Candida albicans Tup1 Is Involved in Farnesol-Mediated Inhibition of Filamentous-Growth Induction

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Received 28 September 2007/Accepted 9 April 2008

Candida albicans is a dimorphic fungus that can interconvert between yeast and filamentous forms. Its ability to regulate morphogenesis is strongly correlated with virulence. Tup1, a transcriptional repressor, and the signaling molecule farnesol are both capable of negatively regulating the yeast to filamentous conversion. Based on this overlap in function, we tested the hypothesis that the cellular response to farnesol involves, in part, the activation of Tup1. Tup1 functions with the DNA binding proteins Nrg1 and Rfg1 as a transcription regulator to repress the expression of hypha-specific genes. The tup1/tup1 and nrg1/nrg1 mutants, but not the rfg1/rfg1 mutant, failed to respond to farnesol. Treatment of C. albicans cells with farnesol caused a small but consistent increase in both TUP1 mRNA and protein levels. Importantly, this increase corresponds with the commitment point, beyond which added farnesol no longer blocks germ tube formation, and it correlates with a strong decrease in the expression of two Tup1-regulated hypha-specific genes, IHP1 and RBT1. Tup1 probably plays a direct role in the response to farnesol because farnesol suppresses the haploinsufficient phenotype of a TUP1/tup1 heterozygote. Farnesol did not affect EFG1 (a transcription regulator of filament development), NRG1, or RFG1 mRNA levels, demonstrating specific gene regulation in response to farnesol. Furthermore, the tup1/tup1 and nrg1/nrg1 mutants produced 17- and 19-fold more farnesol, respectively, than the parental strain. These levels of excess farnesol are sufficient to block filamentation in a wild-type strain. Our data are consistent with the role of Tup1 as a crucial component of the response to farnesol in C. albicans.

Candida albicans is the opportunistic fungal pathogen most commonly isolated in humans. C. albicans is part of the normal flora, and it resides in the gastrointestinal and genitourinary tracts, as well as on the skin. However, C. albicans is capable of causing a wide range of diseases, from mild mucosal infections to life-threatening systemic infections termed candidemia (19). Vulnerable patients include those with AIDS and patients undergoing chemotherapy and organ transplantation (19). The annual cost of treating candidiasis in the United States was estimated to be 1 billion dollars, and the mortality rates for patients with candidiasis are 30 to 50%, even with antifungal treatment (28), indicating a need for new antifungal drugs.

The ability of C. albicans to cause disease has been strongly linked to its conversion between two distinct morphological forms, yeast and filamentous. Recently, our research has focused on farnesol, the first quorum-sensing molecule discovered in a eukaryote (17). Farnesol is a virulence factor (35) that is excreted continuously by C. albicans (17), and when it accumulates beyond a threshold level, it blocks the yeast to filament conversion (17). Stationary-phase cultures of C. albicans have accumulated 2 to 4 μM farnesol (17), and the 50% inhibitory concentration value for blocking germ tube forma-
nals include 10% serum, 10 mM l-proline, 2.5 mM N-acetylgulo- 
somes, or the combination of N-acetylgulosamine and l-proline, all at 37°C (17). Thus, farnesol may individually 
block each of the morphogenic signaling pathways and/or act at a 
common control point in morphogenesis. Tup1 repression of 
filament-specific genes is an attractive candidate for a common 
control point that may be regulated by farnesol (21).

The *C. albicans* Tup1 protein is a transcription regulator that 
plays two key roles in the cell: (i) regulation of phase switching, 
and (ii) inhibition of filamentous growth. Tup1 interacts with 
the corepressor protein Snf6 or Tcc1. These complexes func-
tion with DNA binding proteins to repress gene expression (23, 
42). At least three DNA-binding proteins have been identified 
that function with Tup1: Nrg1 (homologous to the *Saccharo-
myces cerevisiae* Nrg1p protein), Rfg1 (homologous to the *S.
cerevisiae* Rox1p protein), and Migl (homologous to the *S. 
cerevisiae* Mipl protein). Homozygous tup1 mutants are un-
able to grow as yeast and instead remain locked in the fila-
mentous form, in all media tested (5). Deletion of *TUP1* results 
in the up-regulation of approximately one-third of *C. albicans* 
genes (32, 33), and these mutants are also avirulent in a murine 
model of infection. The activation of Tup1 transcription re-
pressor complexes results in the repression of filament-specific 
gene expression (5, 6, 32, 33).

Here, we tested the hypothesis that the *C. albicans* response 
to farnesol involves Tup1. The morphological response to far-
nesol was tested with wild-type and *tup1/tup1, tup1/TUP1, nrg1/
rg1*, and *rgf1/rgf1* strains to assess the requirement for these 
genes in the farnesol response. The morphological response 
and gene expression pattern for *MIG1* were not determined 
because the Migl protein does not play a role in the filamen-
tous growth of *C. albicans* (32). The gene expression patterns 
of *TUP1, NRG1, RFG1*, and *EFG1*, as well as genes under their 
control, were examined in the presence or absence of farnesol 
by quantitative Northern and Western analyses. Finally, we 
compared farnesol production levels in *tup1, nrg1*, and *rgf1* 
homozygous mutants relative to that in wild-type cells.

**MATERIALS AND METHODS**

**Strains and media.** *Candida albicans* SC5314 is an independent clinical isolate and the reference strain for the *Candida* gene sequence (1). *C. albicans* strains CAF-2 ([ura3::mn34/AUR3] and CAF-4 ([ura3::mn34/AUR3::mn34]) are de-

*SC5314* by gene replacement (16). Strains BCA2-9 (*tup1/tup1* in CAI-4 [3]), BCA2-10 (*tup1/tup1*, frameshift disruption fragment in CAI-4 [5]), DU152 (*nrg1/migl* in CAI-4 [5]), DU129 (*rgfl/rgf1* in CAI-4 [22]), BCA65, which expresses *TUP1* ectopically (*tup1/nup1, MAL3::p455 in CAI-4 [3, 5]), and BCA2-3 (*TUP1/ 
tag/tup1* in CAI-4 [5]) were obtained from Alexander Johnson, University of Cali-

*ifornia, San Francisco, CA. Strain MEN was provided by Richard Cannon, Uni-

versity of Otago, Dunedin, New Zealand.

Resting cells were obtained by growing cells in modified glucose salts biotin 
media (mGSB) overnight, washing them three times with 50 mM phosphate (pH 
6.5), resuspending them in 10 ml of 50 mM phosphate, and storing them at 4°C, 
to be used within a month. The defined glucose-salts medium GPP (pH 4.8) contained 
(per liter of dis-
tilled water) glucose, 20 g; l-proline, 1.15 g; NaHPO₄, 3.2 g; KH₂PO₄, 4 g; MgSO₄, 0.5g; CuSO₄, 0.1 mg; MnSO₄, 0.1 mg; MnCl₂, 0 mg; FeSO₄, 0 mg; biotin, 20 mg; pyridoxine · HCl, 200 mg; thiamine · HCl, 200 µg. The glucose (20% [wt/vol]) and l-proline (100 mM) were autoclaved separately and 
added aseptically, as were the filter-sterilized vitamins (27). Modified GPP 
(mGPP) also contained 2.5 mM N-acetylgulosamine (17). GPP (pH 6.8) con-
tained 3.2 g/liter Na₂HPO₄ instead of NaH₂PO₄. For maltose phosphate proline 
(MPP) medium, filter-sterilized maltose replaced the glucose. Cremallar 
agar (Difco, Detroit, MI) was also used. Solid medium included 2% (wt/vol) agar. All 
media for CAI-4 included uridine at 40 µg/ml.

**Microscopy.** Differential interference contrast images were produced with an 
Olympus BX51 microscope, and colony morphology photographs were made 
with an Olympus SZX12 microscope.

**Quantitative Northern blotting analysis.** To measure mRNA accumulation, 
SC5314 resting cells were inoculated in mGPP to an optical density at 600 nm of 0.5 
to 0.6 and allowed to equilibrate at 37°C for 5 min, whereupon 20 µM 
farnesol was added to half of the flasks. Cells were grown at 37°C for 0, 20, 40, 
60, and 80 min, until the cells were harvested, and total RNA was extracted by 
the hot phenol method (24). Equal amounts of RNA (15 µg) were resolved on 
1.0% agarose-formaldehyde gels, and the RNA was transferred to GeneScreen 
Plus (NEN Life Science Products, Inc., Boston, MA), using the capillary blot 
transfer protocol recommended by the manufacturer. The Northern blots were 
probed with radiolabeled DNA probes. The probe DNA used for synthesis 
was prepared by PCR with MEN genomic DNA. The probes were labeled with 
[³²P]dCTP (GE Health Sciences, Piscataway, NJ), using an oligo labeling kit, 
RadPrime DNA labeling system, following the protocol recommended by the 
manufacturer (Invitrogen, Carlsbad, CA). Northern blots were phosphorimaged 
with a Storm phosphorimager (Amersham Pharmacia Biotech Inc., Piscataw-
ay, NJ) and quantified using ImageQuant software (version 5.0; Molecular Dynam-
ics, Sunnyvale, CA). mRNA abundance measurements were done using a min-
imum of three independent Northern blots.

**Western blotting analysis.** Western blots were prepared as previously de-
scribed (2), and Tup1 and Act1 proteins were detected with a Supersignal 
Western Pico chemiluminescent substrate, using the manufacturer’s protocol (Pierce, 
Rockford, IL), except that blocking was done with 5% nonfat dried milk. Rabbit 
monoclonal antibodies against Tup1 were previously described (18). Mouse mono-
clonal anti-Act1 antibodies and horseradish peroxidase-labeled anti-rabbit immu-
noglobulin G antibodies were from Amersham Pharmacia Biotech, Inc. (Pisca-
taway, NJ). Horseradish peroxidase-labeled anti-mouse antibody was from 
Perkin-Elmer (Boston, MA).

**Analysis of farnesol levels.** Extracellular farnesol was extracted from cell-free 
 supernatants of cultures grown in mGPP at 30°C and analyzed by gas chroma-
ography-mass spectrometry as described previously (17).

**RESULTS**

The *tup1/tup1* and *nrg1/nrg1* mutants lack a morphological 
response to farnesol, while the *rgf1/rgf1* mutant responds to 
*farnesol*. The juxtaposition of farnesol’s ability to inhibit dif-
ferentiation and the role of Tup1 as a transcription repressor 
for filamentation genes suggest that farnesol could function by 
avivating Tup1 and/or one of its coregulators, Nrg1 or Rfg1. 
Consequently, we examined the effect of farnesol on the mor-
phology of null mutants lacking *TUP1*, *NRG1*, and *RFG1*. As 
a control, the wild-type *C. albicans* SC5314 in filament-inducing 
mixed growth as yeast in the presence of 20 µM farnesol and as 
filaments in media lacking farnesol, demonstrating a positive 
response to farnesol (Fig. 1). The *rgf1/rgf1* mutant responded 
to 20 µM farnesol in a manner similar to that of SC5314 (Fig. 
1). Unlike SC5314 and *rgf1/rgf1*, the *tup1/tup1* and *nrg1/nrg1* 
mutants lacked a detectable response to farnesol and remained 
filamentous in the presence of 20 µM farnesol (Fig. 1). The 
filamentous-only cell morphology is the phenotype expected 
for these known mutants (5, 7, 25, 32, 33). However, in this 
regard, the *tup1/tup1* and *nrg1/nrg1* mutants differ from 
the great majority of filamentous-only mutants recovered from a 
previous study, 96% of which reverted to a smooth (yeast) 
colonies morphology on yeast malt (YM) agar plates with 50 µM 
farnesol (20). For the *tup1/tup1* mutant, the lack of response to 
*farnesol* was specific for the loss of Tup1 because we found that 
ecotropic expression of *TUP1* (5) restores the strain’s ability to 
respond to farnesol (data not shown).

*TUP1* mRNA levels increase in the presence of farnesol, 
while *RFG1* and *NRG1* mRNA levels were not affected by 
farnesol. We analyzed the effect of farnesol on *TUP1* mRNA 
levels over time in *C. albicans* SC5314 cells that had been
induced to commence germ tube formation (GTF) by growth at 37°C in mGPP. We previously showed (31) that under these conditions, the first germ tubes appeared at 30 min and the process was complete by 110 min. Furthermore, farnesol no longer blocked GTF when it was added at 60 to 90 min after inoculation (27). Here, our analysis was designed to evaluate changes in TUP1 mRNA just before GTF, when the cells were still responsive to farnesol. Filamentation was induced by transferring resting cells into mGPP (pH 4.8) at 37°C in the presence and absence of 20 μM farnesol, and mRNA levels were determined at 0, 20, 40, 60, and 80 min following induction. In all experiments, the TUP1 mRNA levels decreased over the first 20 min and then increased (Fig. 2A). This pattern is consistent with the single-time-point result of Toyoda et al. (45), who showed that TUP1 mRNA levels increased slightly at 180 min after induction of filamentation. In the presence of farnesol, we found that TUP1 mRNA consistently increased 2.5-fold ± 0.6-fold (n = 4) from 20 to 60 min. Importantly, this is the time period just prior to that at which the cells become committed and are no longer responsive to farnesol (31). In contrast, in the absence of farnesol, there was very little increase (1.4 ± 0.3; n = 4) in TUP1 mRNA levels from 20 to 60 min (Fig. 2A). Thus, farnesol (20 μM) causes a consistent increase in TUP1 mRNA levels during the precise time period when it blocks GTF. This increase of 2.5-fold in TUP1 mRNA corresponded to an increase in SC5314 Tup1 protein levels at 60 min following induction (Fig. 3). Tup1 protein in SC5324 was increased in all three replicate experiments by an average of 2.5-fold.

Since Tup1 functions with DNA binding proteins such as Rfg1 and Nrg1, and in C. albicans strain JCM9061 the NRG1 mRNA levels decreased during filamentation (45), we tested the effect of farnesol on the RFG1 and NRG1 mRNA levels during differentiation from yeast to filamentous form. Like TUP1 mRNA, the RFG1 mRNA levels initially decreased and then increased (data not shown). However, unlike TUP1 mRNA, the timing and magnitude of the RFG1 mRNA level changes were similar in the presence and the absence of farnesol (Fig. 4, data not shown). Under the same conditions, the NRG1 mRNA levels did not change during development, and they too were the same in the presence and absence of farnesol (Fig. 4). Thus, we conclude that farnesol does not affect the RFG1 or NRG1 mRNA levels.

Expression of the Tup1-regulated filamentation genes HWP1 and RBT1 is inhibited by farnesol. To determine whether the increased TUP1 expression in the presence of farnesol was biologically significant, we examined the expression of two Tup1-regulated genes, HWP1 and RBT1 (Fig. 2B and C [12]). In the absence of farnesol, the HWP1 and RBT1 transcripts were undetectable at time zero, but they were strongly expressed from 40 to 80 min (Fig. 2B and C). Farnesol delays and dramatically reduces the magnitude of HWP1 and RBT1 mRNA expression (Fig. 2B and C). At 80 min, HWP1 and RBT1 levels were 30- and 7.6-fold lower, respectively, in farnesol-treated cells than in untreated cells. Similar results were observed by Davis-Hanna et al. (11) for HWP1 mRNA at 2 h after treatment with 75 μM farnesol. Thus, there is a strong correlation between elevated TUP1 expression in response to farnesol and the expression of Tup1-regulated genes.

**EFG1 mRNA levels remain unaffected by farnesol.** Efg1 is a transcription regulator for genes required for filamentation. EFG1 mRNA levels are downregulated at the initiation of filamentation, and farnesol delays the expression of EFG1. This suggests that farnesol does not inhibit the expression of Tup1-regulated genes by directly affecting the EFG1 promoter.
filament development and then increase as filament formation progresses (44). *HWP1* and *RBT1* are activated by Efg1 during filamentation (6). Therefore, we determined whether farnesol also affected *EFG1* mRNA levels (Fig. 4). The *EFG1* mRNA levels were high at time zero, decreased to a minimum at 20 min, and then increased steadily throughout the remaining time (data not shown). However, farnesol had no influence on *EFG1* mRNA levels, since the timing and magnitude of the changes were similar in the presence and the absence of farnesol (Fig. 4 and data not shown).

**Farnesol suppresses the haploinsufficient phenotype of a *TUP1/tup1* heterozygote.** Braun and Johnson (5) showed that BCa2-3, a *TUP1/tup1* heterozygote, is haploinsufficient in that these cells develop a higher proportion of filaments than

FIG. 2. *TUP1* mRNA levels increased, while two Tup1-regulated genes, *HWP1* and *RBT1*, were downregulated in the presence of farnesol (FOH). *C. albicans* SC5314 resting cells were inoculated into mGPP (pH 4.8) medium in the presence or absence of 20 μM farnesol and incubated at 37°C. Cells were then harvested at 0, 20, 40, 60, and 80 min postinoculation. Northern blots were prepared with total RNA from cells incubated in the presence or absence of farnesol. Shown is a phosphorimage of a representative Northern blot probed with radiolabeled *TUP1* DNA (A), *HWP1* DNA (B), and *RBT1* DNA (C) and a plot of average mRNA levels from a minimum of three independent experiments. *ACT1* mRNA levels were used as a loading control.

![Graph](image)

**FIG. 3.** Tup1 protein levels are higher in the presence of farnesol. Total protein extracts were prepared from SC5314 and *TUP1/tup1* (BCa2-3) resting cells inoculated into mGPP (pH 6.8) medium at 37°C in the presence or absence of 20 μM farnesol and incubated at 37°C for 60 min. The average change (fold) in Tup1 protein accumulation for farnesol-treated cells relative to that of untreated cells is shown. Act1 levels were used as a loading control.

![Graph](image)

**FIG. 4.** Farnesol does not affect the expression of *RFG1* or *NRG1*, which encode DNA binding proteins that function with Tup1, or *EFG1*, which encodes a transcription activator of hypha-specific genes. Quantitative Northern blotting analysis was used to measure the *TUP1*, *NRG1*, *RFG1*, and *EFG1* mRNA levels in SC5314 at 60 min after the inoculation of resting cells under conditions that promote GTF in the presence and absence of 20 μM farnesol. The results are averages of three independent experiments.
TABLE 1. The tup1/tup1 and nrg1/nrg1 null mutants do not respond to farnesol but overproduce farnesol

| C. albicans strain | Farnesol response | Farnesol production (µg/g dry wt of cells) ± SD | Fold increase in farnesol |
|--------------------|-------------------|---------------------------------|-------------------------|
| CAI-4              | Positive          | 1.6 ± 0.36                      | 1.8                     |
| CAF-2              | Positive          | 2.0 ± 1.30                      | 2.6                     |
| tup1/tup1 (BCa2-10)| Negative          | 30.6 ± 6.40                     | 17                      |
| nrg1/nrg1 (DU152)  | Negative          | 34.5 ± 12.2                     | 19                      |
| rfg1/rfg1 (DU129)  | Positive          | 4.8 ± 2.0                       | 2.6                     |

a Farnesol responses on GPP agar with and without 20 µM farnesol, incubated at 37°C for 48 h. A positive response indicates smooth colony morphology (yeast cells) in the presence of farnesol and rough colony morphology (filamentous cells) without added farnesol. A negative response to farnesol indicates rough colony morphology in the presence and absence of farnesol.

b Farnesol production values (µg/g dry weight of cells) ± standard deviation (SD) were the averages of three measurements.

c Values are based on fold increases over 1.8, the average value for strains CAI-4 and CAF-2.

wild-type cells on most media (5). Presumably, these cells do not make enough Tup1 to compensate for the reduced gene copy number. We hypothesized that farnesol might suppress this phenotype because it increases TUP1 expression 2.5-fold in SC5314 and 4.2-fold in TUP1/tup1 (Fig. 3). This increase should restore Tup1 to roughly wild-type levels. To test this hypothesis, we examined the effect of farnesol on C. albicans BCa2-3 on cornmeal agar plus Tween 80, under a coverslip for 25 h at 25°C. Under these conditions, the TUP1/tup1 mutant was more filamentous than the wild-type colonies but less filamentous than the tup1/tup1 mutant (BCa2-10 [4]; see Table 2). As a control, the TUP1/tup1 mutant was shown to respond to farnesol because, although it forms filamentous cells when grown in mGPP medium, the addition of 20 µM farnesol results in growth as yeast (Fig. 1), and Tup1 protein levels were ca. 4.2-fold higher in the TUP1/tup1 mutant treated with farnesol. In contrast to the haploinsufficient phenotype observed with the absence of farnesol, in the presence of farnesol, the TUP1/tup1 mutant looked identical to the wild-type C. albicans (see Table 2). Thus, farnesol suppresses the haploinsufficiency phenotype of the TUP1/tup1 heterozygote.

The tup1/tup1 and nrg1/nrg1 mutants produce excess farnesol. Jensen et al. (20) tested the farnesol production levels for several filament-only mutants. A subset of these mutants produced levels of farnesol significantly higher than those of the wild-type strains. This overproduction suggests that the ability to respond to farnesol may be linked to the regulation of farnesol production. Here, we tested farnesol production levels in the CAI-4, CAF-2, tup1/tup1, nrg1/nrg1, and rfg1/rfg1 strains. Farnesol production levels were dramatically increased in the tup1/tup1 and nrg1/nrg1 mutants (Table 1), which were unable to respond to farnesol (Fig. 1). The tup1/tup1 and nrg1/nrg1 mutants produced ca. 17- and 19-fold more farnesol, respectively, than did CAF-2 and CAI-4. In contrast, the farnesol-responsive rfg1/rfg1 mutant produced only ca. 2.6-fold more farnesol than the wild-type strains (Table 1). Thus, the two mutants that are unable to respond to farnesol (tup1/tup1 and nrg1/nrg1) produced much higher levels of farnesol than did strains that do respond to farnesol.

**tup1/tup1 overproduction of farnesol inhibits SC5314 filamentation.** We tested the biological significance of farnesol overproduction by sequentially plating tup1/tup1 and SC5314 next to one another and observing the resultant colony morphologies. When SC5314 was plated and followed 1 day later by another streak with SC5314, a small area of filament inhibition was observed (Fig. 5B). In contrast, when tup1/tup1 was plated first, followed by SC5314, a much larger area of filament inhibition was observed (Fig. 5A). These results are consistent with the tup1/tup1 overproduction of farnesol. As controls, whenever tup1/tup1 was plated second, no filament inhibition was observed (Fig. 5C and D).

**DISCUSSION**

C. albicans responds to farnesol, in part, by changing gene expression (8, 15). We hypothesize that some of these changes are mediated by changes in the activity of the signaling pathways regulating morphogenesis. Here, we show that the tup1/tup1 and nrg1/nrg1 null mutants are strictly filamentous strains and that the cells remain filamentous in the presence of added
farnesol (Fig. 1). In these cases, the total farnesol levels are actually much higher than the added farnesol because the mutants themselves produce elevated levels of farnesol (Table 1; see below). Furthermore, Tup1 mRNA and protein levels increased in the presence of farnesol, while mRNA levels of two Tup1-regulated genes, *HWP1* and *RBT1*, decrease (Fig. 2, and 3). Importantly, the timing of this increase (40 to 60 min, Fig. 2) corresponds with the commitment point, beyond which added farnesol no longer blocks GTF (31). Finally, we believe that Tup1 is part of the farnesol response pathway because farnesol suppresses the haploinsufficient phenotype of the *TUP1/tup1* strain (Table 2).

Cell synchrony, farnesol concentration, and timing were all important considerations for our experimental design. Previous work examining farnesol-dependent changes in the global transcription profiles of developing biofilms (8, 15) and during resumption of growth following stationary phase (15) were done with mixed cell populations that differed in their ability to respond to farnesol. Furthermore, the effect of adding farnesol on the global gene expression during biofilm formation was determined at a single time point, 24 h after the addition of farnesol (8). This point is significant because such a study could measure only stable long-term farnesol-dependent changes in gene expression. Timing is also important because of the commitment phenomenon. This is the point at which a switch in the environmental stimulus no longer causes the expected switch in gene expression including that of *Nrg1*. Soto et al. (41), who also found no change in *Nrg1* mRNA levels at a single time point with added farnesol (41). Together with our results, this suggests that farnesol does not regulate *EFG1* mRNA levels at this single time point with added farnesol (41). Together with our results, this suggests that farnesol does not regulate *EFG1* mRNA levels, but at this time, we cannot exclude the possibility that posttranslational regulation of Efg1 is affected by farnesol.

Two other farnesol-related findings regarding filamentous growth can have profound effects on the genes it regulates. For example, we have shown that nonsense-mediated mRNA decay in *S. cerevisiae* regulates the accumulation of the mRNA for Adr1, a transcription regulator of the genes responsible for making acetyl-coenzyme A and NADH from nonfermentable substrates. In particular, the respiratory impairment seen with nonsense-mediated mRNA decay mutants is due, in part, to the overexpression of Adr1 (43). The change in *ADR1* mRNA levels is small (2.6-fold) but sufficient to affect expression of Adr1-regulated genes. Thus, even though the change in *Tup1* expression is relatively small, it can have a profound effect on the expression of the genes it regulates.

Table 2. Farnesol suppression of the *TUP1/tup1* heterozygote

| C. albicans strain | Cell morphology at the colony periphery in response to: | No farnesol | 20 μM farnesol |
|--------------------|-----------------------------------------------------------|-------------|----------------|
| Wild type (SC5314) | Yeast plus a few filaments | Yeast plus a few filaments | |
| *TUP1/tup1* (BCa2–3) | Yeast plus filaments | Yeast plus a few filaments | |
| *tup1/tup1* (BCa10) | Filamentous form | Filamentous form | |

*Farnesol suppressed the haploinsufficient phenotype of the *TUP1/tup1* heterozygote. Cells were plated on a cornmeal agar plus Tween 80 plate, under a coverslip, and grown at 25°C for 25 h. The phenotype for these strains grown on a plate with cornmeal agar plus Tween 80 under a coverslip without farnesol was also previously reported (5).*
HST7 mRNA levels. CPF1 is a transcription factor that regulates filamentous growth, and HST7 is a mitogen-activated protein kinase kinase involved in filamentous growth. Both are downregulated by Tup1 and, thus, their downregulation by farnesol (41) is consistent with a secondary effect of farnesol on Tup1. Additionally, Chkl, a histidine kinase shown to be required for the farnesol response (26), is also encoded by a Tup1-repressed gene; CHK1 was elevated 6.5-fold in the tup1/ tup1 mutant (21). Taken together, these findings indicate that Tup1 is involved in mediating the C. albicans response to farnesol.

ACKNOWLEDGMENTS

We thank Alexander Johnson and Richard Cannon for providing us with C. albicans strains and the Tup1 antibody. We also thank Jessica A. Wiles for assisting with the germ tube assays and RNA work.

This work was supported by grants from the National Science Foundation (MCB-0110999), the University of Nebraska Tobacco Settlement Biomedical Research Enhancement Fund, and the Farnesol and Candida albicans Research Fund, University of Nebraska-Lincoln.

Any opinions, findings, conclusions, or recommendations expressed in this report are ours and do not necessarily reflect the views of the National Science Foundation.

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