Control of Filament Formation in *Candida albicans* by the Transcriptional Repressor TUP1

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The pathogenic yeast *Candida albicans* regulates its cellular morphology in response to environmental conditions. Ellipsoidal, single cells (blastospores) predominate in rich media, whereas filaments composed of elongated cells that are attached end-to-end form in response to starvation, serum, and other conditions. The *TUP1* gene, which encodes a general transcriptional repressor in *Saccharomyces cerevisiae*, was isolated from *C. albicans* and disrupted. The resulting *tup1* mutant strain of *C. albicans* grew exclusively as filaments under all conditions tested. *TUP1* was epistatic to the transcriptional activator *CPH1*, previously found to promote filamentous growth. The results suggest a model where *TUP1* represses genes responsible for initiating filamentous growth and this repression is lifted under inducing environmental conditions.

The yeast *Candida albicans* is an opportunistic pathogen of humans, causing common superficial infections as well as life-threatening disseminated and organ infections. Fungal pathogens such as *C. albicans* are of increasing concern because of the rising incidence of immunosuppression brought about by AIDS, diabetes, cancer therapies, organ transplantation, and other conditions (*1*).

Typically, *C. albicans* grows as single ellipsoidal cells called blastospores (also called blastoconidia). In the presence of inducing environmental signals, *C. albicans* can assume filamentous forms in which cells remain attached to each other after dividing and thereby form long branched strings of connected cells. These filamentous forms range from pseudohyphae (where cells that form filaments are elongated, but still ellipsoidal) to true hyphae (where highly elongated cells that form the filaments are cylindrical and are separated by perpendicular septal walls). The ability of *C. albicans* to adopt these different morphologies is thought to contribute to colonization and dissemination within host tissues, and thereby to promote infection (*2*, *3*). All morphological forms can be found within infected tissues. In the laboratory, environmental conditions influence the morphological state of *C. albicans*. Serum causes blastospores to sprout true hyphae (termed germ tubes at their initial appearance). High temperature (37°C), high ratio of CO_2 to O_2, neutral pH, and nutrient-poor media also stimulate hyphal growth. Conversely, low temperatures, air, acidic pH (4 to 6), and enriched media promote blastospore growth (*2*, *4*). Intermediate conditions can induce various pseudohyphal forms as well as true hyphae (We use "filamentous" to refer to both pseudohyphae and hyphae).

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One pathway that regulates cell morphology in *C. albicans* has been discovered. The gene products of *CPH1*, *HST7*, and *CST20* are the *C. albicans* homologs of the *S. cerevisiae STE12*, *STE7*, and *STE20* products, respectively. *Candida albicans* strains mutant in any of these genes show retarded filamentous growth but no impairment of serum-induced germ tube and hyphae formation (*5*, *6*). These results suggest that a kinase signaling cascade, similar to that leading to *STE12* activation in *Saccharomyces cerevisiae*, plays a part in stimulating the morphological transition between blastospore and filamentous forms in *C. albicans*.

We now describe another regulator of filamentous growth, the *TUP1* gene, whose function has been studied in *S. cerevisiae*, where it represses transcription of many different genes (*7*–*9*). Targets of *TUP1* regulation include glucose-repressed genes, oxygen-repressed genes, DNA damage–induced genes, **a**-specific mating genes, haploid-specific genes, and flocculation genes. These sets of genes are each regulated by a distinct upstream DNA-binding protein, and each DNA-binding protein recruits to the promoter a complex containing the *TUP1* gene product. Several lines of evidence indicate that the *TUP1* gene product plays the principal role in bringing about transcriptional repression by mechanisms still not well understood (*10*).

In our search for homologs of *S. cerevisiae TUP1*, we isolated a gene from the closely related yeast *Kluyveromyces lactis*, which has the ability to complement a *tup1* deletion mutation in *S. cerevisiae* cells. The *K. lactis TUP1* gene was similar to *S. cerevisiae TUP1*, and we used the shared sequence information to design degenerate PCR (polymerase chain reaction) primers to amplify conserved regions in the COOH-terminus of *TUP1* from other organisms including *C. albicans*. The principal PCR product generated from *C. albicans* genomic DNA was cloned, sequenced, and used as a probe to isolate a full-length gene from a *C. albicans* genomic library (*11*). Sequencing and conceptual translation revealed an open reading frame similar to that of *TUP1* from *S. cerevisiae* (67% identity over the entire amino acid sequence) (Fig. 1A). Major conserved features were the seven WD40 repeats at the COOH-terminus of *TUP1* (which anchor *TUP1* to DNA-binding proteins) and the NH_2-terminus, including a proximal glutamine-rich segment (Fig. 1B). WD40 amino acid sequence repeats are found in many other proteins, including β subunits of heterotrimeric G proteins (*12*).

To determine whether the *C. albicans TUP1* gene had functional as well as structural similarity to *S. cerevisiae TUP1*, we obtained expression of *C. albicans TUP1* under galactose control in *tup1 S. cerevisiae* cells. The overexpressed *C. albicans* gene restored repression of a genomic **a**-specific gene reporter, *Mfa2:lacZ* to that in wild-type (*13*). In addition, *tup1 S. cerevisiae* cells overexpressing the *C. albicans TUP1* were not flocculent, were not temperature sensitive, exhibited wild-type cell shape, and grew rapidly, indicating that several other phenotypes characteristic of *tup1* cells had also been corrected by the *C. albicans* gene. Thus in these two species, *TUP1* apparently has the same molecular function: It is recruited to DNA by various DNA binding proteins, and it represses transcription.

To determine which pathways are controlled by the *TUP1* repressor in *C. albicans*, which is diploid, we disrupted both copies of the gene in two rounds (*14*). The disruption consisted of a large deletion that excised most of the *TUP1* gene as well as 330 bp of DNA upstream of the open reading frame. To ensure that the phenotypes described below resulted from loss of *TUP1* function rather than loss of the upstream DNA or other features of the locus separate from the *TUP1* open reading frame, we performed a second round of disruption with a DNA fragment that carried *tup1 C. albicans* with an NH_2-terminal frameshift mutation instead of a large deletion (Fig. 2A). The resulting strains were, in all respects, phenotypically identical to the homozygous mutant strains carrying the large deletions of *TUP1*, which are described below. Wild-type *C. albicans* phenotypes were fully restored by insertion of a wild-type copy of the *TUP1* gene linked to an adjacent *URA3* marker (Fig. 2A) back into the disrupted locus (Fig. 2B, lane 4). Furthermore, insertion of a wild-type copy of the gene under the control of a maltase promoter into the genome also rescued the *tup1* deletion mutant phenotypes in a maltose-dependent manner (*13*).

Differences were observed when *tup1* and wild-type C. *albicans* were compared under the microscope (Fig. 3) (*15*). For A to D, both strains were grown under conditions (YEPD) (*16*) that favor the blastospore form of growth, and, as expected, the wild-type strain exhibited the blastospore form under these conditions (Fig. 3, A and C). In contrast, the homozygous *tup1*/*tup1* mutant strain was completely filamentous (Fig. 3, B and D). The mutant strain formed only filaments on all media tested, including common and specialized media, namely, YEPD, YD, Saboraud, corn meal with or without Tween 80, Spider, 20% calf serum, Lee's defined, and minimal S medium with a variety of fermentable and nonfermentable carbon sources (*16*). On most media, mutant cells grew as pseudohyphae rather than as true hyphae; but under certain hyphal-inducing conditions, they attained elongated and straight-walled shapes indistinguishable from those of true hyphae (Fig. 3, B and D; and Fig. 4). Some of these conditions included growth on nutrient-poor media such as corn meal agar, and micro-aerobic growth under glass coverslips. The distinction between true hyphae and pseudohyphae is based on cell shape and cell division timing, and a spectrum of intermediate morphologies is observed in wild-type C. *albicans* cells (*2*, *4*, *6*).

Closer examination of homozygous *tup1* mutant cells revealed that, apart from their overall altered morphology, they resembled filamentous wild-type cells in most respects (Fig. 3, E and F). In particular, DNA was centrally located in non-mitotic cells, filaments branched several septal compartments behind the growing hyphal tip, and branches were situated near the apical septa, as is normally seen in wild-type C. *albicans*. One minor difference was that the mutant cells often had slightly misshapen cell walls (Fig. 3F).

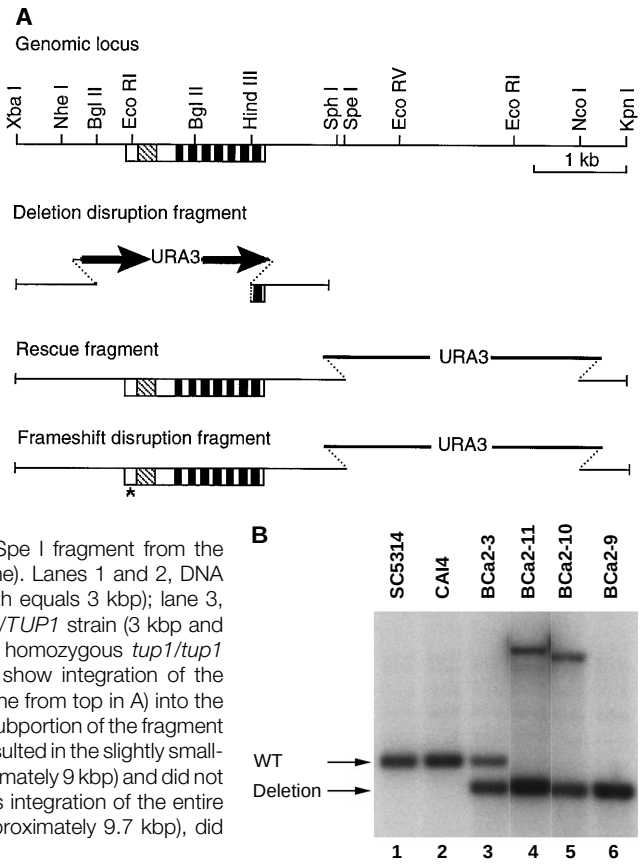

Fig. 2. Disruption of *C. albicans TUP1*. (**A**) The open reading frame of the *C. albicans TUP1* locus shows as a box containing conserved sequence elements (as in Fig. 1B). The top line represents the original genomic clone, the insert of plasmid p371. The second line represents the disruption fragment contained on p383C. The third line represents the rescuing fragment carried on p405, and the last line corresponds to the frameshift mutant (p418), created by filling in the indicated Eco RI site of p405. (**B**) A DNA blot of *C. albicans* genomic DNA (cut with Nhe I–Spe I) was probed with the Hind III–Spe I fragment from the *TUP1* genomic locus (A, top line). Lanes 1 and 2, DNA from *TUP1*/*TUP1* strains (length equals 3 kbp); lane 3, DNA from a heterozygous *tup1*/*TUP1* strain (3 kbp and 2.3 kbp); lane 6, DNA from a homozygous *tup1*/*tup1* mutant strain. Lanes 4 and 5 show integration of the p405 rescuing fragment (third line from top in A) into the *TUP1* locus. Integration of the subportion of the fragment with *URA3* but without *TUP1* resulted in the slightly smaller band shown in lane 5 (approximately 9 kbp) and did not restore TUP1 function, whereas integration of the entire fragment, shown in lane 4 (approximately 9.7 kbp), did restore TUP1 function.

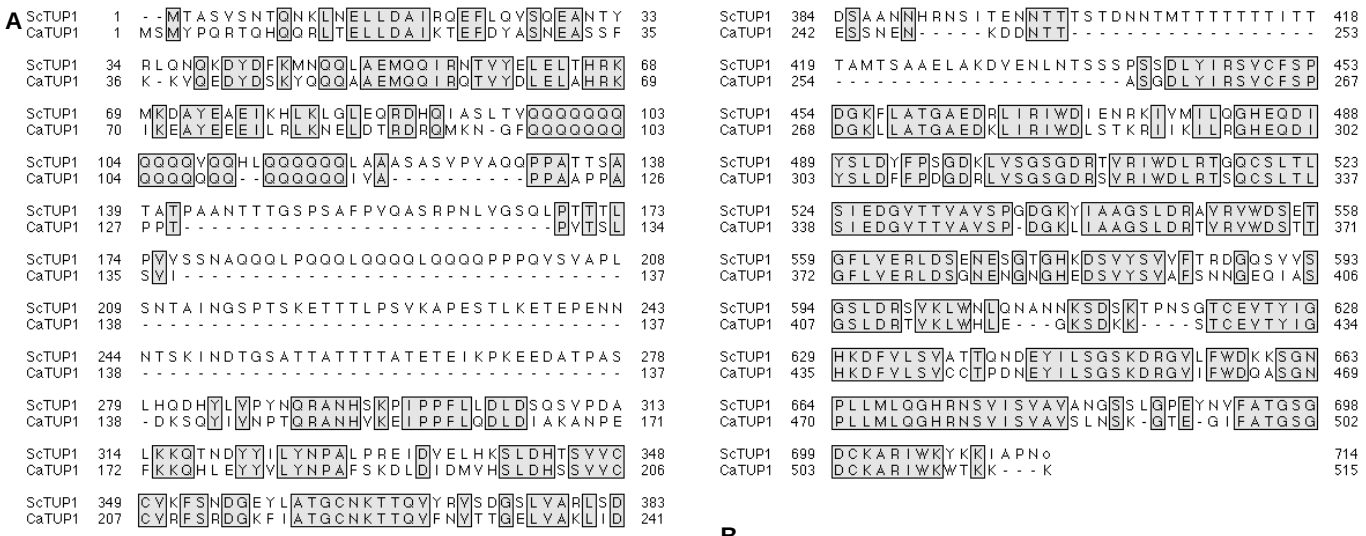

Fig. 1. Sequence of *C. albicans TUP1*. (**A**) *TUP1* gene products from *C. albicans* and *S. cerevisiae*, as conceptually translated from their respective DNA sequences, are compared. Alignment was performed by the program pileup (GCG, Inc.), and identities are highlighted. (**B**) The two *TUP1* gene products and the relative arrangement of their NH_2-terminal conserved domains (hatched) and their COOH-terminal WD40 repeated motifs (filled). Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K. Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Heterozygous *TUP1*/*tup1* strains showed a morphological phenotype intermediate between the wild-type and homozygous strains. Although their cells resembled wild-type cells in morphology, on most media heterozygous colonies developed a higher proportion of filaments compared to wild-type colonies (Fig. 4B), confirming the filament-repressing role of *TUP1* and suggesting that its gene product is present in limiting amounts.

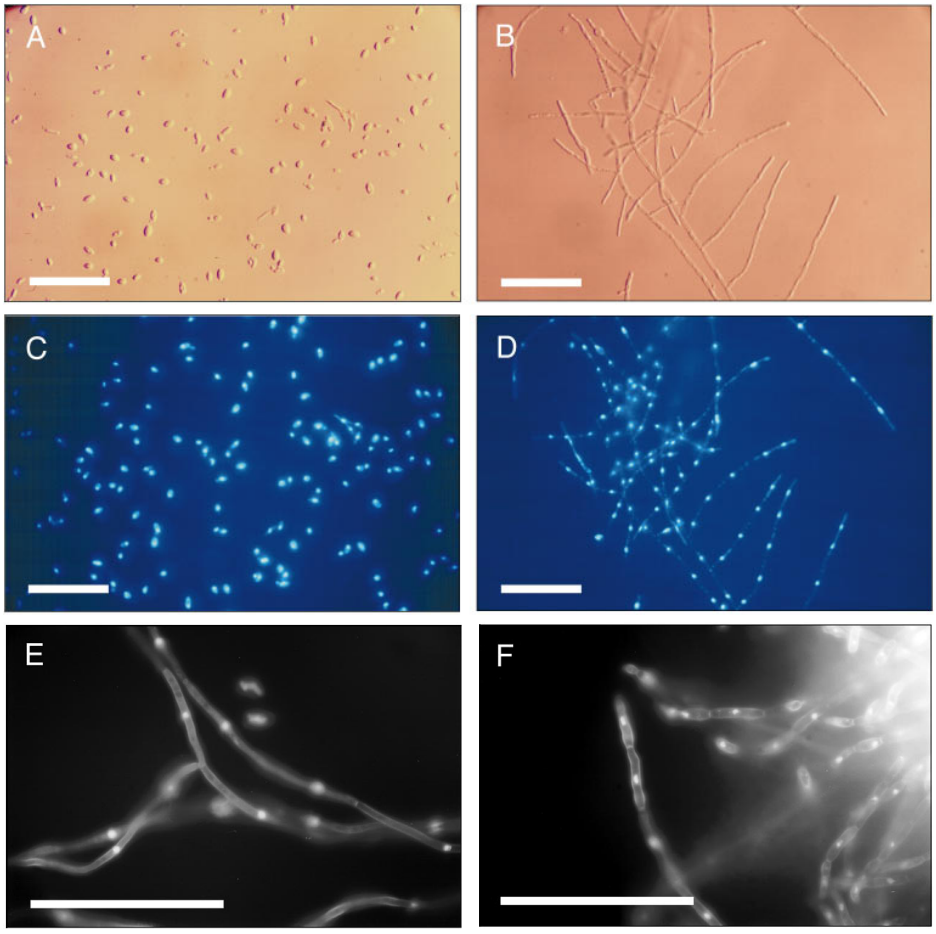

Fig. 3. Morphological characteristics of *tup1 C. albicans*. (**A** and **C**) Wild-type cells (SC5314) and (**B** and **D**) *tup1* cells (BCa2-10) were grown in YEPD at 30°C to late log phase and stained with DAPI (*15*) to highlight the DNA before being photographed at 40× through differential interference contrast (A and B) and fluorescence optics (C and D). (**E**) Wild-type cells (SC5314) and (**F**) *tup1* cells (BCa2-10) were grown in Lee's medium, pH 6.7, at 37°C, conditions that promote germ tube formation and hyphal growth, and then stained with calcofluor and DAPI to highlight both the cell walls and DNA before being photographed at 100× through fluorescence optics. Scale bar, 50 μM.

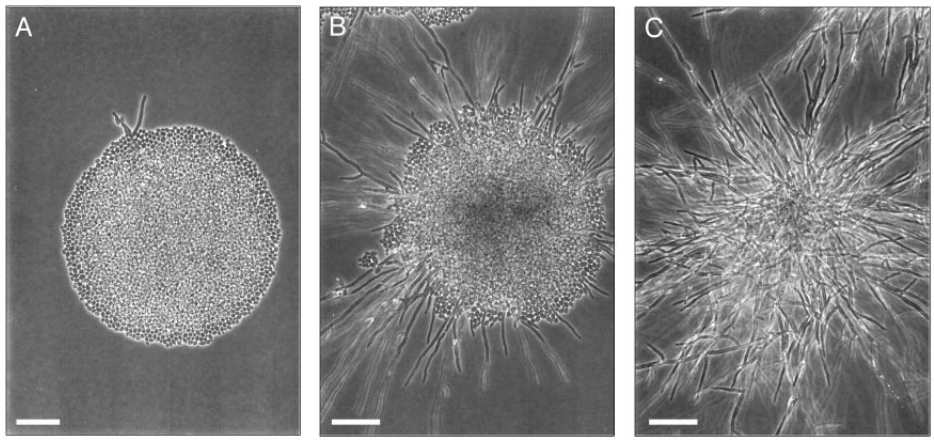

Fig. 4. Colony growth of homozygous and heterozygous *tup1* strains. (**A**) Wild-type (SC5314), (**B**) heterozygous (BCa2-3′), and (**C**) homozygous (BCa2-10) cells were placed on a cornmeal agar plus Tween 80 plate under a coverslip and grown for 25 hours at 25°C before being photographed at 40× with phase optics. Scale bar equals 50 μM.

Whereas deletion of the *TUP1* gene caused constitutive filamentous growth in *C. albicans*, there was a surprising lack of response of *tup1* cells to some strong germ tube and filamentous growth inducers such as mammalian serum and Lee's medium. Germ tube formation from the blastospore state is a special property of *C. albicans* and as such is used for clinical identification. Wild-type and *TUP1*/*tup1* heterozygous blastospores exhibited rapid germ tube formation progressing to true hyphae on YEPD or minimal media containing 10 to 20% calf serum (*2*, *3*). However, in these same media the homozygous *tup1* mutant cells showed no detectable change in filamentous morphology; in particular, they showed no sign of germ tubes or of increased transformation toward true hyphae. The blastospore to hypha transition can also be experimentally manipulated with the defined medium developed by Lee *et al.* (*17*) which, depending on the pH and temperature of incubation, promotes blastospore growth or germ tube formation and filamentous growth. As on serum, *tup1* mutant cells were unaffected by Lee's medium and grew with the same filamentous morphology regardless of pH and temperature (Fig. 4F). One hypothesis to explain these observations is that initiation of the pathway blastospore to germ tube to hyphae requires the blastospore cell type. Since this cell type is absent in the *tup1* homozygote, the pathway would, according to the hypothesis, fail to initiate. Another hypothesis is that serum induction normally operates through *TUP1*.

tup1 mutants of *S. cerevisiae* show various phenotypes including sensitivity to 37°C, slow growth, lack of glucose repression, poor growth on glycerol, inability of the α cell type to mate, inability to sporulate, flocculence, and irregular cell shape (*7*, *9*). We therefore examined the *tup1* strains of *C. albicans* for additional phenotypes. Differences in the growth rate between wild-type and *tup1* *C. albicans* were examined under numerous growth conditions. After a slightly longer lag time, growth of the homozygous *tup1* mutant strain (BCa2-10) was virtually as rapid as the wild-type cells in rich YEPD media (doubling times of 64 and 58 min in log phase, respectively, as assayed by optical density at 600 nm). Growth of the mutant cells was arrested at 42°C but was normal at 37°C, whereas wild-type cells grew at both temperatures. No auxotrophies were detected, and growth on most carbon sources was similar. Growth of the strains on sucrose, glucose, galactose, and

acetate was comparable.

One of the few metabolic phenotypes identified in *tup1* C. *albicans* was a faster growth rate and accumulation to higher density on glycerol when compared to wild-type. During growth on glycerol and acetate, *tup1* mutant cells exhibited the shortest cell length of all conditions tested. Short chains of stubby cells were typical, with poor cell-to-cell attachment marked by occasional single elongated cells. A formal notation, termed morphological index (Mi) has been developed to describe C. *albicans* cell shape (*18*). According to this system, where blastospores rank at 1 and true hyphae rank near 4, the *tup1* mutant has values of 3.0 to 3.5 when grown on YEPD, and values of 1.5 to 2.5 when grown in minimal medium with glycerol.

The foregoing observations indicate that *tup1* C. *albicans* has several mutant phenotypes; some (temperature-sensitive growth, for example) are similar to those of *tup1* mutants of *S. cerevisiae*. In most respects, however, the effects of a *TUP1* deletion appeared different in the two species. Since *S. cerevisiae* is capable of filamentous growth we also determined the effects of a *TUP1* deletion on filamentous growth in *S. cerevisiae*.

Saccharomyces cerevisiae exhibits filamentous growth (exclusively in the form of pseudohyphae) in response to nitrogen starvation in diploid cells and in response to unknown inducers in haploid cells (*19*, *20*). *Saccharomyces cerevisiae* strains that do exhibit filamentous growth (*21*), and **a**/α diploid homozygous *tup1* derivatives were constructed (*22*). The resulting strains exhibited typical *tup1* phenotypes, such as flocculence, temperature-sensitive growth, and an inability to sporulate; however, when grown on pseudohyphal growth-inducing media (SLAHD), they showed a marked reduction of pseudohyphal growth. Haploid cell types of *S. cerevisiae* show a different type of filamentous growth, termed invasive growth (*20*). Invasive growth was reduced in haploid *tup1 S. cerevisiae* strains derived from the diploids described above. The interpretation of these observations is complicated by the multiple defects of *tup1* mutant strains, especially since *TUP1* is required to maintain the **a**/α and α cell types of *S. cerevisiae*. However, *TUP1* does not repress filamentous growth in *S. cerevisiae* as it does in C. *albicans*.

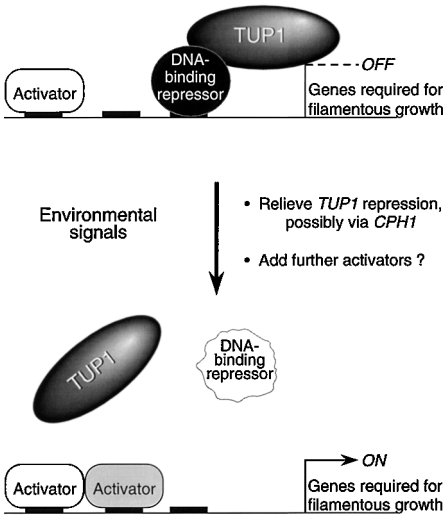

Fig. 5. Model for control of filamentous growth in *C. albicans* by *TUP1*. Repression by *TUP1* is regulated by environmental signals through a postulated DNA-binding protein. One regulator of this DNA-binding protein may be *CPH1*, which is placed upstream of *TUP1* based on epistasis of *TUP1* to *CPH1*. In the absence of *TUP1* repression, filamentous growth is constitutive and still responds to some environmental signals, suggesting the presence of both regulated and constitutive activators at genes controlled by *TUP1*.

CPH1 is a transcriptional activator that positively regulates filamentous growth in C. *albicans*. Filamentous growth is reduced in *cph1* cells under certain conditions (*5*). We constructed a double mutant to investigate the interaction of *TUP1* and *CPH1*. This *tup1*/*tup1 cph1*/*cph1* strain was indistinguishable in its morphological characteristics from strains containing the *tup1* mutations alone. The *cph1* mutant strains that were heterozygous for *TUP1*, however, resembled wild-type cells in their tendency to form filaments in some media, such as pH 7.0 Spider plates. That is, they were intermediate between *cph1* mutants (which induce more poorly than wild type) and double *tup1 cph1* mutants (which are constitutively induced for filamentous growth), suggesting that both genes participate in the same pathway.

If we assume that the C. *albicans* blastospore is the default state, the finding that deletion of *TUP1* activates a filamentous morphology regardless of external conditions indicates that *TUP1* is a repressor of filamentous development. In the yeast *S. cerevisiae*, *TUP1* is a transcriptional repressor and two lines of evidence indicate that *TUP1* has the same molecular function in C. *albicans*: (i) the two proteins show a high degree of amino acid sequence conservation (Fig. 3A) and (ii) expression of the C. *albicans TUP1* gene fully complemented *S. cerevisiae* cells carrying a *tup1* deletion. On the basis of these results, we offer a simple model for the involvement of *TUP1* in the blastospore to filamentous growth transition of C. *albicans* (Fig. 5).

According to this model, TUP1 represses genes whose expression is required to initiate or maintain filamentous growth. *TUP1* is brought to the DNA upstream of these genes by postulated DNA-binding protein (or proteins) whose synthesis or activity is down-regulated by filamentous growth-inducing environmental conditions. In the absence of *TUP1*, its target genes are always expressed, leading to constitutive filamentous growth. In this model the activator of filamentous growth, *CPH1*, is placed upstream of *TUP1* since the phenotypes of the double mutant *tup1*Δ*cph1*Δ resemble those of the *tup1*Δ mutant. More complicated models are also consistent with our observations. For example, *TUP1* might participate in one of several redundant pathways through which genes required for filamentous growth can be turned on. Another possibility is that the absence of *TUP1* might alter cell physiology (via a stress response or metabolic defect, for example) in a general way that makes blastospore formation impossible. Although we believe that the specific morphological phenotype of the *tup1*Δ mutant cells as well as their general vigor argues for a direct role of *TUP1* in regulating filamentous growth, these alternative models are formally possible. The fact that serum and pH activation of germ tube formation and hyphal growth were absent in the *tup1*Δ mutant cells suggests that *TUP1* may also have a role in this pathway (*23*).

A comparison of the phenotypes produced by a *TUP1* deletion suggests that *TUP1* controls genes in C. *albicans* that are different from those it controls in *S. cerevisiae*. For example, disruption of *TUP1* in C. *albicans* results in constitutive filamentous growth and enhanced growth in glycerol, two phenotypes not seen in a *S. cerevisiae tup1*Δ strain. Likewise, flocculence and defects in glucose repression are properties of *S. cerevisiae tup1*Δ strains, but are not seen in C. *albicans tup1*Δ strains. Given that the C. *albicans* gene complements a *S. cerevisiae tup1*Δ mutant, it seems likely that *TUP1* serves as a transcriptional repressor in both species and that interactions between the *TUP1* gene product and the DNA-binding proteins to which it binds have been conserved. Therefore, it appears that the regulation of these DNA-binding proteins and the identity of the genes to which they bind have changed since *S. cerevisiae* and C. *albicans* diverged from a common ancestor.

REFERENCES AND NOTES

1. R. H. Rubin, *Eur. J. Clin. Microbiol. Infect. Dis.* **12** Suppl. 1, S42 (1993); M. N. Dudley and R. T. Schiefe, Eds., *Pharmacotherapy* **10**, 133 (1990).
2. F. C. Odds, *Candida and Candidosis* (Baillière Tindall, London, ed. 2, 1988).
3. ———, *Am. Soc. Microbiol. News* **60**, 313 (1994); *J. Am. Acad. Dermatol.* **31**, S2 (1994).
4. ———, *Crit. Rev. Microbiol.* **12**, 45 (1985); N. A. Gow and G. W. Gooday, *Sabouraudia* **22**, 137 (1984).

5. H. Liu, J. Kohler, G. R. Fink, *Science* **266**, 1723 (1994).
6. S. J. Kron and N. A. Gow, *Curr. Opin. Cell. Biol.* **7**, 845 (1995); J. R. Köhler and G. R. Fink, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13223 (1996); E. Leberer *et al.*, *ibid.*, p. 132217.
7. F. E. Williams and R. J. Trumbly, *Mol. Cell. Biol.* **10**, 6500 (1990).
8. C. A. Keleher, M. J. Redd, J. Schultz, M. Carlson, A. D. Johnson, *Cell* **68**, 709 (1992).
9. J. F. Lemontt, D. R. Fugit, V. L. Mackay, *Genetics* **94**, 899 (1980).
10. D. Tzamarias and K. Struhl, *Nature* **369**, 758 (1994); K. Komachi, M. J. Redd, A. D. Johnson, *Genes Dev.* **8**, 2857 (1994); M. Wahi and A. D. Johnson, *Genetics* **140**, 79 (1995); D. G. Edmondson, M. M. Smith, S. Y. Roth, *Genes Dev.* **10**, 1247 (1996).
11. PCR primers used to amplify a fragment of *C. albicans TUP1* were: 5′GGGGTACCYTTCCADATNCKNGCYTTRCARTCNCC, coding in reverse for COOH-terminal GDCKARIWK, [A. Cornish-Bowden, *Nucleic Acids Res.* **13**, 3021 (1985)]; and 5′GGCTGCAGGGNCAYGARCARGAYATHTAYTC, coding for NH_2-terminal GHEQDIYS. Cycling parameters were 1 min at 95°C, 1 min at 55°C, 1 min ramp to 73°C, and 3 min at 73°C (Perkin-Elmer Cetus 480 cycler). The resulting 659-bp PCR fragment was cloned via the Pst I and Kpn I sites on the ends into Bluescript-derived pVZ1 to produce p348. A λ library of *C. albicans* genomic DNA was provided by N. Agabian and colleagues (UCSF), and was screened with labeled insert from p348. A 7-kbp *TUP1*-containing Kpn I–Xba I fragment from λ363 was cloned into pVZ1 to form p371. Both strands of the *TUP1* open reading frame were sequenced. The *C. albicans TUP1* DNA and protein sequences have been deposited in GenBank (AF005741).
12. M. A. Wall *et al*., *Cell* **83**, 1047 (1995).
13. The *C. albicans TUP1* open reading frame was amplified with Pfu polymerase (Stratigene) and the primers: 5′CGCGGATCCCCACCAGCAATGTCCATGTAT; 5′GCGGGTACCGCGATGTTGACGGGTGCTGT. The product was cloned into the *CEN/ARS/URA3/Gal1-10* expression vector pRD53 (provided by R. Deshaies, California Institute of Technology) to form the *S. cerevisiae* expression plasmid pMH1. *C. albicans TUP1* contains no CUG codons, which encode serine in *C. albicans*, but encode leucine in *S. cerevisiae* and elsewhere [T. Ohama, *et al*., *Nucleic Acids Res.* **21**, 4039 (1993)]. The same PCR product was cloned into pDBV52 (provided by C. Kumamoto and D. Brown, Tufts) to form the maltose-regulated expression plasmid p455, which was transformed into BCa2-9. pAJ181 has been described (*8*). To assess *TUP1* function, β-galactosidase activity was assayed from *tup1 S. cerevisiae* (KKY110) carrying the plasmids described above. KKY110 (*Matα*, *tup1*, *mfa2*::*lacZ*, *leu2*, *ura3*, *trp1*, *his4*; provided by K. Komachi, UCSF) had a β-galactosidase reporter gene under α2/*MCM1*/*TUP1* control integrated at the *MFA2* gene. On glucose, the vector (pRD53) conferred 82 ± 16 units, (no repression); pAJ181 (*S. cerevisiae TUP1*) conferred 3.8 ± 0.9 units; and pMH1 (Gal-driven *C. albicans TUP1*) conferred 32 ± 5 units. On galactose, the vector conferred 83 + 27 units (no repression); pAJ181, 0.7 ± 0.5 units; and pMH1, 0.5 ± 0.4 units (full repression).
14. W. A. Fonzi and M. Y. Irwin, *Genetics* **134**, 717 (1993). The *C. albicans URA3* gene, flanked by tandemly repeated DNA sequences, was inserted in place of *TUP1* within the genomic clone (Fig. 2A) to form p383C, which was treated with Sph I to remove the vector and transformed into a *ura3 C. albicans* cell (CAI4). The *URA3* transformants were screened by DNA blotting for disruption of one *TUP1* gene by homologous recombination. After being selected on 5-FOA for *ura3* "pop-out" revertants, a second cycle of transformation was performed. DNA blotting revealed the successive disruption of both copies of the *TUP1* gene (Fig. 2B, compare lanes 2, 3, and 6). Transformations of *S. cerevisiae* were done by a modified lithium acetate technique [R. D. Gietz, R. H. Schiestl, A. R. Willems, R. A. Woods, *Yeast* **11**, 355 (1995); J. Hill, K. A. Donald, D. E. Griffiths, G. Donald, *Nucleic Acids Res.* **19**, 5791 (1991)]. Transformation of *C. albicans* was identical, except that DMSO was omitted, incubation times at 30°C and 42°C were extended to 3 hours and 1 hour, respectively, and uridine at 25 μg/ml was added to the plating solution. All *C. albicans* strains shared the SC5314 background. The *C. albicans* allele *tup1*:*hisG* described is referred to as *tup1*Δ-*1*.
15. Cells were fixed for microscopy with 70% ethanol, rinsed twice in water, and incubated in 4′,6-diamidino-2-phenylindole hydrochloride (DAPI) (250 ng/ml) or calcofluor white M2R (a boshork) (500 μg/ml) for 10 min at room temperature. DAPI-stained cells were rinsed once before mounting in 50% glycerol, and calcofluor-stained cells were rinsed four times. Fluorescence and differential interference contrast micrographs were taken on a Nikon Optiphot microscope with 40× and 100× objectives with DAPI-specific illumination and filters. Micrographs of cells on plates were taken on an Olympus BX40 microscope, with 10× and 40× objectives with phase. We used the Dalmau plate technique to investigate filamentous growth from colonies of *C. albicans* [M. R. McGinnis, *Laboratory Handbook of Medical Mycology* (Academic Press, New York, 1980)].
16. Standard yeast media such as yeast extract with peptone and dextrose (YEPD) were made as described [F. Sherman, in *Guide to Yeast Genetics and Molecular Biology*, C. Guthrie and G. R. Fink, Eds. (Academic Press, San Diego, 1991), vol. 194, p. 3] Uridine (25 μg/ml) was added to 5-fluoroorotic acid plates used to counter-select against *URA3* in *C. albicans*. SLAHD (*19*), Spider (*5*), and Lee's (*17*) media were made as described. Saboraud dextrose agar and cornmeal agar (Difco) plates were made to manufacturer's directions, with added 0.33% Tween 80 (*16*).
17. K. L. Lee, H. R. Buckley, C. C. Campbell, *Sabouraudia* **13**, 148 (1975).
18. L. A. Merson-Davies and F. C. Odds, *J. Gen. Microbiol.* **135**, 3143 (1989).
19. C. J. Gimeno, P. O. Ljungdahl, C. A. Styles, G. R. Fink, *Cell* **68**, 1077 (1992).
20. R. L. Roberts and G. R. Fink, *Genes Dev.* **8**, 2974 (1994); R. M. Wright, T. Repine, J. E. Repine, *Curr. Genet.* **23**, 388 (1993).
21. Obtained from G. Fink and colleagues.
22. *S. cerevisiae* disruptions were carried out with marked fragments from the plasmids pFW40 [URA3 (*7*)] and pCK36 [LEU2 (*8*)]. All *S. cerevisiae* strains but KKY110 shared the Σ1278b pseudohyphal-competent background (*19*). KKY110 (K. Komachi; UCSF) derives from EG123. Pseudohyphal-competent strains L5684 and L5487 (provided by G. Fink and colleagues; Whitehead Institute) were mated by micromanipulation to create diploid BB8, which was sequentially transformed to disrupt both copies of *TUP1*. Alterations at the locus were assayed by whole-cell PCR with appropriate oligonucleotides.
23. Preliminary results indicate that mutant *tup1 C. albicans* (BCa2-10;*tup1*/*tup1*, *URA3*/*ura3*) are far less infectious in mice than are the parental wild-type (SC5314) cells. This lack of infectivity could be due to constitutive filamentous growth, lack of germ tube formation, or other defects of the mutant strain (P. L. Fidel, Jr., B. R. Braun, and A. D. Johnson, unpublished data).
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