Candida albicans Hyphal Formation and Virulence Assessed Using a Caenorhabditis elegans Infection Model

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Candida albicans colonizes the human gastrointestinal tract and can cause life-threatening systemic infection in susceptible hosts. We study here C. albicans virulence determinants using the nematode Caenorhabditis elegans in a pathogenesis system that models candidiasis. The yeast form of C. albicans is ingested into the C. elegans digestive tract. In liquid media, the yeast cells then undergo morphological change to form hyphae, which result in aggressive tissue destruction and death of the nematode. Several lines of evidence demonstrate that hyphal formation is critical for C. albicans pathogenesis in C. elegans. First, two yeast species unable to form hyphae (Debaryomyces hansenii and Candida lusitaniae) were less virulent than C. albicans in the C. elegans assay. Second, three C. albicans mutant stains compromised in their ability to form hyphae (efg1Δ/efg1Δ, flo8Δ/flo8Δ, and cph1Δ/cph1Δ efg1Δ/efg1Δ) were dramatically attenuated for virulence. Third, the conditional tet-NRG1 strain, which enables the external manipulation of morphogenesis in vivo, was more virulent toward C. elegans when the assay was conducted under conditions that permit hyphal growth. Finally, we demonstrate the utility of the C. elegans assay in a screen for C. albicans virulence determinants, which identified several genes important for both hyphal formation in vivo and the killing of C. elegans, including the recently described CAS5 and ADA2 genes. These studies in a C. elegans-C. albicans infection model provide insights into the virulence mechanisms of an important human pathogen.

Candida albicans is the most common human fungal pathogen; however, our knowledge of its virulence mechanisms is incomplete, and our best antifungal agents are often ineffective in treating severe candidiasis (3). Infections with Candida species account for 70 to 90% of all invasive mycoses (32) and can be associated with devastating consequences, particularly in intensive care units where mortality rates reach 40% (24, 34). The drug resistance of pathogenic fungi exacerbates this problem and often limits therapeutic options (35). The identification of virulence pathways that can be targeted with novel antifungal therapies is urgently needed (31, 38, 46).

One approach to understand the genetic mechanisms of virulence is to use invertebrates, such as the nematode Caenorhabditis elegans, as model hosts (43). Studies of C. elegans infection with Pseudomonas aeruginosa and Cryptococcus neoformans, for example, have led to the identification of evolutionarily conserved mechanisms of host immunity and microbial virulence (1, 21, 50). However, efforts to design an accurate nonmammalian model of C. albicans pathogenesis have been stymied, in part because it has been difficult to capture the role of Candida dimorphism in these systems.

Morphogenesis in C. albicans is intricately related to pathogenesis and thus has been intensively studied. C. albicans hyphae are important for tissue destruction and host invasion (3). As such, C. albicans mutants and non-albicans Candida species that are unable to form true hyphae are attenuated for virulence (3, 37). However, C. albicans yeast cells also have virulence attributes (4, 33) that are likely involved in dissemination of the fungus through the bloodstream, and the establishment of infection at distant sites. To date, genetic screens to identify the determinants of Candida morphology have been conducted in vitro. Determining the role of these genes in virulence has traditionally involved separate and often laborious studies in mammals. Therefore, an expedient system to study morphogenesis of C. albicans in vivo and accurately model pathogenesis would offer many important advantages.

Here, we study C. albicans pathogenesis using the invertebrate host C. elegans. C. albicans yeast cells are ingested into the gastrointestinal tract. In liquid media, the yeast cells form hyphae, which result in an aggressive infection that ultimately kills the nematode. Fungal hyphae destroy worm tissues and pierce the collagenuous cuticle of the animal, a phenotype that is easily visible using a dissecting microscope. By studying mutants and genetically engineered C. albicans strains, we show that hyphal formation is required for full virulence in this system. Finally, we illustrate the utility of the C. elegans-C. albicans infection assay in a screen for genes involved in Candida morphogenesis and virulence.

MATERIALS AND METHODS

Strains and media. The fungal strains used in the present study are listed in Table 1. Yeast strains were grown in liquid yeast extract-peptone-dextrose (BD) broth or on brain heart infusion (BHI; BD) agar containing 45 μg of kanamycin/ml at 30°C. To determine hyphal formation in vitro, C. albicans strains were grown in Spider medium for 20 h at 37°C (36) and photographed using Nomarski optics on a Zeiss Axiosmager microscope.

The C. elegans glp-4; sek-1 strain was used for all experiments as described previously (6, 40, 45). In brief, the rationale for using the glp-4; sek-1 mutant nematodes instead of wild-type animals is that wild-type C. elegans produces many offspring which confounds killing assays, both because it is difficult to determine progeny from adults and because larvae often hatch inside the nematode, leading to death of the worm by a mechanism not directly related to
pathogen exposure (so-called matricidal killing). C. elegans gfp-4 mutant animals are unable to produce gonads or progeny at 25°C and thus are suited for these studies; however, sterile animals are long-lived compared to wild-type animals (39). C. elegans sek-1 encodes a conserved mitogen-activated protein (MAP) kinase kinase involved in the innate immune response, and sek-1 animals are relatively immunocompromised (28). Worms were propagated on nematode growth medium on lawns of Escherichia coli OP50 by using standard methods (8).

C. albicans-C. elegans transcription factor mutants in the

The mutant library was constructed using a split-marker transposon insertion protocol for infecting C. elegans with C. albicans in a liquid medium pathogenesis assay (6) was modified for these studies. Freshly grown C. albicans cells were inoculated into 1 ml of yeast extract-peptone-dextrose and allowed to grow overnight in a roller drum at 30°C. The following day, 100 μl of yeast was spread into a square lawn on a 10-cm plate containing BHI agar and kanamycin (45 μg/ml), followed by incubation for approximately 20 h at 30°C.

Synchronized adult C. elegans gfp-4; sek-1 nematodes grown at 25°C were carefully washed from plates containing their normal food source (E. coli OP50 strain) using sterile M9 buffer. Approximately 400 to 500 washed animals were then added to the center of the C. albicans lawns. The plates were incubated at 25°C for 4 h. Worms were then carefully washed into a 15-ml conical tube using 6-ml of sterile M9. Great care was taken to minimize the transfer of yeast into the conical tube. Worms were washed three or four times with sterile M9. Sixty to seventy worms were then pipetted into a single well of a six-well tissue culture plate (Corning, Inc.) containing 2 ml of liquid medium (80% M9, 20% BHI) and kanamycin (45 μg/ml). Kanamycin was added to prevent growth of any residual E. coli OP50 strain carried on the nematode during the experiment. For control experiments using only E. coli OP50, kanamycin was omitted from the liquid medium. Worms were scored daily into one of three categories: alive, dead with hyphae piercing the cuticle, and dead without hyphae piercing the cuticle. Worms were considered to be dead if they did not move in response to mechanical stimulation with a pick. Dead worms were removed from the assay. Microscopy of nematodes was performed by using Nomarski optics on a Zeiss Axio-Imager microscope.

RESULTS

C. albicans hyphal formation is a key virulence determinant in the C. elegans killing assay. Our laboratory has previously shown that C. albicans is pathogenic toward C. elegans (6). In the original infection assay, which was designed to identify novel antifungal compounds, yeast cells were ingested by nematodes on solid medium and, after transfer to liquid medium, a minority of worms died with true hyphae piercing through the body of the worm (6). We were intrigued by this observation and sought to develop further a C. elegans infection assay for the detailed study of C. albicans pathogenesis.

We found that several variables, including the age of the worms and the pathogen exposure time, affected the degree of C. albicans hyphal formation within infected nematodes. Specifically, when we exposed adult animals, rather than nematodes in the fourth larval stage (L4), to pathogen for 4 h (rather than 2 h) prior to liquid medium transfer, we noted that a majority of infected worms displayed hyphae after 60 to 70 h of incubation in liquid medium (data not shown). By increasing the number of infected worms with C. albicans hyphae piercing their cuticles, it was possible to make statistically significant comparisons of C. albicans mutant strains.

We began our studies of C. albicans pathogenesis in the liquid medium assay by examining nematode infection with the C. albicans laboratory reference strain DAY185 and the C. albicans clinical isolate SC5314 (Fig. 1A). We found that more than half of the worms infected with these strains died within the first 48 h (Fig. 1A) and interestingly every worm that was killed during this period had visible hyphae piercing the cuticle (Fig. 1B and data not shown). This rapid initial decline in worm survival was followed by a second phase, in which worms
died more slowly (Fig. 1A). In these latter animals, we never observed any hyphae either in the intestine or piercing the cuticle among hundreds of infected nematodes examined. We considered the possibility that the development of hyphae in this assay was occurring postmortem and thus was a marker of aggressive infection, rather than a determinant of pathogenesis. We therefore photographed animals at various stages of infection with *C. albicans* DAY185. Hyphae start to accumulate within the intestines of live animals 12 to 16 h after infection and these animals die within 48 h (Fig. 1B and data not shown). In the studies presented here, we wanted to characterize *C. albicans* hyphal-mediated killing of the nematode and therefore, we focused our experiments on the first 72 h after infection.

To understand the role of *Candida* dimorphism in worm infection, we studied two fungal species closely related to *C. albicans* (Debaryomyces hansenii [Candida famata] and *C. lusitaniae*) that are thought to be incapable of hyphal formation. We observed a dramatic difference in *C. elegans* killing after infection with *C. albicans* DAY185 and *C. albicans* SC5314 compared to the two control organisms (Fig. 1A). *D. hansenii* is a hemiascomyceteous yeast that is closely related to *C. albicans* (19) but is an uncommon human pathogen (17, 18, 51). As seen in Fig. 1C, no hyphae were observed in *C. elegans* after infection with *D. hansenii*. The organism was still capable of killing the nematode; however, the killing was slow and involved pathogenic distention of the worm intestine (Fig. 1C and data not shown). These features suggest *C. elegans* killing by *D. hansenii* occurred through a yeast-dependent process.

*C. lusitaniae* is also a relatively rare human pathogen that does not readily employ hyphal formation as a virulence mechanism in mammals (2, 25). During nematode infection with the *C. lusitaniae* clinical isolate 6856-2, only yeast cells were observed within the worm intestine (Fig. 1D), and killing was significantly slower compared to the *C. albicans* clinical isolate SC5314 (Fig. 1A). Consistent with these data, *C. lusitaniae* was...
also less pathogenic than *C. albicans* in a murine model of fungal infection (2). Taken together, these data suggest that *Candida* hyphal formation is a key virulence determinant toward *C. elegans*, leading to an early, rapid decline in worm survival.

Conservation of *C. albicans* virulence determinants toward mammals and *C. elegans*. *C. albicans* is an opportunist that can grow in a myriad of microenvironments partly because of its ability to respond specifically to environmental cues and adapt by altering its morphology (3, 23). Several conserved signal transduction cascades regulate this response. Specifically, a cyclic AMP-mediated cascade utilizes the transcription factors Efg1p and Flo8p to control hyphal formation in response to a myriad of microenvironments partly because of its ability to respond specifically to environmental cues and adapt by altering its morphology (3, 23). Several conserved signal transduction cascades regulate this response. Specifically, a cyclic AMP-mediated cascade utilizes the transcription factors Efg1p and Flo8p to control hyphal formation in response to a cyclic AMP-mediated cascade utilizes the transcription factors Efg1p and Flo8p to control hyphal formation in response to a variety of environmental stimuli. Accordingly, *C. albicans* efg1Δ/efg1Δ and flo8Δ/flo8Δ mutants have hyphal formation defects and are attenuated for virulence in animal models of candidiasis (10, 37). Likewise, when we infected adult *C. elegans* animals with the efg1Δ/efg1Δ and flo8Δ/flo8Δ mutants, we saw a marked attenuation of virulence compared to the isogenic wild-type *C. albicans* strain SC5314 (Fig. 2A). Furthermore, worms that were infected with these mutants did not develop any hyphae throughout the course of the experiment (Fig. 2B).

We also examined the role of the *C. albicans* MAP kinase signal transduction cascade in virulence toward *C. elegans* by studying a mutant of the *CPH1* gene (36). This pathway coordinates dimorphism in *C. albicans*, but homozygous mutation of the *CPH1* gene results only in a modest hyphal formation defect in vitro and does not affect the virulence potential of *C. albicans* toward a mammal (37). Similarly, we found that the *cpk1Δ/cph1Δ* mutant was able to make hyphae within *C. elegans* and remained fully virulent in our assay (Fig. 2). Disruption of both the cyclic AMP-mediated signaling pathway and the MAP kinase cascade in a *cpk1Δ/cph1Δ efg1Δ/efg1Δ* double mutant, however, resulted in dramatic attenuation of both in vivo hyphal formation and virulence toward *C. elegans* (Fig. 2), findings that are consistent with studies of this double mutant strain in a murine model of hematogenously disseminated candidiasis (37) and a murine model of gastrointestinal colonization and candidemia (29). As observed for *D. hansenii* and *C. lusitaniae*, the *flo8Δ/flo8Δ, efg1Δ/efg1Δ* and *cpk1Δ/cph1Δ efg1Δ/efg1Δ* mutant strains eventually killed the worms by a yeast-mediated process (data not shown).

For additional confirmation that *C. albicans* hyphal formation contributes to virulence in the nematode, we used a genetic tool that allows the external manipulation of morphogenesis. Saville et al. engineered a *C. albicans* strain in which one copy of the *NRG1* gene (a hyphal growth regulator) is under the control of a doxycycline-regulatable promoter (47). In the presence of doxycycline, this strain can form hyphae. Worms were infected with this modified strain and then transferred to liquid medium with or without doxycycline. As was observed in murine studies of this strain (47), we found that the presence of doxycycline in the media was sufficient to allow hyphal formation and manipulate the virulence potential of the *C. albicans* strain (Fig. 3). A total of 60% of infected nematodes died after 66 h in liquid medium containing doxycycline compared to just 11% mortality in the control well (*P* < 0.001) (Fig. 3). Doxycycline was not toxic to the worms and did not alter the ability of the *C. albicans* wild-type strain DAY185 to kill the worm (data not shown).

A *C. elegans*-*C. albicans* assay can be used to screen a library of *C. albicans* mutants for genes involved in hyphal formation in vivo. Given the importance of *C. albicans* hyphal formation in mammalian and nematode pathogenesis, we sought to determine whether this simple model could be used to identify...
Candida virulence determinants. A C. albicans mutant library containing homozygous mutations in 83 transcription factors (44) was screened for mutant clones attenuated both in their ability to form hyphae in vivo and kill C. elegans. We identified five mutants. Interestingly, two of the genes we recovered in this screen (RIM101 and NRG1) have previously described roles in hyphal development and are required for the full virulence potential of C. albicans in a murine model of candidiasis (Table 2). The identification of these genes from our screen provides internal validation for this approach.

![Graph showing percent alive and percent hyphal killing over time with and without doxycycline](image)

**FIG. 3.** Hyphal formation in a genetically modified C. albicans strain drives virulence. Nematodes were infected with the conditional tet-NRG1 strain and transferred to liquid medium either with or without doxycycline (20 μg/ml). Doxycycline increased the killing of nematodes infected with the tet-NRG1 strain (A) by permitting hyphal formation (B) (P < 0.001 for both comparisons). The data in panel A are from a single experiment representative of three independent biologic replicates. The error bars in panel B represent the standard errors of the mean for three independent biologic replicates. Worms infected with the tet-NRG1 strain in liquid media supplemented with doxycycline (C) and without doxycycline (D) are shown. The scale bar in panel C represents 20 μm.

| Library strain | Disrupted gene | Description (reference) | Virulence role in a murine model (reference) |
|----------------|----------------|-------------------------|---------------------------------------------|
| CJN267         | *RIM101*       | Required for alkaline-induced hyphal growth (15, 20) | Yes (14) |
| CJN322         | *NRG1*         | Transcriptional repressor of hyphal genes that acts with *TUP1* (5, 41) | Yes (41) |
| CJN432         | *CAS5*         | Acts with Ada2p to promote cell wall integrity (9) | Yes (12) |
| CJN863         | *ADA2/CAS3*    | Transcriptional coactivator involved in cell wall integrity, metabolic processes and stress responses (9, 48) | Yes (48) |

* Nobile and Mitchell (44).
We identified two other genes in our screen (CAS5 and ADA2) without defined roles in hyphal formation but whose molecular function has been previously studied (9, 48). Interestingly, these genes are also important for mammalian molecular function has been previously studied (9, 48). In addition, our screen recovered C. albicans strain CJN878, which carries a homozygous insertion in ORF19.2458. However, testing of several independent orf19.2458::UAU1/orf19.2458::URA3 insertion mutants failed to confirm the phenotype. Strain CJN878 may therefore have a secondary mutation that affects hyphal growth in the C. elegans pathogenesis assay.

Thus, our screen successfully identified four of the five genes in the transcription factor library that have previously described roles in mammalian virulence (RIM101, NRG1, ADA2, and CAS5). We did not identify the tec1::UAU1/tec1::URA3 insertion mutant, but it is interesting that this strain was also fully virulent in a Drosophila melanogaster model of C. albicans pathogenesis (12).

Ada2p and Cas5p are important for C. albicans hyphal development in vivo and for pathogenesis toward C. elegans. Identification of the ADA2/CAS3 (hereafter referred to as ADA2) and CAS5 genes in our screen of C. albicans transcription factors required for C. elegans killing generates interesting hypotheses regarding their contributions to C. albicans pathogenesis. ADA2 and CAS5 are each important for the cell wall damage response and were previously identified in a screen for mutants hypersusceptible to the antifungal agent caspofungin (9). ADA2 encodes a transcriptional coactivator, which functions as part of the conserved Spt5-Ada-Gcn5 acetyltransferase (SAGA) coactivator complex that regulates numerous metabolic activities in eukaryotes by coordinating posttranslational modifications of histones (48). Cas5p is a transcription factor that is the functional equivalent of the Saccharomyces cerevisiae protein Rlm1 (9).

We confirmed the findings from our screen by studying a prototrophic ada2Δ/ada2Δ deletion mutant and a reconstituted strain. The ada2Δ/ada2Δ mutant strain was significantly attenuated in its virulence toward C. elegans (Fig. 4A) and had similar killing kinetics to mutant strains containing lesions in two genes, RIM101 or NRG1, which have well-defined roles in hyphal development (data not shown). We also observed a striking reduction in the ability of the C. albicans ada2Δ/ada2Δ mutant to produce hyphae in vivo (Fig. 4B and C) and found that this defect was completely restored with ADA2 complementation of the mutant gene (Fig. 4B and D). The ada2Δ/ada2Δ mutant eventually killed the worm, but through a separate pathogenic process since we never observed any hyphae within ada2Δ/ada2Δ mutant-infected nematodes (Fig. 4C and data not shown).

Thus far, an association between ADA2 and hyphal development has not been described. To confirm further these in vivo observations, we incubated the ada2Δ/ada2Δ mutant and its reconstituted strain under hypha-inducing conditions in vitro (Spider media at 37°C). We observed that the ada2Δ/ada2Δ strain is unable to produce hyphae under these conditions (Fig. 4F) compared to its parent strain (Fig. 4E). This phenotype was complemented by reintroduction of the wild-type gene (Fig. 4G). These observations indicate that the genetic network controlling hyphal growth in the ada2Δ/ada2Δ strain is compromised and suggest that Ada2p also coordinates the transcription of genes involved in Candida dimorphism. This hypothesis is strengthened by recent results reported by Sellam et al. (48), who identified 200 genes regulated by Ada2p, 49 of which have roles in C. albicans morphogenesis.

We also found that a C. albicans strain with homozygous disruption of CAS5 was attenuated for virulence and did not form hyphae in vivo as readily as C. albicans DAY185. We confirmed these observations by studying a cas5Δ/cas5Δ deletion mutant and a reconstituted strain (Fig. 4A and B). Interestingly, the cas5Δ/cas5Δ strain was able to make hyphae in a small percentage of worms (~8%, P < 0.001 compared to C. albicans DAY185) (Fig. 4B), and their appearance was similar to the wild-type control (data not shown). Furthermore, the cas5Δ/cas5Δ mutant made hyphae equivalent to a wild-type control strain when cultured in vitro (Spider medium at 37°C) (data not shown). These findings argue that the cas5Δ/cas5Δ mutant still contains the genetic architecture to program wild-type hyphal formation and suggests that there must be a different mechanism to account for the observed in vivo hyphal defects.

**DISCUSSION**

Nonmammalian infection models have been remarkably useful in the study of host-pathogen interactions (42). We therefore developed a system for the study of C. albicans pathogenesis using the nematode C. elegans. We show that C. albicans hyphal formation is required to efficiently kill the worm and is a phenotype that predicts virulence in murine models of candidiasis. We also demonstrate the utility of this system in a screen of C. albicans transcription factor mutants, which identified both established and novel virulence determinants related to C. albicans hyphal formation.

An advantage of the C. elegans-C. albicans pathogenesis system is that it reflects several stages of mammalian infection. In our assay, C. albicans yeast cells are ingested into the digestive tract of the nematode. C. albicans hyphae then aggressively penetrate through host tissues and ultimately pierce the collagenous cuticle of the worm. Likewise, humans carry C. albicans in the gastrointestinal tract and from this location the fungus can disseminate in a susceptible host. In patients with candidiasis, hyphae are often seen at areas of tissue invasion and thus are thought to be involved in dissemination of disease (3, 37, 47). It is also interesting that these assays are conducted at a temperature that does not normally induce hyphal formation (25°C). Thus, studies of C. albicans morphogenesis in C. elegans also enable the analysis of hyphal growth triggers that are independent of temperature. We hypothesize that environmental factors within the nematode gastrointestinal tract, which may have mammalian counterparts, induce hyphal development in C. albicans.

Invertebrate models of candidiasis using D. melanogaster and larvae of the greater wax moth Galleria mellonella have been developed, and their utility has been demonstrated (7, 11). The C. elegans-C. albicans system, however, offers several advantages over these models. First, hyphal formation in nematodes can be easily visualized under a dissecting microscope, allowing direct and expedient evaluation of Candida dimorphism in vivo. Furthermore, the transparency of the worm allows examination of fungal cells within the intestine at all
FIG. 4. ADA2 and CAS5 are required for the full virulence potential of *C. albicans* in the *C. elegans* infection model. Compared to infection with *C. albicans* DAY185, the ada2Δ/ada2Δ and cas5Δ/cas5Δ mutant strains were attenuated for virulence (A) and in vivo hyphal formation (B) in a *C. elegans* assay (*P* < 0.01 for both mutant strains compared to *C. albicans* DAY185). The virulence and hyphal formation defects of the ada2Δ/ada2Δ and the cas5Δ/cas5Δ mutants were restored to wild-type levels in corresponding reconstitution strains (the *P* values were not significant for both reconstitution strains compared to the wild type). The data in panel A are from a single experiment representative of three independent biologic replicates. The error bars in panel B represent the standard errors of the mean for three independent biologic replicates.

Nematodes infected with the ada2Δ/ada2Δ mutant (C) and the ada2Δ/ada2Δ +pADA2 reconstitution strain (D) are shown to demonstrate the marked hyphal formation defect of the ada2Δ/ada2Δ mutant in vivo. Compared to the *C. albicans* reference strain DAY185 (indicated as ADA2/ADA2) (E), the ada2Δ/ada2Δ mutant (F) also showed attenuated hyphal formation in vitro (Spider medium for 20 h at 37°C). This phenotype was restored in the ada2Δ/ada2Δ +pADA2 reconstitution strain (G). The scale bars given in panels C and E represent 20 μm.
stages of infection. Although dimorphism is important for Candida pathogenesis in the D. melanogaster system, direct visualization of hyphae is not possible. Lastly, the portal of entry for Candida infection in the nematode is through the gastrointestinal tract, which is a physiologically accurate representation of human infection. In both fly and larva models, yeast must be injected into the animal to establish infection. However, the innate immune system of D. melanogaster is sophisticated and thus studies in both systems will likely be highly complementary.

The C. elegans-C. albicans infection model is an important new tool that is complementary to existing assays. Nobile et al. created a library of 83 mutants, each with a homozygous insertion in a transcription factor, and tested the strains for their ability to form biofilm on a silicone pad (44). That study found that two genes, TEC1 and BCR1, are required for wild-type in vitro biofilm formation. Interestingly, our screen of this transcription factor mutant library in a C. elegans-C. albicans liquid medium infection assay identified five mutants that were not found to be defective in biofilm formation in vitro. In addition, neither the tec1::UAU1/tec1::URA3 nor the bcr1::UAU1/bcr1::URA3 insertion mutant strains (44) had virulence or hyphal-formation defects in the C. elegans assay (data not shown).

The identification of ADA2 in our screen for C. albicans virulence determinants offers a provocative link between chromatin remodeling via the SAGA coactivator complex and C. albicans hyphal formation. We found that strains carrying a mutation in the ADA2 gene were hypovirulent in our C. elegans-C. albicans assay (Fig. 4A). Phenotypic analyses of the ada2Δ/ada2Δ deletion mutant in vivo and under hyphal-inducing conditions in vitro suggest that the hyphal formation program in this mutant strain is compromised (Fig. 4). Ada2p functions as a key component of the SAGA coactivator complex, a conserved transcription regulatory system involved in histone modification. In S. cerevisiae, the SAGA system modulates the expression of ca. 10% of the genome and specifically affects the transcription of genes upregulated in response to several types of stress (26). The function of this conserved system in C. albicans biology and pathogenesis is now being elucidated.

Sellam et al. determined the genome-wide occupancy of Ada2p by using chromatin immunoprecipitation and found that this protein is recruited to the promoters of 200 genes involved in many different metabolic processes and stress responses (48). Interestingly, our analysis of these data revealed that 49 of these 200 genes have a role in Candida morphogenesis. Seventeen Ada2p-regulated genes are required for wild-type filamentous growth, and twenty-two of these genes are also controlled by transcription factors that are regulators of C. albicans dimorphism. In addition, several Ada2p-regulated genes have particularly prominent roles in morphogenesis. For example, the transcription factor RIM101 (15); a key regulator of hyphal formation, Hsp90 (49); the MAP kinase kinase and hyphal growth regulator HST7 (30); and Swi1, a component of the Swi/Snf chromatin remodeling complex (analogous to the SAGA coactivator complex), were all identified to be controlled by Ada2p (48). Taken together, the data from our study and from Sellam et al. suggest that activation of a transcriptional network governing dimorphism in C. albicans involves the coordination of chromatin remodeling.

The phenotype of the cas5Δ/cas5Δ mutant in our C. elegans assay informs hypotheses regarding Candida virulence mechanisms toward both nematodes and mammals. C. elegans uses two principle defenses against ingested pathogens, a muscular pharyngeal grinder organ and secreted antimicrobial peptides (13, 27). We speculate that defects in the fungal cell wall damage response of the cas5Δ/cas5Δ strain increases yeast susceptibility to these defenses. Consistent with this hypothesis, the cas5Δ/cas5Δ mutant strain is able to make wild-type appearing hyphae but only in a small percentage infected worms (Fig. 4B). We also did not observe any defects in hyphal formation in the cas5Δ/cas5Δ mutant strain in an in vitro assay. Thus, we suggest that the hyphal formation program in the cas5Δ/cas5Δ mutant is intact and that these yeasts are more easily cleared from the C. elegans digestive tract. It is notable that the cas5Δ/cas5Δ mutant is also hypovirulent in a murine model of candidiasis and that sections of kidneys from animals infected with the mutant strain demonstrate very few hyphae (12). Taken together with the data from our study, it seems likely that C. albicans are exposed to analogous host defenses or mechanical forces during mammalian infection, which accounts for the virulence defect of the cas5Δ/cas5Δ mutant in the murine model. Thus, the C. elegans-C. albicans system offers a simple assay to study the ability of mutant strains to form hyphae in vivo, a phenotype that can predict pathogenicity in mammals.

In summary, we describe and validate a system for the identification and characterization of C. albicans virulence determinants. We demonstrate the utility of this model in a screen of transcription factor mutants and show that the C. elegans-C. albicans assay can be used to study the virulence mechanisms of an important human pathogen.

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