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Regulatory mechanisms controlling morphology and pathogenesis in *Candida albicans*

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Candida albicans, a major human fungal pathogen, can cause a wide variety of both mucosal and systemic infections, particularly in immunocompromised individuals. Multiple lines of evidence suggest a strong association between virulence and the ability of *C. albicans* to undergo a reversible morphological transition from yeast to filamentous cells in response to host environmental cues. Most previous studies on mechanisms important for controlling the *C. albicans* morphological transition have focused on signaling pathways and sequence-specific transcription factors. However, in recent years a variety of novel mechanisms have been reported, including those involving global transcriptional regulation and translational control. A large-scale functional genomics screen has also revealed new roles in filamentation for certain key biosynthesis pathways. This review article will highlight several of these exciting recent discoveries and discuss how they are relevant to the development of novel antifungal strategies. Ultimately, components of mechanisms that control *C. albicans* morphogenesis and pathogenicity could potentially serve as viable antifungal targets.

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Introduction

Candida albicans is a major human fungal pathogen and a leading cause of hospital-acquired bloodstream infections in the U.S, with an attributable mortality rate of 35–60% [1,2]. *C. albicans* is also responsible for a wide variety of mucosal infections, including oral and vaginal thrush [3–6]. Immunocompromised individuals, including organ transplant recipients, HIV/AIDS patients and cancer patients are especially vulnerable to infections [5–7]. HIV/AIDS patients are particularly susceptible to mucosal infections whereas cancer patients and organ transplant recipients are susceptible to both mucosal and systemic infections [8–13].

The ability of *C. albicans* to undergo a reversible morphological transition from yeast (single oval cells) to filaments (elongated cells attached end-to-end) is promoted in response to a variety of host environmental conditions and has long been associated with virulence and pathogenesis [14–17]. Indeed, *C. albicans* filaments are known to play an important role in the establishment of biofilms, invasion of epithelial cell layers, breaching of endothelial cells and macrophages, tissue invasion, as well as contact sensing (thigmotropism) [17–19]. Initial studies showed that strains locked in either the yeast or filamentous form were highly attenuated for virulence in a mouse model of systemic candidiasis [20–22]. More definitive evidence came from a subsequent study which demonstrated that allowing a genetically engineered strain to transition from yeast to filaments at different time points during the course of an infection was sufficient to promote virulence [23]. A complementary experiment showed that inoculating mice with yeast cells of a strain that has been engineered to rapidly undergo the yeast-filament transition and promote strong hyphal growth was sufficient to enhance virulence in a mouse model of systemic candidiasis [24]. Additional evidence supporting a strong association between filamentation and pathogenesis came from the demonstration that a *C. albicans* strain deleted for *HGC1*, encoding a cyclin-related protein specifically important for hyphal growth, was highly attenuated for virulence in the mouse systemic model [25]. Finally, a recent large-scale functional genomics screen has indicated that most *C. albicans* mutants defective for filamentation are also defective for virulence [26••]. Interestingly, however, this study also showed that filamentation is not required for macrophage lysis and an independent genetic study has identified *C. albicans* mutants that are defective for kidney infectivity but not morphogenesis [26••,27]. While these studies suggest that the relationship between morphology and virulence in *C. albicans* may be more complex than expected, the large majority of evidence indicates a strong association between the yeast-filament transition and pathogenesis. Also consistent with this notion, several small molecule compounds have recently been identified that strongly inhibit *C. albicans* filamentation and biofilm formation as well as virulence and pathogenicity [28,29,30••]. These findings are important because they suggest that targeting

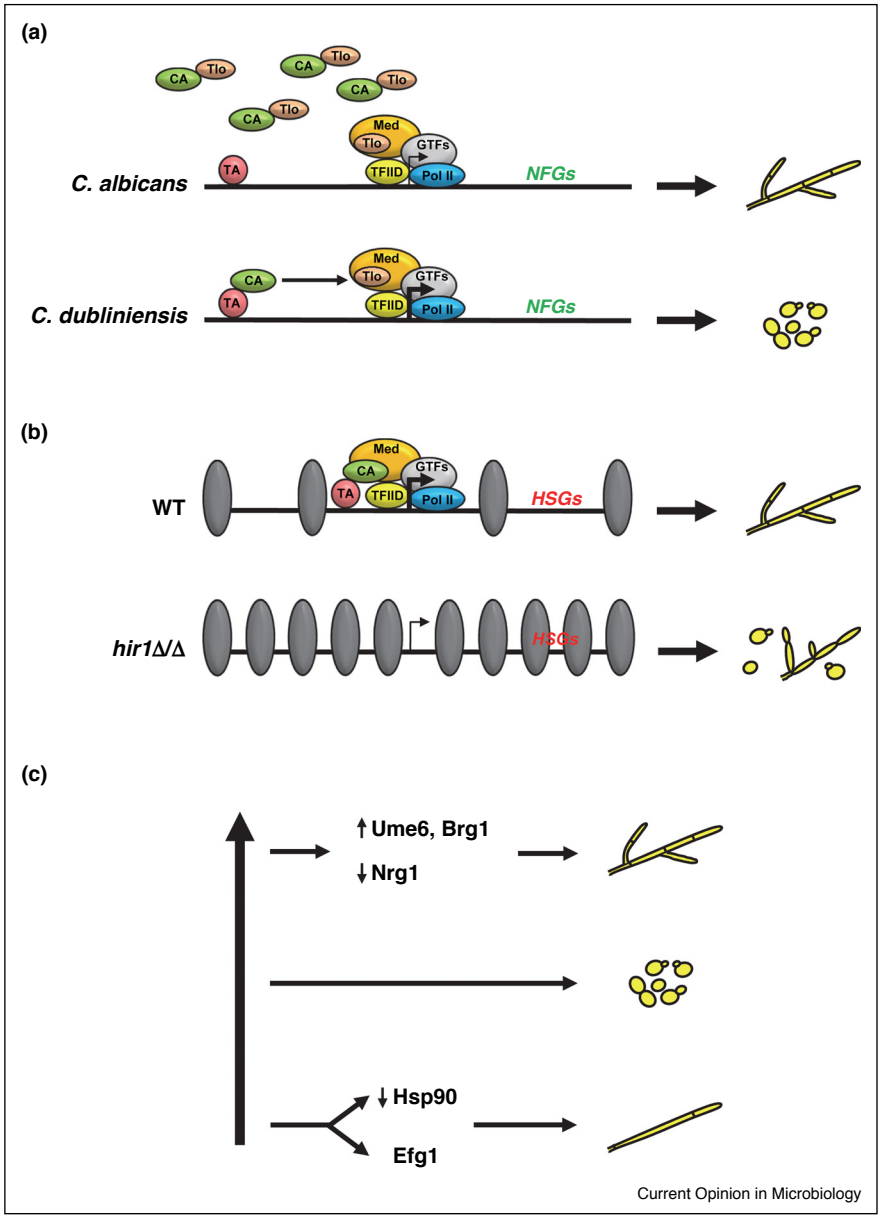
mechanisms that promote the *C. albicans* yeast-filament transition may serve as a viable strategy for the development of novel and more effective classes of antifungals [31]. Over the past several years, many new and exciting advances have been made in identifying and characterizing such mechanisms. This review article will serve to highlight several recently discovered global transcriptional and translational mechanisms important for the *C. albicans* yeast-filament transition as well as certain previously known biosynthesis pathways that have been shown to play novel roles in this process. New insights and perspectives into whether components of these mechanisms and pathways may serve as promising targets for novel antifungals will also be provided.

Global transcriptional mechanisms that control the *C. albicans* morphological transition

A variety of transcription factors have been shown to regulate the *C. albicans* yeast-filament transition [32,33]. Whole-genome transcriptional profiling experiments have demonstrated that these factors generally control large sets of target genes, several of which encode components of the *C. albicans* filamentous growth program [34–36]. While most of these transcription factors are promoter-specific DNA-binding proteins, considerably less is known about the role of general transcription machinery components in regulating *C. albicans* morphology and virulence. However, recent studies have shed new light in this area and also provided some insight into the evolution of morphology in *Candida* species. Whole-genome sequencing of multiple *Candida* species revealed that *C. albicans* showed a significant expansion in the *TLO* gene family compared to other *Candida* species, which are generally less pathogenic and do not filament as readily [37–40]. *TLO* genes encode fungal-specific components of the Mediator, a large multi-subunit complex that functions as a general transcriptional co-activator in eukaryotes [38,41,42,43•]. Liu *et al.* have recently found that overexpression of *TLO2* in *Candida dubliniensis* led to a significant increase in filamentation that was associated with a large 'free' pool of Tlo2 protein [43•]. Interestingly, nuclear localization of the *C. dubliniensis* Tlo2 activation domain was sufficient to promote filamentation. Med3, a component of the Mediator complex, was shown to be important for nuclear localization of Tlo proteins in both *C. dubliniensis* and *C. albicans* and *C. albicans med3*Δ/Δ mutants were attenuated for virulence in a mouse model of systemic candidiasis. Liu *et al.* suggest that 'free' Tlo2 activation domain competes with DNA-bound transcriptional regulators for association with Mediator components, which, in turn, results in the activation of genes important for filamentation [43•] (Figure 1a). The genomic expansion of the *TLO* gene family in *C. albicans* leads to a larger pool of 'free' Tlo proteins, which may partially account for the significantly increased filamentation ability of *C. albicans* compared to other *Candida* species, as this evolutionary difference can be overcome simply by overexpressing *TLO2* in *C. dubliniensis*. These findings are important because they suggest a novel mechanism by which increased expression of a Mediator complex component can promote both filamentation and pathogenicity in *C. albicans*. Indeed, overexpression of certain *C. albicans TLO* genes, including *TLOβ2*, is sufficient to promote *C. dubliniensis* pathogenicity in a *Galleria* infection model [44]. As Tlo proteins are fungal-specific, they may also represent promising targets for development of new antifungal strategies. In future work, it will be interesting to determine whether overexpression of *TLO* genes in other non-*albicans Candida* species is sufficient to promote strong filamentation. If so, this would strengthen the argument that enhanced filamentation and pathogenicity of *C. albicans* can be attributed to *TLO* gene expansion.

A novel chromatin-mediated transcriptional mechanism has also recently been shown to control *C. albicans* morphogenesis in response to a variety of environmental signals, including growth in serum, GlcNAc and Spider medium at 37°C. This mechanism is mediated by the evolutionarily conserved HIR histone chaperone complex, which facilitates chromatin assembly in a replication-independent manner [45–47]. Deletion of *HIR1*, which encodes a key component of this complex, resulted in a reduction in both filamentous growth and sensitivity to morphogenesis signals [48•]. Interestingly, genes associated with filamentation in *C. albicans* were still induced in the *hir1*Δ/Δ mutant, although the transcriptional amplitude of induction was significantly reduced. Hir1 likely functions downstream of the cAMP/PKA pathway to promote the expression of filament-specific transcripts during the early stages of the yeast-filament transition. In support of this hypothesis, the *hir1*Δ/Δ deletion strain phenocopies a strain deleted for Efg1, an important downstream target of the cAMP-PKA signaling pathway, and the *hir1*Δ/Δ filamentous growth defect can be rescued by overexpression of Ume6, a transcriptional regulator important for maintaining hyphal filament extension during the later stages of filamentation [35,48•,49]. How exactly does the HIR histone chaperone complex affect filament-specific gene expression in *C. albicans*? Jenull *et al.* have shown increased histone densities at the promoters of several filament-induced genes in the *hir1*Δ/Δ mutant [48•], suggesting that the HIR complex functions to generate an open chromatin structure at these promoters, thereby enhancing filament-specific gene expression (Figure 1b). Importantly, this complex appears to function as part of a novel fine-tuning mechanism to carefully modulate both transcriptional and, subsequently, phenotypic responses to filament-inducing signals. Although the HIR complex is highly conserved in fungal species, at this point it remains unclear whether this complex plays a similar role in modulating both filamentous growth and filament-specific gene expression in other fungal pathogens. Because HIR histone chaperone complex

Figure 1



Models for control of *C. albicans* morphology by global transcriptional mechanisms. **(a)** *C. albicans* shows a significant genomic expansion in members of the *TLO* gene family, which encodes fungal-specific subunits of the Mediator (Med) transcriptional complex, compared to the less pathogenic *C. dubliniensis*. Increased levels of Tlo proteins in *C. albicans* versus *C. dubliniensis* results in a large 'free' pool of Tlo protein, which competes with DNA-bound transcriptional activators (TA) for binding to co-activators (CA). As a consequence, certain genes encoding negative filamentous growth regulator genes (*NFGs*) may not be activated and cells grow as filaments. In *C. dubliniensis* there is no free pool of Tlo proteins, thus allowing transcriptional activators to make contact with the Mediator complex, which may result in activation of *NFGs* and promotion of growth in the yeast form. Alternative mechanisms involving indirect transcriptional regulation are also possible. GTFs, general transcription factors. Pol II, RNA polymerase II. **(b)** In a wild-type (WT) strain, upon exposure to appropriate filament-inducing conditions transcriptional activators (TA) promote assembly of a transcriptional pre-initiation complex, which increases the expression of hyphal-specific genes (*HSG*s) leading to hyphal growth. In the absence of Hir1, a key component of the replication-independent histone chaperone complex, histone (gray) density is increased, leading to a reduction in the amplitude of *HSG* expression and reduced filamentation. **(c)** Hsf1, a key *C. albicans* transcriptional regulator that responds to heat shock, promotes filamentation at high levels by increasing expression of positive filamentous growth regulators and reducing expression of negative regulators. Low levels of Hsf1 also promote filamentation through an Efg1-dependent pathway and by compromising function of the Hsp90 chaperone; filaments generated by Hsf1 depletion have distinct features and are multinucleate with reduced septa. Intermediate levels of Hsf1 result in yeast growth (adapted in part from Ref. 53•,73).

components are also evolutionarily conserved in higher eukaryotes and involved in embryonic development [50], it appears unlikely that these components could serve as viable antifungal drug targets, unless a critical fungal-specific protein domain is identified.

Hsf1, a key regulator of *C. albicans* global transcriptional changes during heat shock, has also recently been shown to play an important and novel role in controlling *C. albicans* morphogenesis in response to temperature [51,52,53•]. Depletion of Hsf1 compromises the function of Hsp90, a critical molecular chaperone, thereby increasing filamentation (Figure 1c). Interestingly, overexpression of Hsf1 also promotes the *C. albicans* yeast-filament transition through an Hsp90-independent mechanism by increasing the expression of several positive regulators of morphogenesis, such as *UME6* and *BRG1*, as well as reducing the expression of *NRG1*, an important negative regulator of this process [53•]. Careful fine-tuning of Hsf1 levels therefore appears to be critical for maintaining the *C. albicans* yeast morphology. Although Hsf1 is an essential protein in *C. albicans*, it is also evolutionarily conserved in higher eukaryotes where it functions as an important regulator of stress response [54]. While antifungals that reduce or abolish Hsf1 function may not be practical, drugs that function to specifically stabilize *HSF1* transcript or protein levels could be effective in blocking the *C. albicans* yeast-filament transition and reducing pathogenicity, as strains locked in the yeast form have previously been shown to have significantly reduced virulence in a mouse model of systemic candidiasis [20,23].

Regulation of *C. albicans* morphogenesis and pathogenicity by translational mechanisms

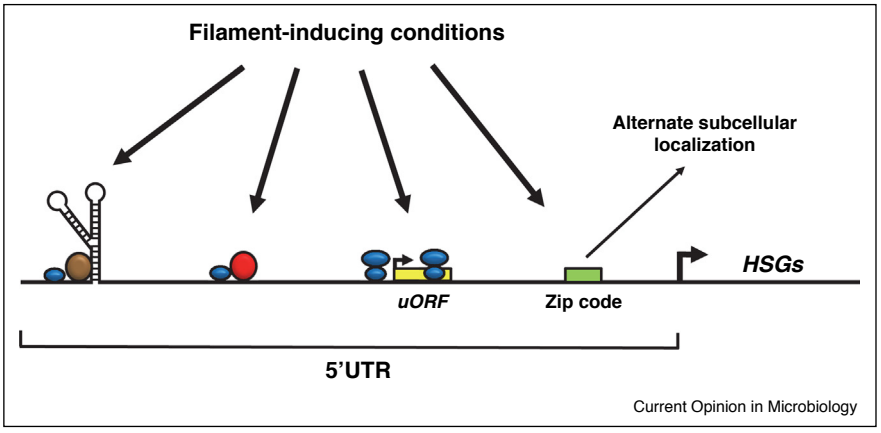
While a variety of transcriptional mechanisms have been shown to regulate the *C. albicans* yeast-filament transition, until recently very little, if anything, was known about translational control of this process. An initial study indicated that *UME6*, a key transcriptional regulator of morphology and virulence, is translationally regulated by an exceptionally long (>3 kb) 5′ untranslated region (UTR) [35,55••]. The 5′ UTR was specifically shown to inhibit translational efficiency of *UME6* and deletion of this element resulted in enhanced filamentation under a variety of filament-inducing conditions. Interestingly, the level of translational inhibition directed by the *UME6* 5′ UTR was modulated in response to different filament-inducing conditions, including temperature, serum, Spider and Lee's medium, pH 6.8 [55••]. A subsequent study has shown that expression of *EFG1* is also controlled by a 5′ UTR-mediated translational efficiency mechanism [56•]. Deletion of the *EFG1* 5′ UTR affected *C. albicans* filamentation. However, unlike the *UME6* 5′ UTR, the *EFG1* 5′ UTR was shown to function as a positive, rather than negative, regulator of translational efficiency.

An RNA-seq analysis has indicated that a variety of additional transcriptional regulators of *C. albicans* morphology and virulence also possess long (>500 bp) 5′ UTR regions [57]. Polysome profiling has demonstrated that transcripts for several of these regulators show increased abundance in monosome versus polysome fractions, suggesting that they are under negative translational control [58]. In addition, a 5′ UTR region has also been shown to control translational efficiency of *WOR1*, a key regulator of *C. albicans* white-opaque switching and mating [58]. Long 5′ UTR regions are present upstream of genes associated with a variety of additional *C. albcans* virulence-related processes, including adhesion, secreted degradative enzyme production and the ability to tolerate oxidative stress [57]. While it is unclear at this point whether all of these 5′ UTRs function to direct translational control, their presence upstream of so many genes associated with virulence and/or virulence-related processes suggests that they are important for pathogenicity. How exactly do 5′ UTR regions control translation? This may occur through a variety of mechanisms (Figure 2) [59,60]. For example, both *UME6* and *EFG1* 5′ UTRs are predicted to form complex secondary structures, which could affect ribosome accessibility. RNA-binding proteins that associate with these structures, or other regions of the 5′ UTR, could also block or facilitate ribosome scanning. Short upstream open reading frames (uORFs) present in 5′ UTR regions can function to inhibit translational readthrough to the main ORF (Figure 2). Finally, zip code sequences in 5′ UTR regions can direct the entire transcript to a location of the cell that is not undergoing active translation. While the precise mechanisms important for controlling translational efficiency of *UME6*, *EFG1* and a variety of other regulators of *C. albicans* morphology and virulence remain unknown, fungal-specific components of these mechanisms are likely to serve as potential targets for the development of novel and more effective antifungals. In support of this notion, several highly effective antibiotics are known to specifically target bacterial translation mechanisms [74].

Functional genomics identifies biosynthesis pathways with new roles in *C. albicans* filamentation

A recent large-scale functional genomics screen has provided an unbiased global approach to identify factors that play an important role in *C. albicans* morphogenesis [26••]. In addition to identifying many previously known regulators of filamentation, this screen also identified two biosynthesis pathways that were found to play unexpected roles in the *C. albicans* yeast-filament transition. The first is the ergosterol biosynthesis pathway, which already represents a known target for the azole class of antifungals [61]. Transcriptional repression of all genes in the early stages of this pathway (up to episterol

Figure 2



Regulation of *C. albicans* morphogenesis by 5′ UTR-mediated translational mechanisms. Translational efficiency of hyphal-specific genes (*HSG*s) could be altered by the formation of RNA secondary structures that block ribosome (blue) access. RNA-binding proteins (brown) that recognize these structures could also function to promote or inhibit ribosome accessibility. Alternatively, certain RNA-binding proteins (red) could block ribosome scanning along the 5′ UTR by steric hindrance. Translation may occur at short upstream uORF sequences in the 5′ UTR, thus preventing readthrough to the main ORF. Finally, a zip code sequence could specify alternative localization of *HSG*s to subcellular compartments that are not actively translated. Filament-inducing conditions may potentially impact translation of *HSG*s through one or several of the indicated mechanisms. Adapted in part from Refs. [55••,73].

biosynthesis) led to significant filamentation defects [26••]. In addition, treatment with antifungal drugs that inhibit various steps of the ergosterol biosynthesis pathway also inhibited filamentation; importantly, cells were grown at drug concentrations that did not inhibit growth. These findings are significant because they suggest that azole drugs are effective against *C. albicans* not only because they inhibit growth at higher concentrations, but also because they inhibit morphogenesis, possibly as a consequence of the accumulation of sterol intermediates [26••]. While the precise mechanism(s) by which ergosterol biosynthesis pathway components function to promote *C. albicans* filamentation remain to be elucidated, investigation of such mechanism(s) is likely to represent an interesting and fruitful avenue for future research.

A second pathway shown by the functional genomics screen to play an important role in *C. albicans* morphogenesis is involved in *N*-linked glycosylation. Glycosyltransferases on the cytoplasmic (but not lumen) side of the endoplasmic reticulum (ER), which are important for linking mannose or *N*-acetylglucosamine to dolichol pyrophosphate, as well as components of the oligosaccharyltransferase complex (important for transferring glycan to polypeptide chains) were specifically found to be required for *C. albicans* filamentation [26••]. Consistent with these observations, treatment of *C. albicans* with subinhibitory concentrations of tunicamycin, which functions to block *N*-linked glycosylation, also inhibited filamentation. These findings suggest that remodeling of polysaccharides on the *C. albicans* cell surface may play a crucial role in morphogenesis.

Conclusions and perspectives

Because the *C. albicans* yeast-filament transition is strongly associated with virulence and pathogenicity, many studies have focused on mechanisms that control this transition. In general, post-translational mechanisms that are mediated by signaling pathways as well as transcriptional mechanisms that work through sequence-specific DNA-binding protein transcription factors have received the greatest attention. This review serves to highlight several recently discovered alternative mechanisms, including those associated with global transcriptional regulation, translation and biosynthesis pathways. What is the relative contribution of each type of mechanism toward controlling *C. albicans* morphology and pathogenesis? While a comprehensive answer to this question is unknown, both post-translational as well as transcriptional mechanisms mediated by DNA-binding proteins are likely to exert substantial control, given the large number of proteins involved in these mechanisms that have been identified by numerous groups [16,33,62]. Although not as well-studied, many DNA-bound transcriptional regulators are likely to direct transcriptional regulation by chromatin-mediated mechanisms. This has been shown directly for certain transcription factors, such as Brg1 and Rep1 [63,64]. In addition, aside from Hir1, deletion of several components of complexes associated with chromatin-mediated regulation (e.g. Set3, Hos2, Hat1, Eaf1, Yaf9) is known to affect *C. albicans* morphology and/or pathogenesis, suggesting that these mechanisms exert significant control [48•,65–69]. While translational mechanisms are likely to be less prevalent than transcriptional and chromatin-mediated mechanisms, they are expected to have a significant impact on both the

regulation and fine-tuning of *C. albicans* filamentation and pathogenesis given that many regulators of these processes, similar to *UME6*, *EFG1* and *WOR1*, possess long 5′ UTR regions [57]. In addition, the *C. albicans* genome encodes for over 300 known or putative RNA-binding proteins (www.candidagenome.org), several of which are likely to play important roles in translational control. Since only a few biosynthesis pathways that control filamentation were identified in the functional genomics screen described above (which covered approximately one-third of the *C. albicans* genome), this type of mechanism is expected to be less common. Overall, however, many of the alternative regulatory mechanisms described in this review are likely to be just as important, if not more so, than previously characterized well-studied mechanisms in controlling *C. albicans* morphology and pathogenicity.

Given that many large-scale screening approaches for compounds with antifungal activity are becoming exhausted [70,71], the discovery of novel mechanisms important for *C. albicans* morphogenesis also opens new and fruitful avenues for antifungal drug development. Rational drug design against fungal-specific targets remains a promising approach. For example, targeting of fungal-specific translation components that play key roles in *C. albicans* filamentation, virulence and pathogenicity is an unexplored and unexploited avenue for the development of new antifungal strategies. Even evolutionarily conserved proteins important for *C. albicans* morphogenesis and pathogenicity could represent promising targets if they possess fungal-specific domains that are critical for function [72]. Ultimately, the range and variety of potential antifungal targets is likely to be significantly expanded by future advances in our understanding of molecular mechanisms that control morphogenesis in *C. albicans* as well as other fungal pathogens.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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