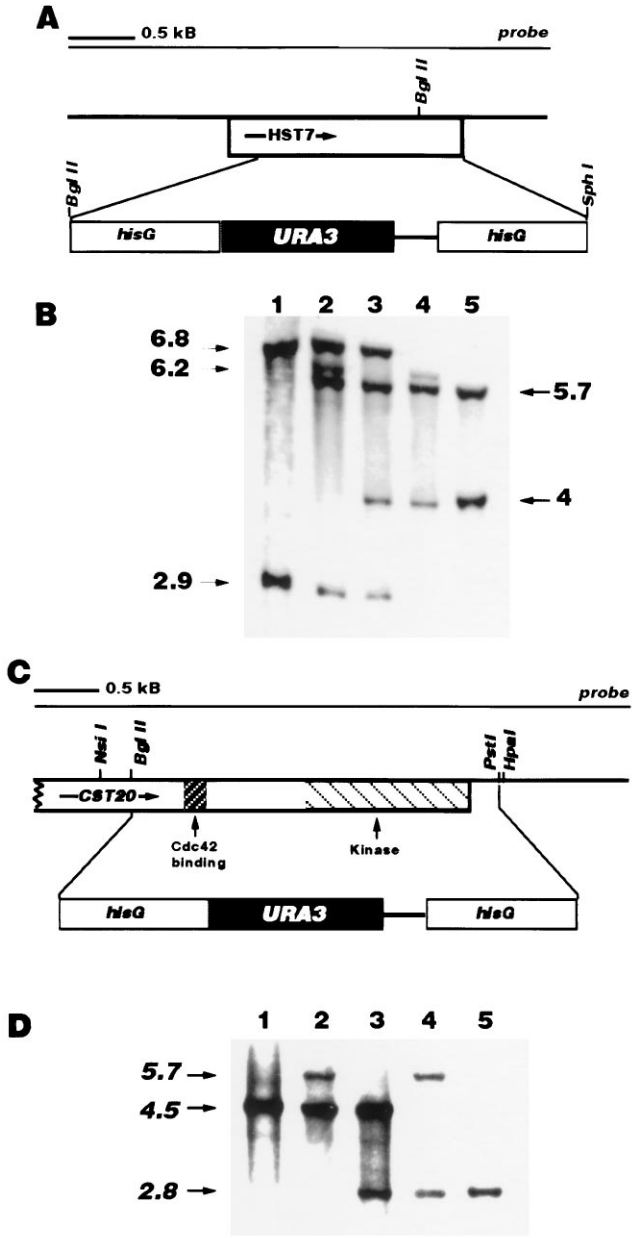

FIG. 3. (*A*) *HST7* deletion construct: map of pJK41. The *Bgl*II site, with which the *hisG-URA3-hisG* cassette was inserted, and the *Sph*I site were created by site-directed mutagenesis and therefore not present in the wild-type gene. (*B*) Southern blot of *HST7* deletion mutants: *Candida* genomic DNA digested with *Bgl*II. Lane 1, wild type. The 6.8-kb band results from digestion at the *Bgl*II site in the *HST7* open reading frame and a site on the chromosome upstream of the gene, the 2.9-kb band results from digestion the *Bgl*II site in the open reading frame and a site on the chromosome downstream of it. Lane 2, *HST7/hst7*::*hisG-URA3-hisG*. Integration of the disruption construct at one of the two copies of *HST7* results in loss of the *Bgl*II site in the open reading frame and gain of a new site in the construct, creating a 6.2-kb band between the new *Bgl*II site and the 3′ chromosomal *Bgl*II site, and a 5.7-kb band between the 5′ chromosomal site and the new *Bgl*II site. Lane 3, *HST7/hst7*::*hisG*. Eviction of *URA3* and one *hisG* repeat results in a decrease in size of the fragment between the new *Bgl*II site and the 3′ site from 6.2 to 4 kb. Lane 4, *hst7*::*hisG-URA3-hisG/hst7*::*hisG*. Integration of the disruption construct into the remaining *HST7* allele results in loss of the wild-type bands. Lane 5, *hst7*::*hisG/hst7*::*hisG*. Arrows indicate kilobases. Fragment sizes are approximate. (*C*) *CST20* deletion construct: map of pJK51. The construct lacks the first 0.3 kb of the open reading frame. (*D*) Southern blot of *CST20* mutants: *Candida* genomic DNA digested with *Nsi*I. Lane 1, wild type. The band results from digestion at the *Nsi*I site in the open reading frame and a chromosomal *Nsi*I site 4.5 kb downstream of it. Lane 2. *CST20/cst20*::*hisG-URA3-hisG*. Lane 3, *CST20/cst20*::*hisG*. Lane 4, *cst20*::*hisG-URA3-hisG/cst20*::*hisG*. Lane 5, *cst20*::*hisG/cst20*::*hisG*. Arrows indicate kilobases. Fragment sizes are approximate.

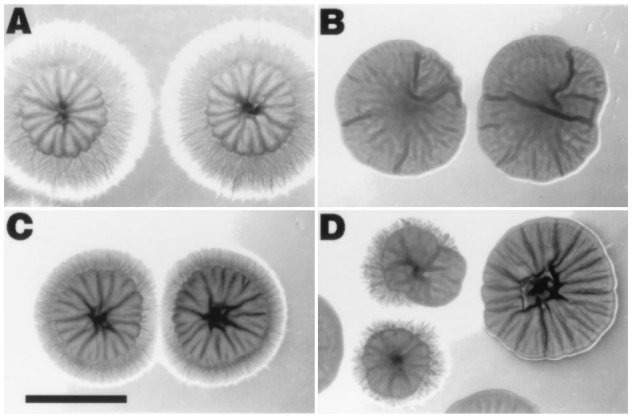

FIG. 4. Filamentous growth defects of *HST7/hst7* heterozygotes. The extent of filamentation varies among strains and from colony to colony. Incubation for 5 days on Spider medium. (*A*) Wild type SC5314. (*B*) JKC48. (*C*) JKC63. (*D*) JKC52. (Bar = 5 mm.)

could be that these genes function in different, but redundant signaling pathways, either of which, when activated by serum, induces hyphal formation. To examine this question, we constructed *hst7/hst7 cph1/cph1* and *cst20/cst20 cph1/cph1* double mutants. The phenotypes of the double mutants on Spider medium resemble that of the single mutant with respect to hyphal formation: *cst20/cst20 cph1/cph1* looked like *cst20/cst20* and *cph1/cph1 hst7/hst7* looked like *hst7/hst7.* However, both the *cst20/cst20 cph1/cph1* and the *hst7/hst7 cph1/cph1* strains still form hyphae in response to serum (data not shown).

DISCUSSION

Our results show that *C. albicans* has many of the elements of the filamentation MAPK cascade that has been described in the budding yeast *S. cerevisiae*. The *Candida* genes are both structural and functional homologs of the *Saccharomyces* genes. Previous reports have shown that *CPH1* and *HST7* are both structural and functional homologs of *Saccharomyces STE12* and *STE7,* respectively (5, 6, 17). The results demonstrate that *Candida CST20* like the *HST7* and *CPH1* genes functions in *Saccharomyces* to complement both the filamentation and mating defects of the cognate *Saccharomyces* mutants. High copy (2 micron) constructs of the *Candida* homologs expressed in *Saccharomyces* bypass upstream but not downstream mutant defects. For example, *CST20* can bypass the defects of a *ste20* mutant, but not *ste11*, *ste7*, or *ste12*, whereas *HST7* bypasses *ste20*, *ste11*, and *ste7*.

Here we show that in *Candida* as in *Saccharomyces* these MAPK genes function to signal filamentation. *cst20/cst20* and *hst7/hst7 Candida* strains have defects in filamentation on solid agar medium, forming only a few short hyphae instead of the florid filaments that emanate from the wild-type parent strain. Despite this defect, none of the null mutants blocks the *Candida* serum response—neither induction of filaments on solid medium nor germ tubes are affected. We examined the possibility that in *Candida* these genes are not in the same pathway, but are different elements of two parallel pathways, either of which can convey the inductive signal from serum. For example, *CST20* might be in one pathway and *CPH1* in another. If this hypothesis were correct then the double mutants (e.g., *cst20/cst20 cph1/cph1)* should have been defective in the serum response. However, the double mutants still

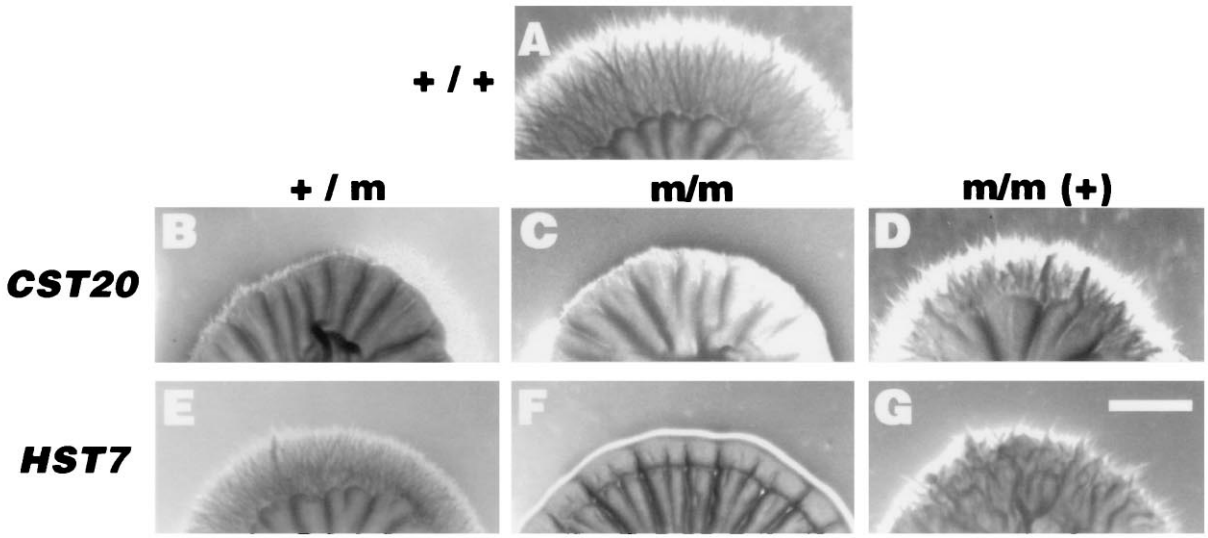

FIG. 5. *HST7* and *CST20* are required for hyphal growth on Spider medium. (*A*) Wild-type strain SC5314 (*CST20/CST20 HST7/HST7 URA3/URA3*). (*B–D*) *CST20* mutants. (*E–G*) *HST7* mutants. (*B*) *CST20/cst20*::*hisG-URA3-hisG* (JKC91). (*C*) *cst20*::*hisG/cst20*::*hisG-URA3-hisG (JKC97).* (*D*) *cst20*::*hisG/cst20*::*hisG/cst20-ura3* (JKC 178). (*E*) *HST7/hst7*::*hisG-URA3-hisG* (JKC63). (*F*) *hst7*::*hisG/hst7*::*hisG-URA3-hisG* (JKC131). (*G*) *hst7*::*hisG/hst7*::*hisG/HST7-URA3* (JKC173). *Candida* strains were incubated for 5 days on Spider medium. (Bar = 2 mm.)

respond to serum. Serum must activate hyphal formation via a distinct signal transduction pathway. Based on the phenotypes of the double mutants, it is likely that these *Candida* genes, *CST20, HST7*, and *CPH1* like their *Saccharomyces* counterparts, are in the same pathway, though our experiments do not rule out more complicated models.

An unexpected feature of our analysis is the finding that all three heterozygotes (*CST20/cst20*, *HST7/hst7*, and *CPH1/cph1*) have obvious defects in filamentation, which suggests that the null alleles are not recessive. Furthermore, the defect in filamentous growth of the homozygote is not fully suppressed by introduction of a functional allele. By contrast, in *Saccharomyces* a single copy of a functional gene suffices to suppress the filamentation defect for any of the steps in the MAPK cascade. This phenomenon may be a reflection of the absence of a haploid phase in *Candida*. The existence of a haploid requires that a single copy of a gene provide sufficient activity to carry out its function. However, this requirement would not apply to obligate diploid organisms such as *Candida*, which has no known vegetative haploid stage. Thus, one interpretation of this data is that in *Candida* hyphal formation is sensitive to the dosage of the MAPK cascade components. The lack of dominance of the wild-type allele to the null allele has also been reported for the *Candida* drug transporter CaMDR and the *Candida MKC1* gene, a homolog of the *Saccharomyces* MAPK *SLT2/MPK1*. The heterozygotes of null mutations of these genes are more sensitive to inhibitors than the wild type (23, 24).

This model, which predicts that $+/+ > +/m > m/m$, agrees reasonably well with the observed phenotypes of strains with these genetic constitutions. However, the transformants $+/m/m$ are overall more filamentous than $+/m$, which does not fit the dosage model. An alternative possibility is that the partial dominance of the null mutation in the heterozygote is a reflection of a more exotic mechanism such as defective pairing between the normal and the deleted allele (25). Whatever the mechanism, the manifestation of mutant phenotypes in the heterozygote may facilitate the identification and isolation of *Candida* mutants.

We thank William Fonzi for the kind gift of *Candida* strain CAI4. We thank Don Higgins, Ekkehard Leberer, and Yona Kassir for kindly providing plasmids. We thank Haoping Liu, Jonathan Loeb, Bonnie Bartel, Judith Bender, and other members of the Fink laboratory for many fruitful discussions and Andrew Diener, Peter Hecht, Hiten Madhani, Rad Roberts, Steffen Rupp, and Eric Summers for helpful comments on the manuscript. J.R.K. thanks Ken McIntosh for support and encouragement. This work was supported by National Institutes of Health Grant GM40266 to G.R.F. J.R.K. was supported by National Research Service Award F32A109236. G.R.F. is an American Cancer Society Professor of Genetics.

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