EUKARYOTIC CELL, Aug. 2011, p. 1004–1012 Vol. 10, No. 8
1535-9778/11/$12.00 doi:10.1128/EC.05030-11
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MINIREVIEWS

Modulation of Morphogenesis in *Candida albicans* by Various Small Molecules▽

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The pathogenic yeast *Candida albicans*, a member of the mucosal microbiota, is responsible for a large spectrum of infections, ranging from benign thrush and vulvovaginitis in both healthy and immunocompromised individuals to severe, life-threatening infections in immunocompromised patients. A striking feature of *C. albicans* is its ability to grow as budding yeast and as filamentous forms, including hyphae and pseudohyphae. The yeast-to-hypha transition contributes to the overall virulence of *C. albicans* and may even constitute a target for the development of antifungal drugs. Indeed, impairing morphogenesis in *C. albicans* has been shown to be a means to treat candidiasis. Additionally, a large number of small molecules such as farnesol, fatty acids, rapamycin, geldanamycin, histone deacetylase inhibitors, and cell cycle inhibitors have been reported to modulate the yeast-to-hypha transition in *C. albicans*. In this minireview, we take a look at molecules that modulate morphogenesis in this pathogenic yeast. When possible, we address experimental findings regarding their mechanisms of action and their therapeutic potential. We discuss whether or not modulating morphogenesis constitutes a strategy to treat *Candida* infections.

Candida albicans, a member of the endogenous human microflora, is the most common human fungal pathogen. In healthy individuals, outgrowth of *C. albicans* results in superficial mycoses of the skin, nails, and mucous membranes (thrush and vulvovaginitis). However, in individuals with immune deficiencies caused by underlying disease, chemotherapy treatment, or immunosuppression following a transplantation, *C. albicans* can cause severe, life-threatening invasive candidiasis. *C. albicans* now ranks as the fourth leading cause of nosocomial infections and is the most common fungal species causing bloodstream infections, with associated mortality rates of 38 to 49% (62, 90, 111, 146). Antifungal drugs currently used for the treatment of *Candida* infections include polyenes, azoles, echinocandins, allyamines, and flucytosine. These drugs exert either fungicidal or fungistatic activities by interfering with essential processes (104). Intensive prophylactic and therapeutic uses of antifungal agents have selected for drug-resistant strains (6, 30, 118). Moreover, the limited arsenal of antifungal drugs is further compromised by severe side effects in patients and the emergence of species refractory to conventionally used agents (90). There is a need to develop new antifungals and to explore novel therapeutic approaches to treat *Candida* infections.

C. albicans has the ability to grow in a variety of morphological forms, including as budding yeast, pseudohyphae, and true hyphae (133). The transition from yeast growth to hyphal growth is induced by a variety of environmental cues reflecting host conditions (temperature of 37°C, neutral or alkaline pH, or presence of serum) that activate a complex network of signaling pathways (15, 19, 41, 145). Although recent findings have demonstrated that the yeast-to-hypha (Y-H) transition is not always required for virulence in systemic candidiasis (99), morphogenesis still belongs to the realm of *C. albicans* virulence factors as demonstrated by several lines of evidence, the first being that strains defective in morphogenesis are attenuated in virulence in systemic candidiasis (83, 121, 152). In addition, hyphal development is necessary for *C. albicans* to evade phagocytes (84), to escape from blood vessels (112), and to colonize medical devices by forming biofilms (97, 98). Moreover, both yeast and hyphal cells are found in *C. albicans*-infected organs (103). Thus, morphogenesis contributes to the overall virulence of *C. albicans*.

To widen the repertoire of antifungal drugs, targets that differ from those of conventional drugs have to be identified. Recently, targeting virulence rather than essential processes has been postulated as a new paradigm for the development of antifungal agents, following the successful development of drugs targeting bacterial virulence in antimicrobial therapy (5, 20, 43). Thus, instead of being killed, a pathogen is maintained in a harmless form by blocking virulence attributes that contribute to its pathogenicity. Moreover, resistance to drugs that target virulence instead of growth is less likely to develop, given that selective pressure is reduced on nonessential targets that are required only to colonize host environments (66). In *C. albicans*, virulence factors that are eligible as targets for the development of new antifungal drugs have been reviewed recently and include secreted aspartic proteases, phospholipases, calcineurin, inositol phosphoryl ceramide synthase, and elastase (20, 43).

Given that morphogenesis is a virulence factor of *C. albicans*, it may also constitute a target for the development of antifungal drugs. Indeed, impairing morphogenesis has been

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▽ Published ahead of print on 3 June 2011.

shown to be a means to treat candidiasis. Using the *tet-NRG1* strain, which can be induced to filament when doxycycline is added to the drinking water of animals, studies have demonstrated that inhibiting filamentation attenuated virulence in a model of systemic candidiasis and served as an effective therapeutic intervention (120, 121). Concurrently, numerous molecules have been reported to modulate the Y-H transition in *C. albicans*. These molecules may harbor interesting therapeutic properties, given that they target a virulence factor. In addition, they are in line with the current trend in antifungal drug development and may constitute novel antifungal agents, which are required to remedy antifungal drug resistance problems and to enhance the arsenal of antifungal agents. In this minireview, we research the literature in an attempt to list the molecules reported to influence morphogenesis in *C. albicans*, while focusing on studies that have addressed the modes of action of specific molecules. When possible, we review experimental findings regarding their effects on *C. albicans* infections. We discuss whether or not modulating morphogenesis constitutes a strategy to treat *Candida* infections.

FARNESOL

Farnesol, a 15-carbon oxygenated lipid made up of isoprene moieties, was the first quorum-sensing (QS) molecule to be identified in eukaryotes (63). Secreted by a number of *Candida* laboratory strains and clinical isolates, farnesol inhibits the Y-H transition in *C. albicans* (63, 64). The QS molecule is active at blocking hyphal growth induced by a variety of morphogenetic cues such as serum and *N*-acetylglucosamine (91). Moreover, farnesol affects various developmental processes in other *Candida* spp. and in pathogenic fungi, including *Aspergillus* spp., *Fusarium graminearum*, and *Paracoccidiodes brasiliensis* (reviewed in reference 76).

Findings regarding farnesol's repressive effects on filamentation and its mode of action have been reviewed extensively (29, 57, 74, 76, 96). To gain insight into the response of *C. albicans* to farnesol, global gene expression analyses were performed (21, 25, 38, 119). Although experimental approaches varied from one study to another, farnesol commonly affected the expression of genes that belonged to functional categories such as stress response, heat shock, drug resistance, amino acid and carbon metabolism, iron transport, cell wall, and cell cycle. One study suggested that farnesol affected the mitogen-activated protein (MAP) kinase pathway, as transcript levels of the *HST7* kinase and the *CPH1* transcription factor were reduced in the presence of the molecule (119). However, farnesol inhibited the Y-H transition in a *cph1*/*cph1* mutant, suggesting that *CPH1* is not a primary but rather a secondary target of farnesol (34). While gene expression analyses generated a wealth of data pertaining to farnesol's transcriptional effects on *C. albicans*, specific cellular targets were not identified.

Other morphogenetic regulators may play a role in the *C. albicans* response to farnesol. *CHK1*, a two-component signal transduction pathway histidine kinase, appeared to be required for the farnesol-mediated inhibition of hyphal growth (75). Chk1p may act either as a sensor or downstream of the sensor for farnesol (74). The histidine kinase may function via the Hog1p MAP kinase, which was shown to be phosphorylated in the presence of farnesol (130). Like *CHK1*, the negative regulators of filamentation *TUP1* and *NRG1* were shown to be involved in the response of cells to farnesol (69). Indeed, *tup1*/*tup1* and *nrg1*/*nrg1* mutants remained filamentous in the presence of farnesol. Farnesol treatment also resulted in an increase in *TUP1* mRNA and protein levels and corrected the haploinsufficient phenotype of a *TUP1*/*tup1* mutant strain (69). Concurrently, the Ras1p-cyclic AMP (cAMP)-protein kinase A (PKA) signaling pathway was identified as an important target of farnesol (34). Several lines of evidence suggest that farnesol inhibits the Y-H transition by downregulating Ras1p signaling. Farnesol repressed hypha formation in a strain that expressed the hyperactive Ras1p^{G13V} variant. Moreover, the addition of dibutyryl cAMP, a cAMP analogue, restored filamentation to farnesol-treated cells. Farnesol treatment also increased mRNA levels of cAMP-repressed genes, suggesting that cAMP levels were reduced in the presence of farnesol.

There are several ways by which farnesol could modulate the Ras1p signaling pathway as well as other factors involved in the Y-H transition. Membrane-bound, Ras1p interacts with the adenylate cyclase Cyr1p in the vicinity of the plasma membrane, thus promoting cAMP synthesis (42, 144). Because farnesol is a highly hydrophobic molecule that localizes to the plasma membrane, it may disrupt the membrane, causing the release of farnesylated Ras1p or affecting the Ras1p-Cyr1p complex, thus impairing cAMP production and the Y-H transition (34, 76, 125). Farnesol may also impede posttranslational modifications of Ras1p, which are required for the protein's localization to the plasma membrane and its biological activity as well as for hyphal development in *C. albicans* (49, 76, 88). Another possibility is that farnesol binds directly to Ras1p or to Cyr1p, thus interfering with cAMP synthesis. The effect of farnesol on *HST7* and *CPH1* expression levels (119) is likely to be mediated by Ras1p, given that the GTPase is a regulator of the MAP kinase signaling pathway (78). Additionally, Tup1p/Nrg1p and Chk1p may also be downstream targets of Ras1p, as regulators of these factors have yet to be identified (76). With the current state of knowledge regarding the molecular networks regulating the Y-H transition, it is impossible to determine whether farnesol has one specific target or modulates simultaneously different targets that vary according to hypha-inducing conditions. Furthermore, a receptor or a binding protein for farnesol has not been identified.

Given its hypha-inhibiting properties, farnesol was purported to harbor therapeutic potential (96), and its effects on various types of *Candida* infections were examined. *C. albicans* cells cocultured with human gingival fibroblasts and epithelial cells were exposed to farnesol. At concentrations tolerated by gingival cells, farnesol blocked the Y-H transition of *C. albicans* (117). In a follow-up study that used a human oral mucosa model, farnesol was shown to promote epithelial cell defense against *C. albicans* by increasing Toll-like receptor 2 (TLR2) expression levels, promoting interleukin-6 (IL-6) secretion, and increasing antimicrobial peptide human beta-defensin 2 (hBD2) levels (35). Likewise, using a mouse model of mucosal candidiasis, farnesol administered orally to infected animals suppressed hyphal development and had an overall protective effect against oral candidiasis (54). Farnesol had contrasting effects in a mouse model of systemic candidiasis. Whether administered intraperitoneally, intravenously, or orally to infected mice, farnesol treatment accelerated the demise of in-

fected animals (94). Moreover, farnesol interfered with the normal progression of cytokine induction by decreasing IL-12 levels, which increased the susceptibility of mice to systemic candidiasis (95). Administered on its own, farnesol was harmless, indicating that it specifically enhanced virulence in systemic candidiasis. Taken together, these findings suggest that farnesol has therapeutic potential in the context of oral mucosal infections but cannot be used to treat systemic candidiasis.

BACTERIAL AND FUNGAL AUTOREGULATORY MOLECULES

In addition to farnesol, *C. albicans* secretes other autoregulatory molecules that influence the Y-H transition (57, 74). The fusel alcohols 2-phenylethanol and tryptophol were the first molecules reported to inhibit hyphal growth (82). Yet, these molecules are not QS molecules, given that they are inhibitory at concentrations the yeast cannot produce naturally (51). An unknown substance termed MARS (morphogenic autoregulatory substance), isoamyl alcohol, and *E*-nerolidol have also been shown to possess hypha-inhibiting activities (51, 52, 87). Farnesoic acid, which is structurally related to farnesol, also blocked the Y-H transition (107). However, it possessed only 3% of farnesol's inhibitory activity when germ tube formation was induced in *N*-acetylglucosamine-containing medium (126). *PHO81*, which encodes a phosphatase, was recently shown to be required for farnesoic acid to inhibit the Y-H transition (26, 27). Additionally, *PHO81* was proposed to be a negative regulator of Ras1p activity, as it appears to act upstream of Ras1p signaling (26). In contrast to these hypha-inhibiting molecules, tyrosol was described as a QS molecule that stimulated germ tube formation (23) and promoted hyphal development in the early stages of biofilm formation (4). Tyrosol also reduced the lag phase of growth in a diluted culture, which may account for its hypha-stimulating properties (23).

As a commensal organism of the mucosal microbiota and a pathogen in different host niches, *C. albicans* encounters microorganisms of the endogenous microflora as well as several opportunistic pathogens. A number of bacteria and yeasts have been reported to secrete molecules that influence the Y-H transition. *Pseudomonas aeruginosa* secretes the 3-oxo-C_{12}-acyl homoserine lactone (3OC12HSL), a 12-carbon backbone molecule structurally related to farnesol, which inhibited the Y-H transition induced in *N*-acetylglucosamine-containing medium and caused filaments to revert to the yeast morphology (61). Similar in structure to farnesol and farnesoic acid, dodecanol and three other QS molecules produced by *Xanthomonas campestris*, *Burkholderia cenocepacia*, and *Streptococcus mutans* also exerted hypha-inhibiting activities (16, 140, 142). Like farnesol, these molecules may block the Y-H transition by inhibiting the Ras1p-cAMP-PKA signaling pathway, potentially through similar mechanisms (57, 60). Additionally, *P. aeruginosa* also had an inhibitory effect on *C. albicans* growth *in vitro*, in burn wounds, and in the lungs of patients with cystic fibrosis (46, 70). Pyocyanin, phospholipase C, and phenazines were the molecules responsible for this inhibitory effect (58, 71). Interestingly, the antifungal activity of *P. aeruginosa* was specifically targeted toward hyphal cells, as bacteria were shown to attach to and kill fungal filaments only (58).

Other microorganisms, including oral and gut residents, have also been reported to exert morphogenesis-modulating activities. *Streptococcus mutans* secreted a 22-amino-acid-containing peptide that inhibited the Y-H transition induced using saliva-coated culture plates containing YNB medium (65). In contrast, cell-free *Streptococcus gordonii* supernatants enhanced hyphal development and biofilm formation induced in the presence of human saliva at 37°C and affected the activation of several MAP kinases, including that of Cek1p, Mkc1p, and Hog1p (11). Although the active compound was not identified, H_2O_2 and autoinducer 2 were proposed to promote filamentation by impacting the oxidative stress response. Culture supernatants from *Lactobacillus rhamnosus* GG and the probiotic yeast *Saccharomyces boulardii* blocked germ tube formation induced in serum-containing medium and in RPMI 1640 medium at 37°C (73, 92, 101). Butyric acid was shown to mimic the effect of *L. rhamnosus* GG spent medium, at concentrations well within the physiological range observed in the colon (101, 116). The hypha-inhibiting activities of butyric acid had previously been reported (18, 56). In the case of *S. boulardii*, capric acid was identified as the active molecule: it inhibited the Y-H transition as well as adhesion and biofilm formation (92). Two other compounds produced by *Acinetobacter baumannii* and *Salmonella enterica* serovar Typhimurium also inhibited filamentation and biofilm formation but have yet to be identified (110, 134).

In complex microbial communities, molecules mediate interspecies interactions (57, 59, 143). With respect to morphogenesis-modulating molecules, these secreted products may be a mechanism by which microbes compete with *C. albicans* by inhibiting hypha formation, limiting attachment, and preventing invasion. It may also be a way for *C. albicans* to respond to the presence of microorganisms. For instance, in the presence of 3OC12HSL, *C. albicans* may block hyphal development as a means to escape being killed by *P. aeruginosa*. Conversely, hypha-inducing molecules may mediate synergistic interactions between bacteria and *C. albicans* by promoting biofilm formation and invasion (11).

FATTY ACIDS, EICOSANOIDS, AND CYCLOOXYGENASE INHIBITORS

Lipids are involved in fungal development and pathogenicity (39, 100, 114, 127). In *C. albicans*, lipid molecules such as fatty acids and eicosanoids have been reported to modulate the Y-H transition. Fatty acids, including butyric, capric, lauric, palmitoleic, oleic, linoleic, conjugated linoleic, and arachidonic acids, inhibited the Y-H transition induced under various conditions (28, 89, 92, 101). The hypha-inhibiting activities of fatty acids were dependent on the medium. For instance, linoleic and oleic acids were shown to have no effect on germ tube formation induced in serum or to block the Y-H transition induced in other conditions, such as Spider medium (28, 101). Such discrepancies may be due to the lipid-binding capacity of serum albumins, which sequester lipidic molecules and reduce their effective concentrations (76). Moreover, although the effects of fatty acids on the Y-H transition have been described, their modes of action have not been studied extensively. Conjugated linoleic acid (CLA) was reported to block the Y-H transition induced in Spider medium by affecting the subcellular localization of Ras1p and reducing its levels,

thereby impeding the activation of Ras1p signaling and blocking the induction of the *TEC1* transcription factor (124). In addition, while the antifungal properties and cytotoxicity of several fatty acids have been reported (13, 22, 37), fatty acids inhibited hyphal growth at concentrations that did not affect cellular growth (124).

In contrast to fatty acids, eicosanoids such as prostaglandin E2 (40, 68, 102) and thromboxane B2 (101) enhanced the Y-H transition. Eicosanoids are oxygenated lipids derived from 20-carbon polyunsaturated fatty acid precursors such as arachidonic acid. In mammalian cells, cyclooxygenases (COX) catalyze the conversion of arachidonic acid into prostaglandins. While *C. albicans* does not possess a COX homolog, the fatty acid desaturase *OLE2* and the multicopper oxidase *FET3* were found to play a role in prostaglandin synthesis (40). Concurrently, several COX inhibitors, including diclofenac sodium, indomethacin, ibuprofen, resveratrol, and eicosatetraynoic acid (ETYA), were shown to block the Y-H transition induced in serum-containing, Lee's or Spider medium at 37°C (3, 44, 108, 136). It is not clear whether or not COX inhibitors affected filamentation by blocking prostaglandin synthesis, given that a direct association between reduced prostaglandin levels and reduced hypha formation was not shown and that *C. albicans* does not encode a COX homolog. Moreover, COX inhibitors appeared to reduce viability of *C. albicans* cells, which may account for their hypha-inhibiting activities (3, 36, 102). For instance, diclofenac sodium and resveratrol blocked hyphal development but also affected cellular growth (44, 108).

PEPTIDES AND PROTEINS

Various peptides and proteins were shown to modulate the Y-H transition in *C. albicans*. At concentrations of 25 $\mu g\ ml^{-1}$, nisin Z, an antimicrobial peptide of the lantibiotic family, reduced germ tube formation induced in serum-containing medium and decreased adhesion of *C. albicans* cells to gingival monolayer cultures (2). However, a previous study had shown that at slightly higher concentrations (100 $\mu g\ ml^{-1}$), nisin Z reduced cellular growth rates and caused ultrastructural disturbances in *C. albicans* cells, casting doubt on the mechanism by which the antimicrobial peptide inhibited filamentation (81). Salivary components statherin and mucin also affected morphogenesis. Hyphae grown overnight in RPMI 1640 medium switched to yeast growth in the presence of statherin (80), while mucin inhibited hypha formation induced in RPMI 1640 medium at 37°C and blocked *RAS1* induction without affecting cellular growth (106). *C*dc42/*R*ac *i*nteractive *b*inding (CRIB) fusion polypeptides containing the CRIB consensus sequence were designed to disrupt the binding of the Rho GTPase Cdc42p to its effectors Cst20p and Cla4p, interactions that are required for hyphal development (77, 79). CRIB polypeptides, which have high-affinity binding to Cdc42p, blocked the Y-H transition induced in Lee's medium in time- and dose-dependent fashions, without impairing cellular growth (132). Additionally, hyphal cells grown overnight in Lee's medium switched to yeast-like forms when they were incubated in fresh medium in the presence of either of the CRIB polypeptides, indicating the peptides can convert hyphal cells back into the yeast-like form.

RAPAMYCIN

Rapamycin, a hydrophobic macrolide produced as a secondary metabolite by the soil bacterium *Streptomyces hygroscopicus*, was initially discovered as an antifungal agent against *C. albicans* (139). Rapamycin inhibits the function of the Tor (target of rapamycin) kinases (53), of which one homolog was identified in *C. albicans* (Tor1p) (32). Tor1p has contrasting roles in regulating morphogenesis which vary according to hypha-inducing conditions. For instance, on solid synthetic low ammonium dextrose (SLAD) medium, alkaline M199 medium (pH 8.0), and Spider medium at 37°C, sublethal concentrations of rapamycin blocked hyphal growth, indicating that Tor1p is a positive regulator of filamentation under nitrogen or nutrient starvation conditions and in response to alkaline growth conditions (12, 33, 86). In contrast, in most liquid hypha-inducing media, rapamycin had no effect on the Y-H transition, indicating that Tor1p does not regulate hyphal development under these conditions (12).

Interestingly, in liquid Spider medium, rapamycin promoted cellular aggregation and flocculation (12). These findings demonstrate that Tor1p negatively regulates cellular adhesion under such growth conditions. Gene expression analysis showed that rapamycin treatment strongly induced the hyphal growth program in *C. albicans*, resulting in the induction of hypha-specific genes (*ALS1*, *ALS3*, *HWP1*, and *ECE1*) and hyphal growth regulators (*TEC1* and *RFG1*). Additionally, rapamycin decreased transcript levels of filamentation repressors *TUP1* and *NRG1*. The transcription factors Efg1p and Bcr1p were required for the rapamycin-mediated cellular aggregation as well as for expression of adhesins. Thus, in liquid Spider medium, rapamycin promotes cellular aggregation by compromising Tor1p function, which results in the activation of Efg1p and Bcr1p and the downregulation of *TUP1* and *NRG1*.

GELDANAMYCIN

Geldanamycin (GdA) is a microbial metabolite that inhibits the function of Hsp90p, a molecular chaperone and heat shock protein (115). Hsp90p regulates the form and function of various client proteins, several of which regulate morphogenesis in *C. albicans* (109, 113, 123). GdA treatment induced filamentation in *C. albicans* cells grown in noninducing conditions (123). The genetic depletion of *HSP90* was also shown to phenocopy the GdA-induced filamentation. Combined, these results suggest that Hsp90p is a negative regulator of morphogenesis. Upstream inputs from the cAMP-PKA signaling pathway were required for cells to filament in the presence of GdA, while the transcription factor Efg1p was not required. These results suggest that the GdA-induced filamentation may be regulated by transcription factors that function downstream of PKA, such as Tec1p, Flo8p, and Sfl1p (122, 123). Hsp90p has been proposed to repress cAMP-PKA signaling, either by (i) interacting with a positive regulator of the pathway and maintaining it in an inactive form (e.g., Tpk1p or Tpk2p, the catalytic subunits of PKA), (ii) stabilizing a negative regulator of the pathway (e.g., Bcy1p, the regulatory subunit of PKA), or (iii) interacting indirectly with the cAMP-PKA pathway via an unknown protein (122). Other models involving different Hsp90p client proteins are also possible. Interestingly, the ge-

netic depletion of *HSP90* attenuated virulence in a mouse model of systemic candidiasis, resulting in clearance of the infection (123). While these findings suggest that modulating morphogenesis by compromising Hsp90p function is a means to treat candidiasis, reduced virulence may have been due to reduced cellular growth rates associated with reduced *HSP90* cellular levels. Nonetheless, a study has shown that harnessing Hsp90p function, either with the use of clinically relevant GdA derivatives or by genetically compromising *HSP90*, improved the therapeutic efficacy of an azole in two animal models of infection (31). Because they enhance the efficacy of existing antifungal drugs, Hsp90p inhibitors such as GdA and structurally related compounds are good candidates for the development of effective combination therapy strategies.

HISTONE DEACETYLASE INHIBITORS

Histone deacetylases (HDACs) are chromatin-remodeling proteins usually involved in transcriptional repression (45). HDACs deacetylate histones globally or at specific promoters, directed by transcription factors or other DNA-binding proteins (67). The HDACs Hda1p, Hos2p, Set3p, and Hst3p have been reported to regulate morphogenesis, yet they have contrasting roles (55, 148, 151). Moreover, several HDAC inhibitors have been shown to modulate hyphal development and virulence (55, 129, 131, 148). For instance, suberoylanilide hydroxamic acid (SAHA) inhibited the serum-induced Y-H transition, which correlated with a minor reduction in *EFG1* transcript levels (129). In contrast, nicotinamide, an inhibitor of Hst3p, induced filamentation of *C. albicans* cells in noninducing conditions (148). Hst3p, a member of a family of NAD^+-dependent histone deacetylases known as sirtuins, is inhibited by nicotinamide, a product of the NAD^+-dependent deacetylation reaction. The pharmacological inhibition of Hst3p using nicotinamide or the genetic depletion of *HST3* not only induced filamentation but also attenuated virulence in a model of systemic candidiasis. Yet, reduced virulence *in vivo* was likely due to reduced growth rates rather than to the modulation of morphology, as nicotinamide treatment or the repression of *HST3* resulted in cell death.

Trichostatin A (TSA), a well-characterized HDAC inhibitor (150), had contrasting effects on morphogenesis that depended on experimental settings, including strains, growth conditions, and TSA concentrations used. Clinical isolates of *C. albicans* pretreated for 48 h with 4 $\mu g\ ml^{-1}$ of TSA were unable to form germ tubes when induced to filament in yeast extract-peptone-dextrose (YPD) medium supplemented with 20% serum (129). In addition, *EFG1* transcript levels were reduced in TSA-treated cells. These results suggest that TSA inhibited a histone deacetylase required for the Y-H transition and downregulated *EFG1* expression. The Hda1p HDAC was proposed to potentially mediate the effects of TSA on hyphal development. In contrast, similar TSA concentrations had no effect on the serum-induced Y-H transition (131). In these experiments, TSA treatment may have been insufficient to affect morphogenesis, given that the HDAC inhibitor was added at the onset of hyphal induction. Another study showed that TSA treatment promoted hyphal development under noninducing conditions (55). Indeed, on solid YPD medium supplemented with 10 $\mu g\ ml^{-1}$ of TSA, colonies displayed filamentous growth after 3 days of incubation at 37°C. The TSA-induced filamentation was phenocopied by the deletion of the *SET3* and *HOS2* histone deacetylase genes. Additionally, both histone deacetylases were shown to be involved in repressing morphogenesis at the level of the Efg1p transcription factor. Thus, the TSA-induced filamentation appears to be caused by TSA inhibiting the Set3p and Hos2p HDACs and relieving the repression of filamentation (55). Interestingly, despite the fact the *set3*/*set3* mutant strain filamented *in vivo*, it was still attenuated in virulence in a model of systemic candidiasis. Moreover, reduced growth rates did not account for reduced virulence, as the generation times of wild-type and *set3*/*set3* strains did not differ significantly (55). Given that TSA inhibits Set3p, the HDAC inhibitor may reduce virulence *in vivo*, but this has yet to be demonstrated.

CELL CYCLE INHIBITORS

Hydroxyurea (HU) and nocodazole (NZ) are cell cycle-perturbing agents. While HU inhibits ribonucleotide reductase, depleting ribonucleotides and inhibiting DNA synthesis, NZ disrupts microtubules and locks cells in mitosis. In addition to causing cell cycle arrest, both drugs also trigger hyperpolarized growth of *C. albicans* cells grown in noninducing conditions (7–9, 145). Upon exposure to HU or to NZ, yeast cells developed hyperpolarized buds, which continued to elongate despite DNA replication being blocked (7–9). HU- and NZ-induced elongated buds displayed pseudohyphal morphological features (constrictions at the neck and width of $>2\ \mu m$) as well as hyphal-like features, in that they maintained polarized growth, demonstrated nuclear movement out of the mother cell and into the elongating filament, and expressed several hypha-specific genes (HSGs) (7, 8). HU-induced elongated buds eventually died after 24 h. However, sublethal concentrations of the inhibitor have also been reported to induce hyperpolarized growth without impacting cellular growth (128). The development of hyperpolarized buds involves different proteins, several of which are cell cycle checkpoints. For instance, the Rad53p effector kinase of the DNA replication checkpoint and the Mad2p spindle assembly checkpoint were required for hyperpolarization of buds induced by HU and NZ treatments, respectively (9, 128). Interestingly, bud elongation appears to be caused by the activation of cell cycle checkpoints by cell cycle inhibitors rather than by cell cycle arrest (128). Moreover, several components of Ras1p signaling, including the GTPase Ras1p and adenylate cyclase Cyr1p, were required for HU-induced elongated buds (7, 8). Given the fact that hyperpolarized growth has been observed only in response to cell cycle-inhibiting agents or in strains in which cell cycle genes are inactivated or overexpressed (reviewed in references 14 and 145), it is not clear whether or not this growth mode is physiologically relevant. Yet, it remains possible that hyperpolarized buds may be important for pathogenicity and/or survival in the host, as they eventually express hypha- and virulence-specific factors (145).

OTHER SMALL MOLECULES

Many other small molecules have been reported to affect morphogenesis in *C. albicans* (135, 136). Propranolol, a calmodulin inhibitor (141), was shown to inhibit serum-induced hypha formation by reducing *EFG1* expression levels (10, 138).

Various inhibitors of actin dynamics, including latrunculin, jasplakinolide, and cytochalasin A, also blocked the Y-H transition induced in serum-containing, Spider, or M199 medium at 37°C (1, 50, 135, 147). 1,4-Diamino-2-butanone (DAB) blocked the Y-H transition induced in RPMI 1640 medium at 37°C by reducing polyamine levels, decreasing *CYR1* mRNA levels, and reducing cAMP cellular levels (137). Sublethal concentrations of azoles inhibited hypha formation induced in M199 medium at 37°C (47, 105). The hypha-inhibiting activities of azoles may be due to increased farnesol production reported in azole-treated cells (64). Two derivatives of propanol from a chemical library reduced *C. albicans*-induced endothelial injury, most likely by preventing hypha formation (135). The cellular targets of these structurally different molecules are not known. In contrast, hydrogen peroxide and the iron chelator bathophenanthrolene disulfonic acid (BPS) both induced hyphal development in noninducing conditions (48, 93). BPS promoted filamentation by increasing *EFG1* mRNA levels.

Additionally, several cytostatic or cytotoxic molecules have been reported to inhibit the Y-H transition in *C. albicans*, including garlic extracts (85), a Mannich ketone compound (72), the monoterpene thymol (17), riccardin D, a macrocyclic bisbibenzyl compound isolated from Chinese liverwort *Dumortia hirsute* (24), and ECC145 and ECC188, two compounds that impair the fatty acid Δ9 desaturase Ole1p and block the biosynthesis of unsaturated fatty acids (149). Lithium affected filamentation on solid galactose- and serum-containing media but also reduced cellular growth in the presence of galactose (86). However, it is unclear whether or not lithium impacted growth rates in the presence of serum. It remains possible that molecules that are toxic to cells cannot be considered to have hypha-inhibiting properties, given that they might impede the Y-H transition merely by impairing cellular growth.

CONCLUSION

Many small molecules have been reported to modulate morphogenesis in *C. albicans*. Yet, an overview of the literature revealed that the mechanisms of few of these compounds have been investigated. Interestingly, studies that have addressed the modes of action of small molecules have shown that many of these compounds target components of the Ras1p signaling pathway (Fig. 1). It is surprising that several structurally different molecules commonly affect *RAS1*, *CYR1*, *EFG1*, *HST7*, and *CPH1* mRNA levels, modulate cAMP levels or Efg1p-dependent transcription, or require components of the pathway to exert their morphogenesis-modulating activities. On one hand, despite being structurally different, it may be expected that these molecules modulate hyphal growth by targeting components of the Ras1p signaling pathway, given the major role this signaling pathway plays in morphogenesis. On the other hand, it is also possible that these common components are not primary targets, but rather secondary targets of various molecules. For instance, while farnesol was reported to downregulate *CPH1* transcript levels (119), the transcription factor was not required for the QS molecule to block the Y-H transition (34). These results suggest that farnesol's effect on *CPH1* expression levels was a consequence of farnesol affecting its primary target, i.e., the Ras1p-cAMP-PKA signaling pathway. Likewise, components of the Ras1p signaling pathway

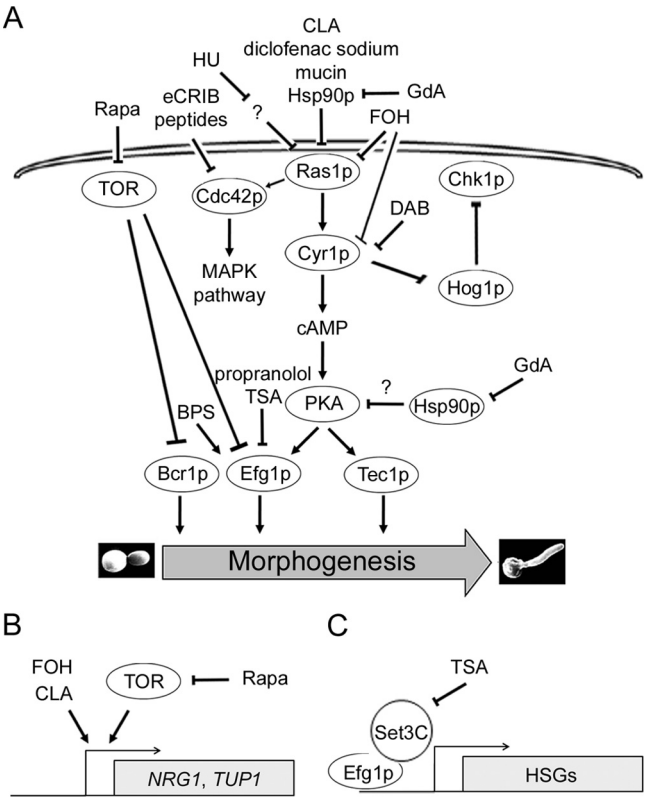

FIG. 1. Summary of the modes of action of selected small molecules that modulate morphogenesis in *Candida albicans*. (A) Several small molecules, including farnesol (FOH), conjugated linoleic acid (CLA), rapamycin (Rapa), geldanamycin (GdA), mucin, diclofenac sodium, 1,4-diamino-2-butanone (DAB), hydroxyurea (HU), propranolol, and bathophenanthrolene disulfonic acid (BPS), modulate morphogenesis by targeting the Ras1p-cAMP-PKA signaling pathway and its downstream effectors. Notably, both CLA and GdA modulate morphogenesis independently of Efg1p. Extended CRIB (eCRIB) peptides interrupt the binding of the GTPase Cdc42p to its effectors Cst20p and Cla4p, and thus prevent the activation of the MAP kinase (MAPK) pathway. (B) Farnesol and CLA block the downregulation of *TUP1* and *NRG1* expression. In contrast, rapamycin induces the downregulation of both repressors by inhibiting TOR. (C) Trichostatin A (TSA) induces filamentation by relieving the Set3C-mediated repression of Efg1p-dependent transcription. TSA may also reduce *EFG1* transcript levels (see text for details). HSGs, hypha-specific genes.

that have been identified as primary targets of morphogenesis-modulating molecules may well be secondary targets.

Proof-of-concept experiments have shown that interfering with the morphogenetic plasticity of *C. albicans* and promoting its growth in a form that does not damage the host attenuates virulence *in vivo* (120, 121). Therefore, does modulating morphogenesis constitute a sound approach to treat candidiasis? First, it is noteworthy to mention that there is an overall lack of evidence demonstrating that impairing the developmental process can be used to treat candidiasis. While many small molecules have been reported to modulate the Y-H transition *in vitro*, very few compounds have been evaluated for their effects on virulence using *in vitro* or *in vivo* infection models. Second, the therapeutic potential of some molecules, including nicotinamide, ECC145, ECC188, and geldanamycin, may stem in part from their cytotoxicity toward *C. albicans* rather than

from their capacity to modulate the Y-H transition, as they target essential gene products. Indeed, the genetic depletion of *HST3*, *OLE1*, and *HSP90* modulated morphogenesis in *C. albicans* and attenuated virulence in a model of systemic candidiasis but also reduced cellular growth rates (123, 148, 149). Third, the studies that have evaluated the effects of small molecules on virulence in various infection models have yielded mixed results. Farnesol and nisin Z were both shown to have a protective effect in mucosal candidiasis (2, 54, 117), while in a model of systemic candidiasis, farnesol enhanced pathogenicity (94, 95).

On the basis of current findings, it appears that modulating morphogenesis is a means to treat mucosal/superficial *Candida* infections. As for systemic candidiasis, the lack of literature renders it difficult to reach similar conclusions. To circumvent this impediment, small molecules that modulate the Y-H transition in *C. albicans* without affecting cellular growth should be evaluated for their therapeutic potential in various infection models. More data are needed to determine whether or not targeting the Y-H transition constitutes a sound therapeutic strategy to treat *Candida* infections.

ACKNOWLEDGMENTS

We thank the reviewers for their careful analysis of the manuscript and for their comments as well as M. Clément for insightful discussions.

J.S. is supported by studentships from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), and the Faculty of Graduate Studies of the Université de Montréal. P.B. is the recipient of the Saputo Research Chair in Biomedical Dairy Products Optimization of the Université de Montréal.

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