Spt3 Plays Opposite Roles in Filamentous Growth in *Saccharomyces cerevisiae* and *Candida albicans* and Is Required for *C. albicans* Virulence

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ABSTRACT

Spt3 of *Saccharomyces cerevisiae* is required for the normal transcription of many genes *in vivo*. Past studies have shown that Spt3 is required for both mating and sporulation, two events that initiate when cells are at G_1/START. We now show that Spt3 is needed for two other events that begin at G_1/START, diploid filamentous growth and haploid invasive growth. In addition, Spt3 is required for normal expression of *FLO11*, a gene required for filamentous growth, although this defect is not the sole cause of the *spt3*Δ/*spt3*Δ filamentous growth defect. To extend our studies of Spt3's role in filamentous growth to the pathogenic yeast *Candida albicans*, we have identified the *C. albicans SPT3* gene and have studied its role in *C. albicans* filamentous growth and virulence. Surprisingly, *C. albicans spt3*Δ/*spt3*Δ mutants are hyperfilamentous, the opposite phenotype observed for *S. cerevisiae spt3*Δ/*spt3*Δ mutants. Furthermore, *C. albicans spt3*Δ/*spt3*Δ mutants are avirulent in mice. These experiments demonstrate that Spt3 plays important but opposite roles in filamentous growth in *S. cerevisiae* and *C. albicans*.

THE *Saccharomyces cerevisiae* transcription factor Spt3 was identified originally by mutations that suppress the transcriptional defects caused by Ty and Ty LTR insertion mutations (Winston *et al.* 1984a; reviewed in Winston and Sudarsanam 1998). Spt3 is required for the normal transcription of ~3% of *S. cerevisiae* genes (Lee *et al.* 2000). Both genetic and biochemical evidence suggest that Spt3 activates transcription by the recruitment of TATA-binding protein (TBP) to particular promoters (Eisenmann *et al.* 1992; Dudley *et al.* 1999; Bhaumik and Green 2001; Larschan and Winston 2001). Other evidence suggests that Spt3 can also repress transcription (Belotserkovskaya *et al.* 2000; Lee *et al.* 2000). The *SPT3* gene is functionally conserved among yeasts and other eukaryotes, including humans (Madison and Winston 1998; Martinez *et al.* 1998; Ogryzko *et al.* 1998; Yu *et al.* 1998).

Spt3 is a component of the SAGA (Spt-Ada-Gcn-Acetyltransferase) coactivator complex (Grant *et al.* 1998b; Winston and Sudarsanam 1998). SAGA is a 1.8-MD protein complex that contains >20 proteins. Both genetic and biochemical evidence have demonstrated that SAGA possesses distinct activities with respect to transcriptional control (Horiuchi *et al.* 1997; Roberts and Winston 1997; Sterner *et al.* 1999). For example, while Spt3 is required to recruit TBP, another SAGA member, Gcn5, has histone acetlytransferase activity (Brownell *et al.* 1996; Grant *et al.* 1997). In addition to Gcn5 and Spt3, SAGA contains several other classes of proteins, including those required for integrity of the complex (Spt7, Spt20, and Ada1) and for recruitment of the complex by transcriptional activators (Tra1; Grant *et al.* 1998a; Brown *et al.* 2001; Roth *et al.* 2001). In this article we focus primarily on the role of Spt3 in filamentous growth in *S. cerevisiae* and *Candida albicans*.

S. cerevisiae and *C. albicans* can each grow in both yeast and filamentous forms. The form of *S. cerevisiae* filamentous growth most intensively studied is diploid pseudohyphal growth (Gimeno *et al.* 1992; Pan *et al.* 2000; Gancedo 2001). Closely related to this form of growth is haploid invasive growth (Roberts and Fink 1994). In addition, a functionally unrelated form of haploid pseudohyphal growth has recently been demonstrated (Hollenhorst *et al.* 2000; Zhu *et al.* 2000). *C. albicans*, the most widespread yeast pathogen of humans, is responsible for clinical problems ranging from thrush and vaginal yeast infections to life-threatening systemic infections in immunocompromised patients (Odds 1988; Fidel and Sobel 1996). *C. albicans* has three forms of growth: one yeast-like form, called blastospores, and two filamentous forms, pseudohyphae and hyphae. The transition from blastospores to the filamentous forms appears to be required for pathogenicity (Kobayashi and Cutler 1998; Mitchell 1998). Several factors have been identified that contribute, either positively or negatively, for the transition from the blastospore to filamentous forms (Braun *et al.* 2001; Kadosh and Johnson 2001; Khalaf and Zitomer 2001; Murad *et al.* 2001; Navarro-Garcia *et al.* 2001). Many of these factors were identified on the basis of their homology to factors

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TABLE 1

***Saccharomyces cerevisiae* strains**

Strain	Genotype	Source
FY293	*MAT*α *spt3-202 ura3-52 lys2-173R2 his4-917*δ	
10560-6B	*MAT*α *ura3-52 leu2 trp1 his3*	Lo *et al.* (1997)
L5684	*MAT***a** *ura3-52 leu2*	Laboratory of G. Fink
YSL86	*MAT***a** *leu2::hisG ura3-52 flo11*Δ*::URA3*	Lo and Dranginis (1998)
L5366	*MAT***a**/*MAT*α *ura3-52/ura3-52*	Liu *et al.* (1993)
L959	*MAT***a** *ura3-52 spt3*Δ*203::TRP1 leu2*	This study
L962	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2/LEU2 his3/HIS3 spt3*Δ*203::TRP1/spt3*Δ*203::TRP1*	This study
L964	*MAT*α *ura3-52 spt3*Δ*203::TRP1 leu2 trp1 his3*	This study
L965	*MAT*α *ura3-52 leu2 trp1 spt7*Δ*402::LEU2*	This study
L970	*MAT***a**/*MAT*α *ura3-52/ura3-52 trp1/TRP1 his3/HIS3 leu2/leu2 spt7*Δ*402::LEU2/spt7*Δ*402::LEU2*	This study
L972	*MAT***a** *ura3-52 leu2 trp1 spt20*Δ*100::URA3*	This study
L973	*MAT*α *ura3-52 leu2 spt20*Δ*100::URA3*	This study
L974	*MAT***a** *ura3-52 leu2 his3 gcn5*Δ*::LEU2*	This study
L975	*MAT*α *ura3-52 leu2 his3 gcn5*Δ*::LEU2*	This study
L976	*MAT***a**/*MAT*α *ura3-52/ura3-52 leu2/leu2 trp1/TRP1 his3/HIS3 gcn5*Δ*::LEU2/gcn5*Δ*::LEU2*	This study
L1006	*MAT***a**/*MAT*α *ura3-52 or* Δ*0/ura3-52 or* Δ*0 trp1*Δ*0::hisG/trp1 leu2/LEU2 his3*Δ*0::hisG/HIS3* (pLP15)	This study
L1007	*MAT***a**/*MAT*α *flo11*Δ*::URA3/flo11*Δ*::URA3 ura3-52/ura3-52 his3*Δ*0::hisG/HIS3 trp1*Δ*0::hisG/trp1* (pLP15)	This study
L1009	*MAT***a**/*MAT*α *spt3*Δ*::kanMx/spt3*Δ*::LEU2 leu2*Δ*0/LEU2 ura3*Δ*0/ura3*Δ*0 trp1*Δ*0/trp1 his3*Δ*0::hisG/HIS3* (pRS416, pLP15)	This study
L1010	*MAT***a**/*MAT*α *ura3*Δ*0/URA3 leu2*Δ*0/LEU2 trp1*Δ*0::hisG/trp1*Δ*0::hisG* (RC4-2)	This study
L1011	*MAT***a**/*MAT*α *spt3*Δ*::kanMx4/spt3*Δ*::LEU2 leu2*Δ*0/LEU2 trp1*Δ*0::hisG trp1*Δ*0::hisG his3*Δ*0::hisG/HIS3 ura3*Δ*0/URA3* (RC4-2)	This study
L1012	*MAT***a**/*MAT*α *flo11*Δ*::URA3/flo11*Δ*::URA3 leu2/LEU2 trp1/trp1* (RC4-2)	This study
L1032	*MAT***a**/*MAT*α *spt3*Δ*::URA3/spt3*Δ*::LEU2 trp1*Δ*0::hisG/trp1 his3*Δ*0/HIS3 ura3*Δ*0/ura3*Δ*0* (pLP15)	This study

required for diploid pseudohyphal growth of *S. cerevisiae* (Kobayashi and Cutler 1998; Brown and Gow 1999; Whiteway 2000; Khalaf and Zitomer 2001). An understanding of the regulatory pathways and transcription factors that control the *C. albicans* transition from blastospore growth to filamentous growth should aid in the development of treatments for *C. albicans* infections.

Two aspects of Spt3 function led us to examine whether Spt3 and other SAGA components are involved in *S. cerevisiae* diploid pseudohyphal growth. First, *spt3*Δ mutants are defective in mating (Hirschhorn and Winston 1988) and sporulation (Winston *et al.* 1984b), which, like filamentous growth, are events that initiate at G_1/START of the cell cycle. Second, it seemed likely that Spt3 is required for transcription of the *FLO11* gene, which is required for diploid pseudohyphal growth (Lo and Dranginis 1996). This possibility became apparent because Spt3 is strongly required for transcription of Ty1 elements (Winston *et al.* 1984b), and Ty1 and *FLO11* transcription are regulated by many of the same factors (Madhani *et al.* 1997; Conte and Curcio 2000).

In this article we show that Spt3 is required for both diploid pseudohyphal growth and haploid invasive growth of *S. cerevisiae*. Consistent with these *spt3*Δ phenotypes, *spt3*Δ mutants have decreased *FLO11* mRNA levels. However, our results demonstrate that the defect in *FLO11* transcription is not the sole cause of the *spt3*Δ filamentous and invasive growth defects. In addition, we have identified the *C. albicans SPT3* gene and have constructed a *C. albicans spt3*Δ*/spt3*Δ mutant. In contrast to the *S. cerevisiae spt3*Δ*/spt3*Δ nonfilamentous phenotype, the *C. albicans spt3*Δ*/spt3*Δ mutant is hyperfilamentous. Furthermore, *C. albicans spt3*Δ*/spt3*Δ mutants are avirulent in mice. Taken together, our data demonstrate that *C. albicans* Spt3 acts as a repressor of filamentous growth and that it is also required for virulence.

MATERIALS AND METHODS

Yeast strains: All *S. cerevisiae* strains used in these studies (Table 1) are in the Σ1278b genetic background (Siddiqui and Brandriss 1988). The *spt3*Δ, *spt7*Δ, *spt20*Δ, and *gcn5*Δ deletion mutations were each recombined into strain 10560-6B (Lo *et al.* 1997) by standard gene replacement methods (Guthrie 1991), using the appropriate restriction fragments. The restriction fragments used were as follows: for *spt3*Δ*203::*

TABLE 2

***Candida albicans* strains**

Strain	Genotype	Source
SC5314	Wild type	Gillum *et al.* (1984)
BWP17	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	Wilson *et al.* (1999)
HLC54	*ura3::λimm434/ura3::λimm434 cph1::hisG/cph1::hisG efg1::hisG/efg1::hisG-URA3-hisG*	Lo *et al.* (1997)
FWC5	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::URA3/SPT3*	This study
FWC6	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::ARG4/SPT3*	This study
FWC7	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* (class 3)	This study
FWC8	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* (class 2)	This study
FWC9	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* (class 1)	This study
FWC10	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* pGEMT-*HIS1-SPT3* integrated (class 1)	This study
FWC11	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* pGEMT-*HIS1* integrated (class 3)	This study
FWC12	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisGarg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* pGEMT-*HIS1-SPT3* integrated (class 3)	This study
FWC13	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisGarg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* pGEMT-*HIS1* integrated (class 2)	This study
FWC14	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisGarg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* pGEMT-*HIS1-SPT3* integrated (class 2)	This study
FWC15	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::ARG4/spt3Δ::URA3* pGEMT-*HIS1* integrated (class 1)	This study
FWC16	*ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG spt3Δ::URA3/SPT3* pGEMT-HIS1 integrated pRSARG4 integrated	This study

TRP1, a 2.5-kb *Ssp*I fragment from pAH100 (Happel 1989); for *spt7Δ402::LEU2*, a 2.7-kb *Mlu*I-*Sph*I fragment from pLG59 (Gansheroff *et al.* 1995); for *spt20Δ100::URA3*, a 2.3-kb *Cla*I-*Xba*I fragment from pSR56 (Roberts and Winston 1996); and for *gcn5Δ::LEU2*, a 3.1-kb *Sph*I-*Sac*I fragment from YCplac111-Δ*gcn5::LEU2* (kindly provided by Kevin Struhl). The deletions were confirmed by PCR. These strains were then tested for several mutant phenotypes previously identified in the S288C background, including inositol auxotrophy, hydroxyurea sensitivity, the ability to use galactose as a carbon source, and slow growth on minimal media. In all cases, the mutant phenotypes correlated well between the two genetic backgrounds. One difference noted between the two genetic backgrounds is that an unusual cell morphology, previously observed in *spt7Δ* and *spt20Δ* mutants, is more severe in the Σ1278B background. The cells appear to be elongated and swollen and the buds do not always separate from the mother cell. However, these cells do not form distinct chains as do pseudohyphal cells (data not shown).

The *C. albicans* strains (Table 2) constructed for this study are derived from strains BWP17 (Wilson *et al.* 1999) and constructed by transformation (Hull and Johnson 1999). The *C. albicans spt3Δ/spt3Δ* homozygous diploid was constructed in two steps. First, the *Xcm*I-*Stu*I fragment of plasmid pLP13, containing the *spt3Δ::ARG4* allele, was used to transform BWP17 to Arg^+. Second, the *Xcm*I-*Stu*I fragment of plasmid pLP12, containing the *spt3Δ::URA3* allele, was used to transform the *SPT3/spt3Δ::ARG4* strain FWC6 to Ura^+ using Sc-Arg-Ura plates. A wild-type copy of *SPT3* was integrated into strains FWC7, FWC8, and FWC9 (*spt3Δ::URA3/spt3Δ::ARG4*) with *Nru*I-digested pLP14, resulting in integration of *SPT3* at *HIS1*. These transformants were selected on SC-Ura-Arg-His medium. The heterozygote strain FWC16 was created by transforming *Nru*I-digested pGEMT-HIS1 and *Cla*I-digested pRSARG4 into strain FWC5. For all *C. albicans* transformants, the correct integration event was confirmed by Southern blot analysis (Ausubel *et al.* 1988).

Media and growth conditions: Rich (YPD), minimal (SD), and synthetic complete (SC) media were prepared as previously described (Rose *et al.* 1990). SLAD media, for testing pseudohyphal growth of *S. cerevisiae* strains, was prepared as previously described (Gimeno *et al.* 1992). For growth of *C. albicans*, YPD was supplemented with 80 mg of uridine per liter. Two types of media were used for testing filamentous growth of *C. albicans*: first, medium 199 (GIBCO BRL, Gaithersburg, MD), made as previously described (Saporito-Irwin *et al.* 1995) and supplemented with 1.35% agar and 80 mg uridine per liter; second, liquid YPD was supplemented with fetal bovine calf serum (Sigma, St. Louis) to a final concentration of 20%. Haploid invasion and pseudohyphal growth assays were done as previously described (Roberts and Fink 1994). For copper induction of *CUP1-FLO11* the SLAD plates were supplemented with 0.05 mM and 0.1 mM $CuSO_4$. *C. albicans* strains were grown on YPD plates for 3 days at 30° and then the colonies were visualized and photographed using a Leica MZFLIII Microscope at ×4 magnification. Colonies grown on medium 199 were visualized and photographed after 1 day at 37° using a Nikon Eclipse E1000 Microscope with a ×20 DIC objective. All other cells were visualized and photographed with a Nikon Eclipse E1000 Microscope with a ×40 DIC objective. For growth in YPD with 20% fetal calf serum, 10 ml of YPD (20% serum) was inoculated and cultures were grown for 4 hr at 37°.

Cloning and DNA sequence analysis of the *C. albicans SPT3* gene: *C. albicans SPT3* was cloned by complementation of the *S. cerevisiae spt3Δ* mutant phenotypes. The host strain, FY293,

contains two Ty1-derived insertion mutations, *his4-917*δ and *lys2-173R2*. In an *SPT3* wild-type strain these insertion mutations confer His^- Lys^+ phenotypes; in an *spt3*Δ mutant such as FY293, the phenotypes are reversed to His^+ Lys^-. To clone *C. albicans SPT3*, strain FY293 was transformed with 0.5 μg of a YEp352 *C. albicans* genomic library (Navarro-Garcia *et al.* 1998). Approximately 80,000 Ura^+ transformants were then replica plated to screen for transformants that had an Spt^+ phenotype (His^- Lys^+). Of 221 candidates, plasmid DNA was isolated from 12 and used to retransform FY293. Eight of the 12 transformants had an Spt^+ (His^- Lys^+) phenotype. The DNA sequences of two candidates were determined by standard sequencing methods and universal M13 and synthetic primers. Sequencing was performed in the Biopolymers Facility in the Department of Genetics, Harvard Medical School. By Southern hybridization analysis, the cloned *C. albicans SPT3* gene hybridizes to *C. albicans* genomic DNA but not to *S. cerevisiae* genomic DNA.

Plasmids: All plasmids were constructed by standard procedures with exceptions noted below (Ausubel *et al.* 1988). Plasmid pLP15, used to overexpress *FLO11*, contains the *FLO11* open reading frame amplified from genomic DNA by PCR using Pfu polymerase (Stratagene, La Jolla, CA). The *Eco*RI-digested *FLO11* DNA was then ligated to *Eco*RI-digested and phosphatased pYSK7 (Butt *et al.* 1984). The ligations were then used to transform yeast, and strains containing the correct orientation of *FLO11* were confirmed by PCR, using primers internal to the *CUP1* and *FLO11* sequences. The resulting yeast strain was used in crosses to segregate the *CUP1-FLO11* plasmid into the strains of interest. Plasmids that contain the *spt3*Δ*::URA3* and *spt3*Δ*::ARG4* deletion constructs were each constructed in three steps. The *spt3*Δ*::URA3* construct was made by using primer pairs to amplify 346 bp upstream of *SPT3* and 371 bp downstream of *SPT3* and another set to amplify *URA3* from pGEMURA3 (Wilson *et al.* 1999). The primers were designed such that the resulting PCR products would overlap in subsequent PCR reactions to create a DNA fragment consisting of *URA3* flanked by sequences adjacent to the *SPT3* open reading frame (Amberg *et al.* 1995). The *C. albicans ARG4* gene was PCR amplified from pRSARG4ΔSpeI (Wilson *et al.* 1999), using primers that contain only 60 bp of homology to *C. albicans SPT3*. Each resulting PCR product was then used to construct a plasmid containing extensive homology to *SPT3* flanking sequences. This was done by mixing each PCR product with *Cla*I-linearized pLP11 (Yep352-*SPT3* from the *C. albicans* genomic library) and using this DNA to transform strain FY293. Ura^+ transformants were screened for an Spt^- phenotype, indicating that the *spt3*Δ construct had recombined to replace *SPT3* on the plasmid. Plasmids were then rescued from yeast and confirmed by standard procedures (Ausubel *et al.* 1988). These plasmids are pLP12 (YEp352-*spt3*Δ*::URA3*) and pLP13 (YEp352-*spt3*Δ*::ARG4*). Plasmid pLP14, used to rescue the *spt3*Δ homozygous mutant phenotype, was constructed from *Sac*I-*Nsi*I-digested pGEMT-HIS1 (Wilson *et al.* 1999) and a *Sac*I-*Nsi*I fragment from pLP11 (*SPT3*).

Overexpression of *FLO11*: The strains containing either the *CUP1-FLO11* plasmid or the *CUP1* vector only were grown overnight in SC-Trp liquid media with 0, 0.05, or 0.1 mM copper. The cultures were then plated for ~100 colonies per plate on SLAD containing 0, 0.05, or 0.1 mM copper. The plates were then incubated at 30° for 10 days and the colonies were scored each day for their pseudohyphal growth using a light microscope.

RNA isolation and Northern analysis: RNA isolation and Northern analysis were performed as previously described (Swanson *et al.* 1991). The haploid strains were grown in YPD to 1–2 × 10^7 cells/ml. To check expression of the *CUP1-FLO11* plasmid in diploid strains, the cultures were pregrown overnight in SC-Trp containing 0, 0.05, or 0.1 mM copper. Then the cultures were diluted back in fresh SC-Trp media with 0, 0.05, or 0.1 mM copper and grown to a cell density of 1–2 × 10^7 cells/ml. The *FLO11* levels were quantified using an *SPT15* normalization probe (Sudarsanam *et al.* 1999). The *FLO11* probe was PCR amplified (Rupp *et al.* 1999). All probes were labeled with [^{32}P]dATP by random priming (Ausubel *et al.* 1988).

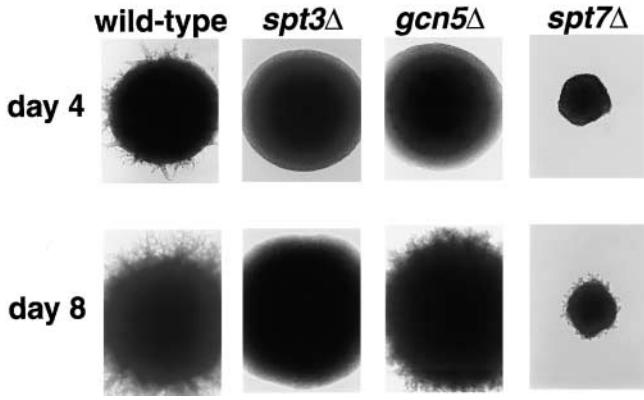

Figure 1.—Pseudohyphal growth of SAGA mutants. Comparison of wild-type (L5366), *spt3*Δ (L962), *gcn5*Δ (L976), and *spt7*Δ (L970) strains grown on SLAD plates at 30° for 4 days and 8 days.

Mouse studies: *C. albicans* strains used in infection experiments were grown in YPD + Uri to OD_{600} between 0.9 and 1, corresponding to 0.5–1.0 × 10^8 cells/ml, depending upon the particular strain. The *C. albicans* strains used were SC5314, HLC54, FWC15, FWC13, FWC11, FWC10, FWC14, and FWC12. *C. albicans* cells were counted and washed once with 0.5 ml of phosphate-buffered saline (PBS) pH 7. BALB/cJ mice 5–6 weeks of age were obtained from Jackson Laboratories and maintained in a barrier facility at Harvard Medical School. All mice were infected by injection of 300 μl of PBS suspension containing 5 × 10^6 cells into lateral tail veins. Four mice were tested for each of the three *spt3*Δ*/spt3*Δ mutant classes, their respective rescue strains, wild type, and a *cph1 efg1* double mutant used as a negative control (Lo *et al.* 1997). Progression of the disease was monitored several times a day in accordance to the Animal Experimentation Protocol approved by the HMS Standing Committee on Animals.

RESULTS

Spt3 is required for both pseudohyphal and invasive growth in *S. cerevisiae*: To study pseudohyphal filamentous growth in SAGA mutants, we first constructed strains that contain deletion mutations of four SAGA genes, *SPT3*, *SPT7*, *SPT20*, and *GCN5*, in a genetic background permissive for *S. cerevisiae* filamentous growth, Σ1278b (Gimeno *et al.* 1992; materials and methods). Then, diploids homozygous for each SAGA mutation to be tested were analyzed after incubation on solid medium that normally induces filamentous growth (SLAD medium). After growth on SLAD plates for 4 days, the wild-type colonies displayed normal filamentous growth, with long chains of pseudohyphae growing outward from the colony (Figure 1). In contrast, the SAGA mutants

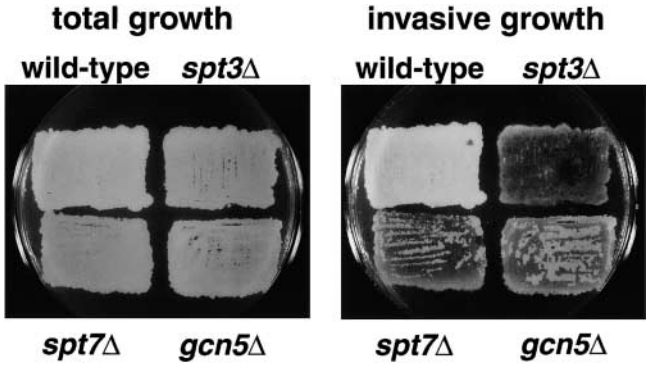

FIGURE 2.—SAGA mutants are defective for haploid invasion. (Left) Wild-type (L5684), *spt3*Δ (L959), *spt7*Δ (L965), and *gcn5*Δ (L974) strains after growing for 3 days at 30° and then an additional 2 days at 25°. (Right) The same strains after washing the cells off the plate under running water.

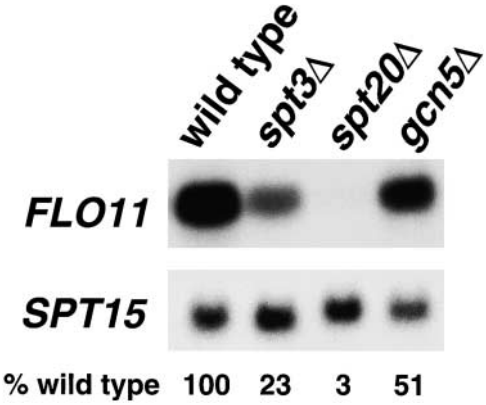

FIGURE 3.—*FLO11* mRNA levels are reduced in SAGA mutants. Total RNA was prepared from wild-type (L5684), *spt3*Δ (L959), *spt20*Δ (L973), and *gcn5*Δ (L975) strains. *SPT15* serves as a loading control. mRNA levels are normalized to the wild-type strain.

displayed a range of filamentous growth defects (Figure 1). The clearest defect was observed for the *spt3Δ/spt3Δ* mutant, which was completely defective for filamentous growth. The colonies had completely smooth edges and all cells examined were in the budding yeast form. The *gcn5Δ/gcn5Δ* mutant showed only a modest defect—after 4 days of incubation it displayed reduced filamentous growth, but after 8 days it was indistinguishable from the wild-type strain. Effects of the *spt7*Δ and *spt20*Δ mutations on filamentous growth were difficult to interpret as both mutants grew poorly and formed irregular cells and colonies under all growth conditions in the Σ1278b genetic background (Figure 1 and data not shown). Our results demonstrate that at least one SAGA component, Spt3, is essential for filamentous growth, while another, Gcn5, may play a minor role.

A process closely related to diploid filamentous growth is haploid invasion, characterized by filament formation and agar invasion (ROBERTS and FINK 1994). Previous studies have demonstrated that both types of filamentous growth are controlled by common factors (ROBERTS and FINK 1994). Therefore, we also tested haploid SAGA mutants for this growth property. Our results (Figure 2) show that, similar to the diploid pseudohyphal growth defects, *spt3*Δ mutants are defective for haploid invasive growth compared to wild type (Figure 2). The *gcn5*Δ, *spt7*Δ (Figure 2), and *spt20*Δ (data not shown) mutants are also defective for haploid invasion, although the defect appears to be less severe than for *spt3*Δ.

Analysis of *FLO11* expression and its relationship to the *spt3Δ/spt3Δ* filamentous growth defect: Expression of the *FLO11* gene is necessary for *S. cerevisiae* filamentous and invasive growth (LO and DRANGINIS 1998). Transcription of both *FLO11* and Ty1 elements depends upon a DNA sequence element called the filamentation response element that is cooperatively bound by the factors Ste12 and Tec1 (LO and DRANGINIS 1996; MADHANI *et al.* 1997; RUPP *et al.* 1999; CONTE and CURCIO 2000). Since several of the Spt proteins in SAGA are required for Ty1 transcription, we reasoned that Spt3, Spt7, Spt20, and Gcn5 may also be required for *FLO11* transcription. We initially attempted to measure *FLO11* mRNA levels in diploid strains. However, *FLO11* mRNA levels are very low in diploids, making accurate measurements difficult (data not shown). Therefore, we measured *FLO11* mRNA levels in haploid strains. Northern analysis (Figure 3) demonstrates that in *spt3*Δ mutants, *FLO11* mRNA levels are reduced approximately fivefold compared to wild-type levels. In *gcn5*Δ mutants, *FLO11* mRNA levels are reduced twofold, while in *spt20*Δ and *spt7*Δ mutants there is a severe reduction in *FLO11* mRNA levels (Figure 3 and data not shown). The relative effects among these three classes of SAGA mutants are similar to other SAGA mutant phenotypes that have been examined (HORIUCHI *et al.* 1997; ROBERTS and WINSTON 1997; STERNER *et al.* 1999).

To determine if the strong *spt3*Δ pseudohyphal and invasive growth defects are conferred solely by reduced *FLO11* mRNA levels, we expressed *FLO11* under the control of an Spt3-independent promoter, *CUP1* (*CUP1-FLO11*), and determined if ectopic *FLO11* expression suppresses the *spt3Δ/spt3Δ* defects. Our results show that *CUP-FLO11* expression is unable to suppress the pseudohyphal growth defect of an *spt3Δ/spt3Δ* mutant (data not shown). As a positive control for *FLO11* expression, *CUP1-FLO11* was shown to complement the filamentous growth defect of the *flo11*Δ mutant. In these experiments *FLO11* mRNA levels were approximately equal in the *flo11*Δ and *spt3*Δ strains and at a level greater than those in wild-type diploids without *CUP1-FLO11* (data not shown). Thus, Spt3's role in filamentous growth is not limited to a role in *FLO11* expression.

Cloning and characterization of the *C. albicans SPT3* gene: Several factors known to be required for *S. cerevis-*

iae filamentous growth are also required for *C. albicans* filamentous growth (Braun and Johnson 1997; Lo *et al.* 1997; Kadosh and Johnson 2001). Therefore, we decided to identify the *C. albicans SPT3* gene and test if it is also required for *C. albicans* filamentous growth. First, we cloned the *C. albicans SPT3* gene by complementation of an *S. cerevisiae spt3*Δ mutation (materials and methods). Sequence analysis identified a 986-bp open reading frame homologous to *S. cerevisiae SPT3*. This sequence is also now present in the *C. albicans* genome sequence database (http://genolist.pasteur.fr/CandidaDB/). The predicted *C. albicans* Spt3 protein is 59% identical and 79% similar to *S. cerevisiae* Spt3. The two histone-fold motifs that are conserved among previously analyzed Spt3 protein sequences from human, *S. cerevisiae*, and other yeasts (Birck *et al.* 1998; Madison and Winston 1998) are also conserved in the *C. albicans* Spt3 protein. The *C. albicans SPT3* gene strongly complements all *S. cerevisiae spt3*Δ mutant phenotypes tested, including the *spt3*Δ/*spt3*Δ pseudohyphal growth defect (Figure 4A), the *spt3*Δ growth defect on galactose as a carbon source, and the *spt3*Δ Spt$^-$ phenotypes (suppression of the *his4-917*δ and *lys2-173R2* insertion mutations; data not shown). These results demonstrate strong functional and sequence conservation between the *S. cerevisiae* and *C. albicans SPT3* genes.

***C. albicans spt3*Δ/*spt3*Δ mutants are hyperfilamentous:** To test the role of Spt3 in *C. albicans* filamentous growth, we constructed an *spt3*Δ/*spt3*Δ mutant. Since all *C. albicans* strains are diploids, we deleted both copies of *SPT3* in *C. albicans* strain BWP17, replacing one copy of *SPT3* with *URA3* and the second copy of *SPT3* with *ARG4* (described in materials and methods). Both gene replacements were confirmed by Southern analysis. The homozygous deletion mutant, *spt3*Δ*::URA3/spt3*Δ*::ARG4* (hereafter referred to as *spt3*Δ/*spt3*Δ), has several obvious growth phenotypes: (1) slow growth compared to wild-type strains in both liquid and solid media (Figure 4B); (2) flocculence in liquid media; and (3) a wrinkled colony morphology on YPD, SD, and SC solid media.

During purification of different initial isolates of *spt3*Δ/*spt3*Δ mutants, we noted that the growth and other mutant phenotypes varied over a moderate range. Fourteen *spt3*Δ/*spt3*Δ isolates that fell throughout this range of phenotypes were studied in greater detail. These isolates define three phenotypic classes (classes 1, 2, and 3). Class 1 mutants (seven isolates) grew as uniformly small, wrinkled colonies on YPD plates; class 2 mutants (four isolates) were more variable, with mostly small colonies, but also some larger smooth colonies; and class 3 mutants (three isolates) gave rise to a larger proportion of large, smooth colonies. As described below, these classes also vary with respect to filamentous growth, and the colony morphologies depended upon the auxotrophies in the strains. The different classes appear to have arisen during the strain constructions and their phenotypes remained stable during purification. Furthermore, these three classes were repeatedly observed when reconstructing the *spt3*Δ/*spt3*Δ homozygous mutant. The different classes might be caused by second-site mutations that modify the severity of the *spt3*Δ/*spt3*Δ-conferred growth defect. To confirm that the phenotypes observed in all three classes are caused by the *spt3*Δ/*spt3*Δ deletions, we integrated a wild-type copy of *SPT3* at the *HIS1* locus in a representative of each class. These transformants all have a wild-type growth rate and colony morphology. They are also wild-type with respect to most of the filamentous phenotypes, as described below. The only phenotype observed was a slightly larger cell size compared to the wild-type strain, a property we also saw for the *SPT3*/*spt3*Δ heterozygote (Figure 5B). Thus, the phenotypes observed in these *spt3*Δ/*spt3*Δ mutants are caused by loss of Spt3 function.

To test whether *C. albicans spt3*Δ/*spt3*Δ mutants have defects in filamentous growth, we first tested them under conditions noninducing for filamentous growth, on

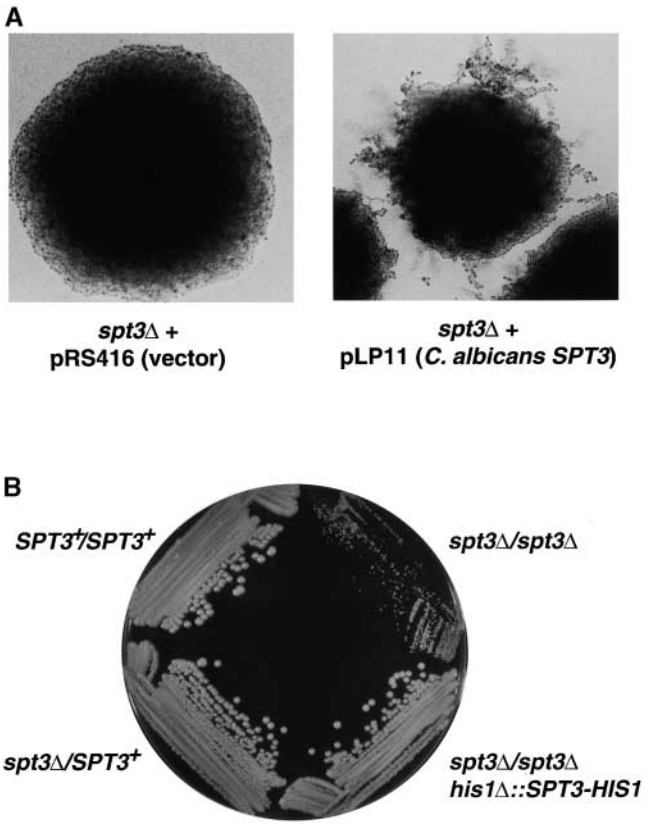

Figure 4.—(A) *C. albicans SPT3* complements the *S. cerevisiae spt3*Δ filamentous growth defect. Plasmid pRS416 (left) and plasmid pLP11 (right) were used to transform strain L962. Transformants were then tested for pseudohyphal growth. Shown are representative colonies after 4 days of incubation at 30°. (B) *C. albicans spt3*Δ/*spt3*Δ mutants have a slow-growth phenotype. Comparison of wild type (SC5314), *spt3*Δ class 1 mutant (FWC15), *spt3*Δ/*SPT3*$^+$ heterozygous strain (FWC16), and an *spt3*Δ class 1 mutant with a copy of *SPT3* integrated at *HIS1* (FWC10) after growth on YPD media at 30° for 2 days.

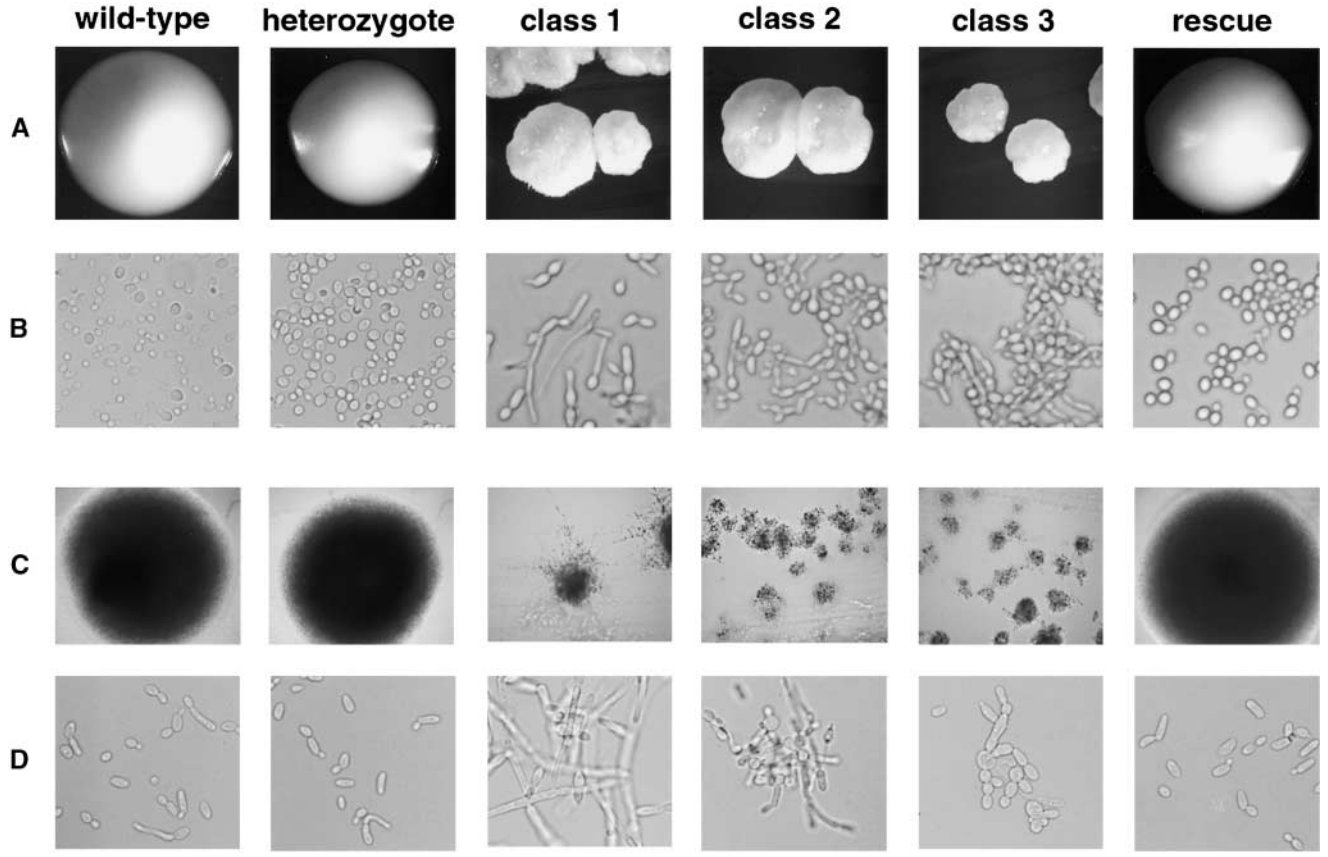

FIGURE 5.—*C. albicans spt3*Δ mutants are constitutive for filamentous growth. Column 1 shows a wild-type strain (SC5314); column 2 shows an *spt3*Δ/*SPT3*$^+$ heterozygous strain (FWC16); column 3 shows an *spt3*Δ class 1 mutant (FWC15); column 4 shows an *spt3*Δ class 2 mutant (FWC13); column 5 shows an *spt3*Δ class 3 mutant (FWC11); column 6 shows an *spt3*Δ class 1 mutant with a copy of *SPT3* integrated at *HIS1* (FWC10; rescue strain). (A) Colonies grown on YPD plates. The strains were incubated at 30° for 3 days and photographed using a ×4 objective. (B) Individual cells from a colony grown on a YPD plate at 30° for 3 days were resuspended in water and photographed using a ×40 DIC objective. (C) Colonies grown on medium 199 plates. The colonies were streaked from the permanent frozen stock and incubated at 37° for 1 day and then photographed using a ×20 DIC objective. (D) Individual cells from a colony grown on a medium 199 plate at 37° for 1 day were resuspended in water. The cells were then visualized using a ×40 DIC objective.

YPD solid media at 30°. For this analysis, we compared strains that were prototrophic, to avoid any possible effects of auxotrophies on growth. When the three classes of *spt3*Δ/*spt3*Δ mutants were in a prototrophic background, we no longer saw the colony size heterogenity that was observed for the original isolates. However, differences in the degree of filamentous growth were still observed, as described below. Under these growth conditions, wild-type *C. albicans* formed smooth and round colonies (Figure 5A) that contained all budding yeast (Figure 5B). In strong contrast, the *spt3*Δ/*spt3*Δ mutants formed wrinkled, irregularly shaped colonies that contained filaments (Figure 5, A and B). The *spt3*Δ/*spt3*Δ class 1 mutants formed colonies that were composed almost entirely of filamentously growing cells. The class 2 and class 3 mutants also formed wrinkled colonies. In these cases, the colonies contained some filaments, but had a greater proportion of budding yeast. The filamentous forms observed in these experiments consisted of both hyphae and pseudohyphae. All of the hyperfilamentous phenotypes observed in the *spt3*Δ/*spt3*Δ mutants on YPD were complemented by the introduction of a copy of wild-type *SPT3* (Figure 5, A and B; see column labeled "rescue"). Thus, although we observed a range in the severity of the hyperfilamentous phenotype, we conclude that the loss of Spt3 function in *C. albicans* results in filamentous growth under noninducing conditions.

We also examined filamentous growth of wild-type and *spt3*Δ/*spt3*Δ strains on M199 media, which induces wild-type *C. albicans* filamentous growth (SAPORITO-IRWIN *et al.* 1995). After one day of incubation at 37°, the wild-type colonies were smooth (Figure 5C) and contained cells that were mostly in the budding yeast form, with some germ tubes beginning to form (Figure 5D). After 2 days, more germ tubes began to appear and true hyphae appeared by 3–4 days of growth (data not shown). To test the *spt3*Δ/*spt3*Δ mutants, cells were plated from YPD-grown cultures that consisted of a mix of budding and hyphal forms for all three classes. In

contrast to the wild-type strain, the *spt3*Δ/*spt3*Δ class 1 mutant exhibited entirely hyphal growth after only 1 day of incubation (Figure 5, C and D). The *spt3*Δ/*spt3*Δ class 2 and 3 mutants exhibited a more intermediate mixture of cell types, containing both filamentous and budding forms. When a copy of wild-type *SPT3* was integrated into representatives of the three classes of *spt3*Δ/*spt3*Δ mutants, we observed complementation of the hyperfilamentous growth (Figure 5D). In conclusion, on inducing media, *spt3*Δ/*spt3*Δ mutants also exhibit hyperfilamentous growth.

***C. albicans spt3*Δ/*spt3*Δ mutants are avirulent in mice:** Previous studies of *C. albicans* mutants that are either nonfilamentous or hyperfilamentous have shown that they are avirulent (Braun *et al.* 2001; Kadosh and Johnson 2001; Murad *et al.* 2001; Navarro-Garcia *et al.* 2001). To test the virulence of *C. albicans spt3*Δ/*spt3*Δ mutants, we used them to infect mice and monitored mouse survival over a period of 30 days. In these experiments, we tested the three classes of *spt3*Δ/*spt3*Δ mutants. We also tested each of these mutants containing a wild-type copy of *SPT3* integrated at *HIS1*. For each infection, 5×10^6 cells were injected in the tail veins of BALB/cJ mice. Four mice were tested for each yeast strain. Our results (Figure 6) demonstrate that all three classes of *spt3*Δ/*spt3*Δ mutants are avirulent as all of the mice infected with the three classes of *spt3*Δ/*spt3*Δ mutants remained viable and healthy over the 30 days. In contrast, infection with the wild-type *C. albicans* strain resulted in the death of all four mice within 2 days. The three classes of *spt3*Δ/*spt3*Δ mutants that also contained a single copy of wild-type *SPT3* integrated at *HIS1* displayed partial complementation with an intermediate level of virulence, suggesting that virulence is sensitive to the level of Spt3. Taken together, these results demonstrate that virulence of *C. albicans* is dependent upon Spt3.

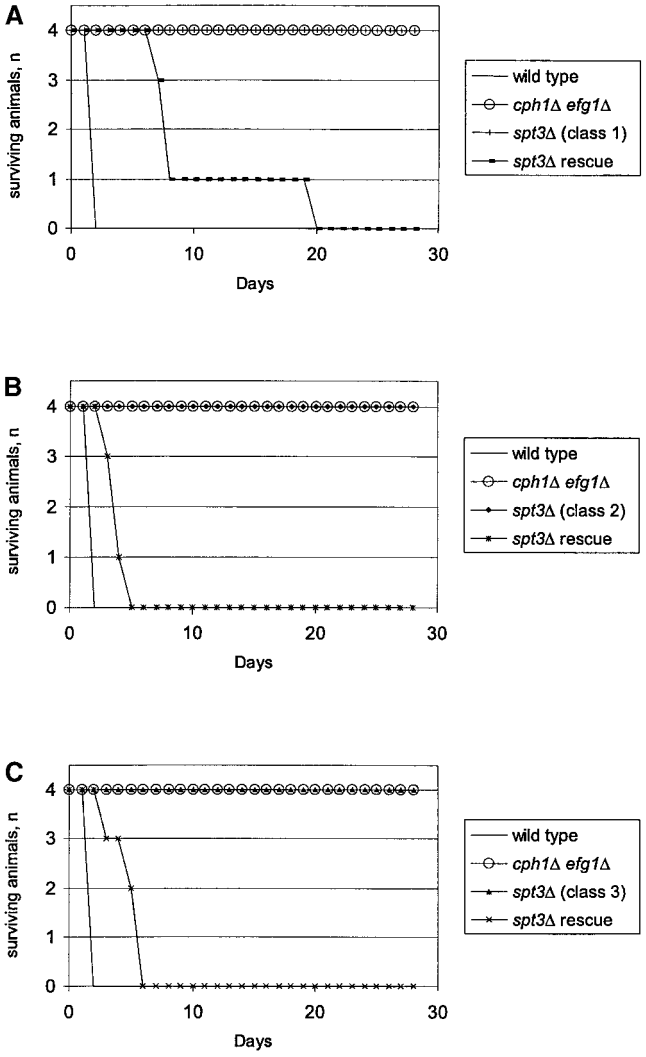

Figure 6.—*C. albicans spt3*Δ mutants are avirulent. Shown are survival plots over 30 days for 6-week-old BALB/cJ mice infected with 5×10^6 *C. albicans* cells. The strains used are as follows: (A) wild type, SC5314; *cph1*Δ *efg1*Δ, HLC54; *spt3*Δ class 1 mutant, FWC15; and *spt3*Δ class 1 mutant rescue (with *SPT3*$^+$ at *HIS1*), FWC10; (B) wild type, SC5314; *cph1*Δ *efg1*Δ, HLC54; *spt3*Δ class 2 mutant, FWC13; and *spt3*Δ class 2 mutant rescue (with *SPT3*$^+$ at *HIS1*), FWC14; (C) wild type, SC5314; *cph1*Δ *efg1*Δ, HLC54; *spt3*Δ class 3 mutant, FWC11; and *spt3*Δ class 3 mutant rescue (with *SPT3*$^+$ at *HIS1*), FWC12.

DISCUSSION

Our results show that Spt3 plays critical, but opposite roles in filamentous growth in both *S. cerevisiae* and *C. albicans*. In *S. cerevisiae*, an *spt3*Δ mutation causes severe defects in both pseudohyphal and invasive growth, suggesting that Spt3 plays a positive role in filamentous growth. In contrast, in *C. albicans*, an *spt3*Δ/*spt3*Δ mutation causes hyperfilamentous growth, strongly suggesting that Spt3 plays a negative role in filamentous growth. Furthermore, a *C. albicans spt3*Δ/*spt3*Δ mutant is avirulent in mice. Since the *C. albicans SPT3* gene can fully complement all tested *S. cerevisiae spt3*Δ mutant phenotypes, including the filamentous growth defect, we conclude that the opposite roles of Spt3 between the two yeasts are not caused by differences between the genes themselves, but rather by differences between the *S. cerevisiae* and *C. albicans* regulatory systems that control filamentous growth. The apparent negative role of Spt3 in *C. albicans* filamentous growth is consistent with evidence that Spt3 plays both positive and negative roles in transcription in *S. cerevisiae* (Belotserkovskaya *et al.* 2000; Lee *et al.* 2000).

Our results suggest that an *S. cerevisiae spt3*Δ mutant has multiple transcriptional defects that impair filamentous growth. In this work, we have shown that Spt3 is required for normal levels of *FLO11* mRNA. Possibly, a direct activator of *FLO11* recruits Spt3, as part of the SAGA complex, to the *FLO11* promoter to allow Spt3-dependent activation. Such an activity for Spt3 has been demonstrated at the *GAL1* promoter (Dudley *et al.*

1999; Bhaumik and Green 2001; Larschan and Winston 2001). Consistent with the idea that Spt3 acts directly at the *FLO11* promoter is genome-wide expression analysis that has shown that mRNA levels for three known regulators of *FLO11* transcription, *TEC1*, *STE12*, and *FLO8* (Rupp *et al.* 1999), are not significantly altered in an *spt3Δ* mutant (Lee *et al.* 2000). However, the *spt3Δ* effect on *FLO11* mRNA levels cannot be the only defect impairing filamentous growth, as ectopic expression of *FLO11* does not suppress the filamentous defect in *spt3Δ/spt3Δ* mutants. Genome-wide expression analysis of *spt3Δ* mutants grown in media that induces pseudohyphal growth will help to identify the extent of the requirement for Spt3 during filamentous growth.

Current evidence suggests that *C. albicans* Spt3 likely functions as the part of a protein complex in *C. albicans* that is similar to the SAGA complex of *S. cerevisiae*. Previous studies have shown that Spt3 and SAGA are conserved throughout eukaryotes (Madison and Winston 1998; Ogryzko *et al.* 1998; Yu *et al.* 1998; Martinez *et al.* 2001). Furthermore, the *C. albicans SPT3* gene fully complements an *S. cerevisiae spt3Δ* mutation, demonstrating strong functional conservation. In addition, sequences homologous to the SAGA genes *SPT7*, *SPT8*, *SPT20*, *ADA2*, and *GCN5* have also been found in the partially completed *C. albicans* genome sequence (http://genolist.pasteur.fr/CandidaDB/). Therefore, it seems likely that a *C. albicans* SAGA complex plays critical roles in transcription and filamentous growth. Since at least one SAGA member, Spt7, is only weakly conserved between yeast and humans (Martinez *et al.* 2001), a *C. albicans* SAGA complex may be a valuable drug target for impairing *C. albicans* growth and virulence.

One complication in our studies of *C. albicans spt3Δ/spt3Δ* mutants was that we observed three phenotypic classes that exhibited different degrees of hyperfilamentous growth. These different phenotypes may be caused by a second mutation in another gene that partially suppresses the *spt3Δ/spt3Δ* mutant phenotype. On the basis of studies in *S. cerevisiae*, the strongest candidates for such a gene are *SPT15* and *SPT8*, as mutations in these genes have been shown to have allele-specific interactions with mutations in *SPT3* (Eisenmann *et al.* 1992, 1994). Unfortunately, the inability to perform standard genetic analysis in *C. albicans* makes this hypothesis difficult to test.

Several different types of genes have been shown to be required for *C. albicans* virulence, including transcription factors that play positive and negative roles in controlling filamentous growth (Navarro-Garcia *et al.* 2001). In addition to Spt3, three other negative regulators of filamentous growth, Tup1, Rgt1, and Nrg1, have been previously identified and shown to be required for virulence (Braun and Johnson 2000; Braun *et al.* 2001; Kadosh and Johnson 2001; Khalaf and Zitomer 2001; Murad *et al.* 2001). At least two of these factors, Tup1 and Rfg1, play a positive role in *S. cerevisiae* filamentous growth and a negative role in *C. albicans* filamentous growth (Braun and Johnson 1997; Kadosh and Johnson 2001), similar to Spt3. However, in *S. cerevisiae* the regulatory roles of Spt3 and the SAGA complex are distinct from Tup1, Rox1, and Nrg1 (DeRisi *et al.* 1997; Lee *et al.* 2000), suggesting that Spt3 defines an independent regulatory pathway in *C. albicans* filamentous growth. Furthermore, the poor growth of *C. albicans spt3Δ/spt3Δ* mutants suggests that Spt3 plays an important role in controlling gene expression in *C. albicans*. Genome-wide expression analysis of *C. albicans spt3Δ/spt3Δ* mutants should help to elucidate further the role of Spt3 in *C. albicans* transcription and filamentous growth.

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