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

Burkhard R. Braun and Alexander D. Johnson

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 blastococci). 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 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 (term meridial tubes at their initial appearance). High temperature (37°C), high ratio of CO2 to O2, 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).

One pathway that regulates cell morphology in C. albicans has been discovered. The gene products of CPH1, HST7, and CST2 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 (62% 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 NH2-terminus, including a proximal glutamine-rich segment (1B). WD40 amino acid sequence repeats are found in many other proteins, including B subunits of heterotrimeric G proteins (12).

To determine whether 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, Mfa2Da2, 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 NH2-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 maltose 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 pseudo-hyphae 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). 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 (length equals 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.
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.

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 \( \alpha \) 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

![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.](image)

![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.](image)
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 α/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 (SLAHG), 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/a and α cell types of S. cerevisiae. However, TUP1 does not repress filamentous growth in S. cerevisiae as it does in C. albicans.

C. albicans strains that are constitutive for filamentous growth and enhanced growth in glycerol, and 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 α/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 (SLAHG), 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/a and α cell types of S. cerevisiae. However, TUP1 does not repress filamentous growth in S. cerevisiae as it does in C. albicans.

C. albicans strains that are constitutive for filamentous growth and enhanced growth in glycerol, and 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 α/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 (SLAHG), 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/a and α cell types of S. cerevisiae. However, TUP1 does not repress filamentous growth in S. cerevisiae as it does in C. albicans.

C. albicans strains that are constitutive for filamentous growth and enhanced growth in glycerol, and 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 α/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 (SLAHG), 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/a and α cell types of S. cerevisiae. However, TUP1 does not repress filamentous growth in S. cerevisiae as it does in C. albicans.

C. albicans strains that are constitutive for filamentous growth and enhanced growth in glycerol, and 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 α/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 (SLAHG), 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/a and α cell types of S. cerevisiae. However, TUP1 does not repress filamentous growth in S. cerevisiae as it does in C. albicans.

The 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 on 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.

**REFERENCES AND NOTES**


**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 on 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.

11. PCR primers used to amplify a fragment of the vector conferred 83 units; and pMH1 (Gal-driven promoter) were: 5′-GGGTTACCAYTTCACCATGCTGATCTTCTTTTGTAA-3′ (F. Sherman, in Methods in Enzymology, 2nd ed. Academic Press, New York, 1980)


13. The same PCR product was cloned into pVZ1 to produce p348. A Bluescript-derived pVZ1 to produce p371. Both strands of the TUP1 open reading frame were sequenced. The C. albicans TUP1 DNA and protein sequences have been deposited in GenBank (AF055741).


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 boshok) (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 Dalmat 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 dextrose (YPED) were made as described (F. Sherman, in Methods in Enzymology, 2nd ed. Academic Press, New York, 1980). To investigate the potential role of C. albicans TUP1 gene, defining a network involved in the maintenance of hepatocyte-specific phenotype.

17. To determine and maintain the hepatic phenotype (P. J. F. Saudou and colleagues; Whitehead Institute) were mated by YEPD (B. R. B.) and by NIH grant GM37049 (A.D.J.).

18. P. J. F. Saudou and colleagues; Whitehead Institute) were mated by YEPD (B. R. B.) and by NIH grant GM37049 (A.D.J.).

19. To determine and maintain the hepatic phenotype (P. J. F. Saudou and colleagues; Whitehead Institute) were mated by YEPD (B. R. B.) and by NIH grant GM37049 (A.D.J.).


