Fungal infections are an increasingly significant cause of human disease and morbidity due to an expanding immuno-compromised population. However, only four main classes of broad-spectrum antifungal drugs are currently available (polyenes, azoles, echinocandins, and 5-fluorocytosine), which target only three cellular components: the cell membrane, cell wall, and nucleotide biosynthesis (55). Compared with the identification of antibacterial drug targets, an obstacle to antifungal drug target identification is the eukaryotic nature of both the fungal pathogen and the host, ensuring a considerably higher degree of conserved genes and pathways. Since a subset of amino acid biosynthetic pathways are not present in humans (46), yet are conserved in fungi, and many are required for survival in vivo and/or virulence (22, 31, 35, 36, 45, 58), various amino acid biosynthetic enzymes are an attractive, unexploited class of antifungal drug targets.

The threonine biosynthetic pathway is of particular interest for antifungal drug targets. Threonine is produced from aspartate, via the intermediate homoserine, in a series of five enzymatic steps, initiated by aspartate kinase (Hom3p). Homoserine is converted to threonine by the sequential actions of homoserine kinase (Thr1p) and threonine synthase (Thr4p). Threonine synthesis is regulated by induction of pathway genes via the general control pathway in response to amino acid starvation (26, 43) and by feedback regulation of aspartate kinase when threonine is abundant (41, 48). Homoserine and threonine are intermediates in the synthesis of methionine and isoleucine, respectively, and we have found that various fungal methionine and isoleucine auxotrophs are unable to survive in vivo and/or are avirulent (31, 36, 45, 58). Myriad phenotypes in addition to auxotrophy have been associated with Saccharomyces cerevisiae threonine biosynthetic mutants, particularly thr1Δ or thr4Δ mutants (2, 8, 14–16, 20, 33, 51) as a result of toxic homoserine accumulation (33), many phenotypes of which may also affect the ability of these mutants to survive in vivo. Since we find that S. cerevisiae hom3Δ mutants are unable to survive in vivo (31) and that C. neoformans hom3Δ and thr4Δ mutations are lethal (34), we further investigated the potential of threonine biosynthetic enzymes as antifungal drug targets by examining the in vivo survival of thr1Δ and thr4Δ mutants constructed in a clinically derived S. cerevisiae strain. Given the severe survival defects of these mutants after only 4 h in vivo, we extended our investigations to Candida albicans, a more clinically relevant pathogen, and observed that C. albicans thr1Δ mutants had attenuated virulence. Consistent with the in vivo defects, we demonstrated that S. cerevisiae thr1Δ, thr4Δ, and C. albicans thr1Δ strains were serum sensitive. We explored the basis of the serum sensitivity and show that low serum threonine concentrations and the accumulation of the biosynthetic intermediate homoserine are key to the rapid death of thr1Δ and thr4Δ mutants in serum.

MATERIALS AND METHODS

Strains, media, and growth conditions. All strains used in this study are listed in Table 1. S. cerevisiae strains were isogenic with clinically derived YJM145 (42), and C. albicans strains were isogenic with strain SC5314 (21). Unless otherwise specified, all strains described are diploid and homozgyous for the gene disruption described. Standard culture media included yeast extract-peptone-dextrose (YPD) and synthetic defined media (SD). Plates were spotted with lawn strategies, and growth was scored after 2 days of incubation at 30 °C. Antifungal and other chemical compounds were purchased from Sigma and used as indicated in the text. Yeast were streaked on YPD plates to perform optimal plating density and approximate colony counts. Standard error bars were calculated and cited only when significant differences were observed. Strains were isogenic with strain SC5314 (21).
flanking the gene of interest. Strains were transformed with the gene-targeting cassette, leaving an FLP recombination target (FRT) site. Multiple deletions in a strain, separate strains containing deletions with different ends 60 bp of sequence homologous to the region immediately upstream and downstream of the deleted region. Trans
genic strains were selected by reversion to prototrophy, and transformants in which the wild-type allele had replaced a disrupted allele were chosen; thus, the introduced gene was expressed from its original chromosomal location. Gene disruptions and mutant complementation were verified by PCR, phenotype where available, and Southern hybridization analysis (see Fig. S1 in the supplemental material).

All primers used in this study are listed in Table S1 in the supplemental material.

**Manipulation of nucleic acids.** DNA was extracted from *S. cerevisiae* and *C. albicans* strains for PCR and Southern hybridization analyses, as described previously (27). To confirm *C. albicans* gene deletions by Southern hybridization analysis, 2 μg of genomic DNA was digested with various restriction enzymes, separated by agarose gel electrophoresis, denatured, and transferred to a nylon membrane (Roche) as described previously (52). Southern hybridization probes were prepared from PCR products (agarose gel purified using the QIAquick gel extraction kit; Qiagen) and labeled with [α-32P]dCTP (Perkin-Elmer) using the random primer labeling procedure. Prehybridization and hybridization were performed in ULTRAhyb buffer (Ambion), blots were washed according to the manufacturer's instructions, and hybridized bands were visualized using a Typhoon 9200 variable mode imager (Molecular Dynamics).

**Serum treatments and sensitivity assays.** To test *S. cerevisiae* for serum sensitivity, strains were typically grown overnight in YPD, washed twice in sterile distilled water, and then added to 1 or 3 ml fetal bovine serum (FBS; Sigma catalog no. F2424) at a concentration of approximately 1 × 10^6 cells/ml. The volume of FBS depended on whether cell viability was estimated by spot dilution or cells were plated for absolute numbers. Strains were either added to independent serum tubes, or differentially marked strains (typically the wild-type, YAG40, and two differently marked strains with the same gene disrupted) were competed in the same tube. Serum was incubated at 37°C, and at various time points, aliquots were removed, serially diluted, and plated to selective media to determine cell viability. To provide an approximate estimate of cell viability, cultures were diluted 10-fold and 5-μl spots were plated. To determine absolute numbers, 100-μl aliquots of appropriate dilutions were plated at least in duplicate. To test *C. albicans* serum survival, the assay was essentially the same, but strains were incubated in serum separately, and the incubation temperature was

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**Table 1. Strains used in this study**

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<tr>
<th>Strain</th>
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**C. albicans**

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30°C to reduce hyphal formation. Survival in other media was assayed similarly. Typically, experiments were performed with two independently constructed strains with the same gene disrupted to ensure reproducibility of results.

Serum was treated in various ways to remove individual components. Serum and YPD pH was adjusted using HCl or KOH, following the addition of 0.05 mM HEPES buffer. Serum was delipidated by using PHM-L Liposorb (Calbiochem) according to the manufacturer’s instructions. To remove proteins, serum was filtered through a <3-kDa Centricron centrifugal filter and then heat inactivated by incubation at 56°C for 30 min, followed by an overnight digestion with proteinase K (InVitrogen) (50 μg ml⁻¹) at 37°C. Calcium and iron were chelated by the addition of 1,2-bis-(2-aminophenoxy)-ethane-N,N',N’-tetraacetic acid (BAPTA; 4 mM) or deferoxamine mesylate (25 μg ml⁻¹), respectively. Other divalent cations were removed by incubation of serum with 5% (wt/vol) Chelex-100 (Sigma) at 4°C for 3 h. Catalase (200 μg ml⁻¹) was added and incubated for 3 h at 25°C to remove serum peroxide. Where specified, amino acids were added to serum at the following concentrations: l-serine (250 μg ml⁻¹), l-threonine (400 μg ml⁻¹), l-methionine (170 μg ml⁻¹), l-isoleucine (120 μg ml⁻¹), l-glycine (230 μg ml⁻¹), and l-valine (410 μg ml⁻¹). Survival in serum (Sigma catalog no. H4522) was also tested.

**Experimental mouse infections.** The in vivo survival of *S. cerevisiae* strains was compared following infection by lateral tail vein injection of 4-week-old male CD-1 mice (outbred, immune competent; Charles River Laboratories), as described previously (10, 22). For each gene disruption tested, 15 mice were infected with 2 × 10⁶ CFU of an equal ratio of one reference strain (YAG40) and two experimental strains, each containing different markers disrupting the same gene, that had been grown to mid-log phase in YPD medium, washed in sterile phosphate-buffered saline (PBS), and resuspended in PBS. To determine the exact proportion of each strain present, the inocula were diluted and plated to selective media. At times 4 h, 1 week, and 2 weeks postinfection, five mice per time point were euthanized by CO₂ inhalation. Since the brain is the predominant organ inhabited by *S. cerevisiae* in CD-1 mice (10), the brains were recovered, homogenized in 5 ml PBS supplemented with 100 μg ml⁻¹ ampicillin and streptomycin, pelleted by centrifugation (700 × g for 10 min), resuspended in 1 ml PBS, and plated to selective media to determine the relative numbers of each strain present. Results at each time point (t – s) were expressed as competitive index (CI) values (7, 9), a measure of (experimental strain/reference strain), where CI values of 0.91 and 0.76 after 2 weeks, respectively; thus, mutants have attenuated virulence.

To test virulence of *C. albicans* strains, 7-week-old male CD-1 mice were given a lateral tail vein injection of 1 × 10⁶ cells suspended in PBS. Mice were observed twice daily, and animals that appeared moribund (>15% loss of body weight, lethargic, or not accessing food) were sacrificed. Mice that remained healthy throughout the course of the experiment were euthanized after 28 days, and their organs were recovered, homogenized in 5 ml PBS plus ampicillin plus streptomycin, and then plated to plates containing YPD plus nourseothricin (YPD + Nat) to determine if the infection had been cleared. Mouse survival data were analyzed using the Kaplan-Meier test.

Mice were fed ad libitum for the course of the experiments. All animal experiments met with institutional guidelines and were approved by the Institutional Animal Care and Use Committee.

**MIC assays.** To determine the MICs of fluconazole (Pfizer), 5-fluorocytosine (Liposome), amphotericin B (Gibco), and caspofungin (Merck) for each strain, the MICs were determined by the microdilution method for each drug concentration. The MIC was defined as the lowest concentration of drug that resulted in a 95% reduction in CFU from the initial inoculums (MFC₉₅).

**RESULTS**

*S. cerevisiae* thr1Δ and thr4Δ mutants are very rapidly eliminated in vivo. Having determined that *S. cerevisiae* hom3Δ mutants are eliminated by 1 week in vivo (31), we decided to assess the in vivo survival requirement of other threonine bio-

![FIG. 1. Survival of *S. cerevisiae* thr1Δ and thr4Δ strains in vivo.](http://ec.asm.org/)
thr1Δ and thr4Δ mutants are extremely serum sensitive.

The considerable difference in survival between the S. cerevisiae thr1Δ and thr4Δ mutants and the wild type and hom3Δ mutants after only 4 h in vivo was far too high to be attributable to differences in growth rate and was not due to auxotrophy per se. The results instead indicate that the in vivo environment is more toxic to thr1Δ and thr4Δ mutants. Since an initial stage in this experimental infection process, and likely most natural infections, involves transit through the bloodstream, we investigated whether S. cerevisiae thr1Δ and thr4Δ mutants were serum sensitive. Two each of the hom3Δ, thr1Δ, and thr4Δ mutants were incubated with the wild type in fetal bovine serum (FBS) at 37°C and then serially diluted and plated to selective media to determine survival. As judged by semiquantitative spot dilution assays (Fig. 3A), the wild-type viability increased over time and hom3Δ mutant viability remained at the inoculated level, while thr1Δ and thr4Δ mutant numbers declined dramatically, with an approximately 100- to 1,000-fold reduction after 4 h. Since thr1Δ (and thr4Δ) mutants were already highly depleted after 4 h, a separate competition experiment to determine how rapidly mutants are killed in serum compared overall numbers of the wild type (YAG40) and two differently marked thr1Δ mutants (YJK498a and YJK506a) surviving serum incubation at earlier time points. As can be seen in Fig. 3B, thr1Δ mutant viability declined rapidly, with average reductions from input values of 8-fold, 279-fold, 863-fold, and 2,309-fold, following 1, 2, 3, or 4 h of incubation, respectively.

To investigate whether serum sensitivity is a conserved phenomenon of thr1Δ mutants, the serum survival rates of C. albicans, thr1Δ (CJK35 and CJK37), hom3Δ (CJK41 and CJK43), and wild-type (SC5314) strains were also compared. All strains were incubated at 30°C to minimize hyphal formation, but microscopic examination at 4 h revealed that the wild-type and the hom3Δ mutants still produced some hyphae. Since even low levels of hyphal formation in serum by the wild-type and hom3Δ mutants resulted in some clumping in serum, their overall cell numbers were greater than those represented by spot dilution. However, no hyphal formation was observed for the thr1Δ mutant. While the C. albicans wild-type and hom3Δ mutant numbers increased over time, thr1Δ mutants were found to also be extremely serum sensitive, with an approximately 100- to 1,000-fold reduction in viability after 4 h (Fig. 3A).

Serum toxicity is due to low threonine levels. Microscopic examination of serum-incubated cells showed that S. cerevisiae thr1Δ mutants were predominantly unbudded cells, thus ruling out cell clumping as an explanation for low numbers of recoverable colonies. Therefore, to identify the toxic feature of serum, S. cerevisiae thr1Δ mutants were incubated in serum that had been treated in various ways to remove serum components, individually and in combination, and viability at different time points was estimated by spot dilution on YPD. Reduction of incubation temperature to 30°C had no influence on S. cerevisiae thr1Δ mutant survival, nor did serum pH, serum source (with FBS and human serum tested), proteins, peroxides, lipids, iron, or other trivalent and divalent ions other than calcium (see Table S2 in the supplemental material). Chelation of calcium resulted in an approximately 10-fold increase in S. cerevisiae thr1Δ mutant survival after 4 h but had no benefit at later time points (Fig. 4). The calcium effect was serum specific, as addition of CaCl2 at levels equivalent to calcium in serum (3.1 mM) had no effect on the viability of S. cerevisiae thr1Δ mutants in SD + Met + Thr (data not shown).

Interestingly, incubation in two defined serum-substitute media, DMEM (plus NaMOPS plus NaHCO3) and RPMI 1640, was also toxic to S. cerevisiae thr1Δ mutants, with an approximately 10-fold reduction in RPMI 1640 and a 100- to 1,000-fold reduction in DMEM after 4 h (see Fig. S2 in the supplemental material). Therefore, the thr1Δ-inhibitory feature of serum is not serum specific. Various versions of DMEM were prepared that were deficient in each inorganic salt individually or in combination, amino acids, or vitamins. Aside from a slight protective effect after 4 h in the absence of calcium chloride, consistent with calcium chelation in serum, the S. cerevisiae thr1Δ mutant died at a similar level in all of the other medium formulations.

The results implied that rather than the inhibitory feature being something that was present in serum and defined serum substitute media, it was in fact the absence or smaller amount of a compound. Since amino acids are present in serum and tissue culture media at levels lower than those added to yeast minimal media (54), threonine, and the threonine-related amino acids serine, glycine, methionine, isoleucine, and valine were added individually to serum at 10-fold-higher concentrations than those present in mouse serum (11). Interestingly, addition of threonine (Fig. 4), but no other amino acid tested (see Table S2 in the supplemental material), completely suppressed S. cerevisiae thr1Δ mutant serum sensitivity. Similarly, when S. cerevisiae thr1Δ mutants were incubated in SD + Met + low Thr (39 μg ml−1, equivalent to threonine levels in mouse serum [11]) medium, viability was reduced at least 100-fold after 8 h of incubation. In addition, when threonine was completely omitted from the medium, S. cerevisiae thr1Δ mutant numbers were reduced at least 100-fold after 4 h of incubation (Fig. 5). Consistent with serum results, incubation for 8 h in threonine-deficient medium had no effect on hom3Δ mutant viability. Therefore, the principal feature of serum toxic to S. cerevisiae thr1Δ mutants is the low threonine concentration.

Similar experiments were also performed to ascertain why C. albicans thr1Δ mutants were serum sensitive. In contrast to S.
S. cerevisiae thr1Δ mutants, calcium chelation had no effect on C. albicans thr1Δ mutant survival (see Table S2 in the supplemental material). Also contrasting with the S. cerevisiae thr1Δ results, buffering the serum pH to 7.5 or below substantially ameliorated C. albicans thr1Δ serum sensitivity, with an at least 100-fold increase in survival after 4 h (Fig. 4). The C. albicans thr1Δ mutants were observed to form hyphae in serum only when pH levels were conducive to survival. The pH sensitivity of C. albicans thr1Δ mutants was medium dependent, since there was no cytocidal effect when mutants were incubated in YPD medium at pH 8 (see Fig. S3 in the supplemental material). Importantly, as with the S. cerevisiae thr1Δ mutant, C. albicans thr1Δ mutant serum sensitivity could be completely overcome by threonine addition (Fig. 4), and mutants were similarly sensitive to incubation in minimal medium lacking threonine (Fig. 5).

**Serum sensitivity is a consequence of intermediate accumulation.** The cidal nature of serum and threonine starvation on thr1Δ mutants in both S. cerevisiae and C. albicans is not merely a consequence of threonine auxotrophy since these phenotypes are not observed in hom3Δ mutants. Furthermore, we have shown that S. cerevisiae thr1Δ and thr4Δ as well as C. albicans thr1Δ mutants are sensitive to exogenous homoserine, and the starvation-cidal phenotype of S. cerevisiae thr1Δ and thr4Δ mu-
the serum sensitivity in both species were suppressed by dis-
SD + Met + Thr medium and at least 30-fold more sensitive in YPD. In both species, since hom3Δ blocked the 5-fluorocytosine sensitivity of thr1Δ and thr4Δ mutants, sensitivity is a consequence of toxic intermediate accumulation. Therefore, even in the presence of abundant exogenous threonine, intermediate accumulation has deleterious, clinically relevant effects.

**DISCUSSION**

The ability to survive in serum represents a crucial aspect for fungal virulence since travel through the bloodstream is necessary for fungal dissemination and systemic infection, and many components and conditions present in the bloodstream likely mimic those found in other body niches occupied during fungal infection. However, serum is a hostile environment for fungal growth due to various components of innate immunity, high pH, ionic composition, and the low concentrations of many nutrients. Consequently, we observed only modest serum proliferation by wild-type S. cerevisiae, a rarely observed phenotype. Conversely, the robustly pathogenic C. albicans proliferates profusely in serum and undergoes a yeast-to-hypha morphological transition upon serum exposure, enabling tissue invasion and escape from the bloodstream, which is mediated by a number of environmental cues, such as pH and temperature (reviewed in references 6, 24, and 39). Not surprisingly, the loss of the ability to survive in serum, mediated by disruption of the calcium-activated phosphatase calcineurin, correlates with attenuated virulence in both C. albicans and Aspergillus fumigatus (5, 13). Despite the importance of serum survival as a virulence factor, much of what is known about the genetic requirements for fungal proliferation and survival in serum is implied through fungal transcriptional responses observed following serum exposure (18, 19). Consistent with a commonly observed lack of correlation between transcription and phenotype (for example, see reference 2), threonine biosynthetic pathway genes were not identified in these screens.

Since some threonine biosynthetic mutants have deleterious phenotypes in addition to auxotrophy (2, 8, 14–16, 20, 33, 51), S. cerevisiae hom3Δ mutants do not survive in vivo (31), and C. neoformans threonine biosynthetic genes are essential (34), we were interested in further exploring the potential of threonine biosynthetic enzymes as antifungal drug targets. To this end, we demonstrated that S. cerevisiae thr1Δ and thr4Δ mutants were not only unable to survive in vivo, they were also highly attenuated after only 4 h postinfection. Consistent with this attenuation, serum incubation for the same period of time resulted in a severe decline in viability of thr1Δ and thr4Δ, but not hom3Δ, mutants. Furthermore, while disruption of HOM3 in the clinically relevant pathogen C. albicans did not influence virulence, C. albicans thr1Δ mutants were considerably attenuated in virulence and were also serum sensitive.

Calcium was shown to cause the serum sensitivity of C. albicans calcineurin mutants (4), and we also identified calcium as a minor contributor to the serum- and DMEM-mediated killing of S. cerevisiae thr1Δ mutants. While S. cerevisiae thr4Δ mutants have been reported to be sensitive to calcium chloride at higher concentrations (0.7 M in YPD) (8), we observed no effect on thr1Δ mutant growth when calcium was added to SD + Met + Thr at levels equivalent to those found in serum (9.1 to 9.5 mg 100 ml⁻¹) (11). Similar results were observed by Blankenship et al. (4), who attributed differences to the presence of a possible calcium chelator in YPD, reducing the effective calcium concentration, or different metabolic rates in the different types of media affecting the calcium response.

High pH was determined to be a major contributor to C. albicans thr1Δ serum lethality. At pHs greater than 7.5, thr1Δ mutants were also deficient in germ tube formation, a process that occurs soon after serum exposure; thus, thr1Δ mutants that managed to survive serum exposure would also be deficient in tissue invasion, a potential clinically relevant benefit of Thr1p inhibitors. The pH of mouse serum can range from 7.3 to 7.55 (11), thus straddling the pH limit to which C. albicans thr1Δ mutants survive in serum and form hyphae, which might explain why these mutants are not completely eradicated in vivo, compared with S. cerevisiae thr1Δ mutants. Any localized pH drop caused by fungal metabolism within a microcolony that managed to establish in vivo, could also provide a protective effect. While lowering serum pH did not support S. cerevisiae thr1Δ mutant survival in serum, thr1Δ mutants have been reported to be pH sensitive (20), and indeed, we observed that incubation of thr1Δ mutants in YPD at pHs above 7 was cidal. Thus, pH could be cidal in combination with an additional component of serum for S. cerevisiae thr1Δ mutants.

The major feature of serum demonstrated to be toxic to both S. cerevisiae and C. albicans thr1Δ mutants was its low threonine concentration (25 to 40 μg ml⁻¹) (11, 12), which equates to approximately one-tenth of the concentration typically added to yeast minimal media (54). Since hom3Δ and hom6Δ mutants are not serum sensitive, and disruption of HOM3 or HOM6 blocks thr1Δ and thr4Δ mutant serum sensitivity, toxicity is not a consequence of altered expression of neighboring genes, threonine auxotrophy, or the loss of a second function of Thr1p or Thr4p; rather, we hypothesize that serum sensitivity is due to the accumulation of the biosynthetic intermediate homoserine, which we find to be toxic (33). Our model predicts that the low levels of threonine present in serum reduce threonine feedback repression of Hom3p (41, 48), increase threonine biosynthesis enzymes as antifungal drug targets. To this end, we demonstrated that S. cerevisiae thr1Δ and thr4Δ mutants were not only unable to survive in vivo, they were also highly attenuated after only 4 h postinfection. Consistent with this attenuation, serum incubation for the same period of time resulted in a severe decline in viability of thr1Δ and thr4Δ, but not hom3Δ, mutants. Furthermore, while disruption of HOM3 in the clinically relevant pathogen C. albicans did not influence virulence, C. albicans thr1Δ mutants were considerably attenuated in virulence and were also serum sensitive.

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Calcium was shown to cause the serum sensitivity of C. albicans calcineurin mutants (4), and we also identified calcium as a minor contributor to the serum- and DMEM-mediated killing of S. cerevisiae thr1Δ mutants. While S. cerevisiae thr4Δ mutants have been reported to be sensitive to calcium chloride at higher concentrations (0.7 M in YPD) (8), we observed no effect on thr1Δ mutant growth when calcium was added to SD + Met + Thr at levels equivalent to those found in serum (9.1 to 9.5 mg 100 ml⁻¹) (11). Similar results were observed by Blankenship et al. (4), who attributed differences to the presence of a possible calcium chelator in YPD, reducing the effective calcium concentration, or different metabolic rates in the different types of media affecting the calcium response.
a toxic threonine analog, inhibiting or misincorporating in processes that utilize threonine as a substrate.

The extreme survival defects of S. cerevisiae thr1Δ and thr4Δ mutants in vivo, the inhibition of hyphal formation, the attenuated survival of C. albicans thr1Δ mutants, and the essentiality of these genes in C. neoformans (34) all validate the potential of Thr1p and Thr4p as antifungal drug targets. Several inhibitors, including phosphohomoserine analogs (17) and the phosphohomoserine analog-containing peptides rhizocticin and dehydroaspartic acid or D,L-2-amino-5-phosphonovaleric acid (data not shown). Nonetheless, the rapidity and profound lethality observed upon exposure of thr1Δ and thr4Δ mutants to serum and threonine starvation conditions indicate that Thr1p and Thr4p inhibitors would be fungicidal in vivo. Such a fungicidal effect would be advantageous over fungistatic agents since fungastic agents require immune function to clear existing fungi from the body so that the infection does not recur despite drug removal, and many fungal infections occur in immunocompromised individuals. Since both S. cerevisiae and C. albicans thr1Δ are sensitive to other inhibitors of amino acid biosynthesis, likely due to general control-mediated increased flux through the threonine biosynthetic pathway that results in increased homoserine production (33), we predict that inhibitors of Thr1p (and Thr4p) would have a therapeutically beneficial synergistic action with inhibitors of other amino acid biosynthetic pathways. For example, thr1Δ mutants are hypersensitive to the herbicide sulfonyluron methyl (33), which targets the isoleucine and valine biosynthetic enzyme acetolactate synthase, also an interesting drug target, since it is also required for fungal virulence and/or survival in vivo (31, 32, 36). An additional virtue for targeting Thr1p and Thr4p is the demonstration that thr1Δ and thr4Δ mutants are hypersensitive to the clinically relevant drug 5-fluoroacytosine, even under conditions of high threonine. Since 5-fluoroacytosine is converted into 5-fluoroouracil, which inhibits RNA and DNA metabolism (25, 28, 29, 53), and thr1Δ and thr4Δ mutants are hypersensitive to DNA-damaging agents (2, 3), 5-fluoroacytosine-induced DNA damage may explain the observed hypersensitivity. Thus, novel Thr1p or Thr4p inhibitors would have great potential as sole (C. neofomrans) and combination (C. albicans) therapies.

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