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Signaling cascades as drug targets in model and pathogenic fungi

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Abstract

Microbes evolved to produce natural products that inhibit growth of competing soil microorganisms. In many cases, these compounds act on fungi, which are eukaryotes closely related to metazoans, including humans. The calcineurin inhibitors CsA and FK506, the Tor inhibitor rapamycin, and the Hsp90 inhibitor geldanamycin all act via targets conserved from yeast to humans. This allows use of genetically tractable fungi as models to elucidate how these drugs and their targets function in yeast and human cells. They also enable studies to harness their intrinsic antimicrobial activities to develop novel antifungal therapies. Extensive studies have revealed a globally conserved role for Tor in regulating growth and proliferation in response to nutrients, and targeting its essential functions results in robust antifungal action. Similarly, a conserved and essential role for calcineurin in fungal virulence has been discovered that could be targeted by inhibitors in therapeutic use in a variety of clinical settings. Finally, the discovery that inhibitors of calcineurin or Hsp90 result in dramatic synergism with either azoles or glucan synthase inhibitors (candins) provides another therapeutic vantage point. Taken together, these fungal targets and their inhibitors provide a robust platform from which to develop novel antimicrobial therapies.

Keywords

Tor; Calcineurin; Hsp90; Rapamycin; FK506; Cyclosporin A; Geldanamycin

Introduction

The limited drug armamentarium and increasing drug resistance to some current antifungal therapies based on 5-flucytosine, polyenes, azoles and candins, has created a need for novel molecular targets and drugs to combat fungal infections. The central roles of the Tor kinase and the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin and its modulator Hsp90 in regulating cell growth and responses to stress in fungi have raised interest in the use of their inhibitors: rapamycin (Tor), FK506 and cyclosporin A (CsA) (calcineurin), and geldanamycin (GD) (Hsp90), as antifungal drugs. The potent immunosuppressive effects of these inhibitors have fueled development of novel, less immunosuppressive analogs, several of which are now under preclinical and clinical study. Recent findings reveal an interesting interplay between

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Tor, calcineurin and Hsp90 in regulating polarized growth and stress responses, which suggests that combination therapies with inhibitor analogs could confer synergistic antifungal effects (Figure 1). Here we present an overview of the Tor, calcineurin and Hsp90 signaling pathways in the model yeast *S. cerevisiae* and pathogenic fungi and discuss recent developments in targeting these pathways for antifungal therapy.

Tor in *Saccharomyces cerevisiae*

The Tor kinases were first identified in *S. cerevisiae* as the targets of the antifungal and immunosuppressive drug rapamycin, a cyclic macrolide produced by the soil bacterium *Streptomyces hygroscopicus* [1]. When rapamycin diffuses into the cell it forms a complex with the FKBP12 prolyl isomerase, which subsequently binds to the Tor kinase and blocks its functions. Both FKBP12 and Tor are ubiquitously conserved in eukaryotic organisms from yeasts to humans.

Aside from the protein kinase domain, the Tor proteins have putative domains for protein-protein interactions, including multiple N-terminal HEAT repeats and the FAT domain [2–4]. Overexpression of the FAT domain is toxic in *S. cerevisiae* and mutations in this domain in *Schizosaccharomyces pombe tor1*$^{+}$ or mammalian Tor renders Tor constitutively active [2,5]. In addition, the Tor proteins feature the highly conserved FRB domain, which serves as the binding site for FKBP12-rapamycin [6].

The Tor proteins populate two multi-protein complexes in *S. cerevisiae*: TOR complex 1 (TORC1) formed by either Tor1 or Tor2 in association with Kog1, Lst8, and Tco89, and Tor complex 2 (TORC2) consisting of Tor2, Lst8, Avo1, Avo2, Avo3, Bit2 and Bit61 [7–9]. Recently, novel components of these complexes have been identified in the fission yeast *S. pombe* and several TORC components have putative homologs in the fungal pathogens *C. albicans* and *Cryptococcus neoformans* [10,11](Table 1). Importantly, except for *S. cerevisiae* and *S. pombe* that possess two Tor homologs, other eukaryotic organisms ranging from fungi to mammals have only one Tor protein. However, where examined, this single Tor protein is capable of forming two distinct protein complexes equivalent to TORC1 and TORC2.

TORC1 is rapamycin-sensitive and essential to promote growth by regulating transcription and translation whereas TORC2 is insensitive to rapamycin and regulates actin cytoskeleton polarization and responses to stress [7]. However, recent studies have shown that mutations in TORC1 components and rapamycin treatment also result in actin depolarization [12–14]. This functional overlap between these two complexes was previously unnoticed and underscores the complexity embedded within this signaling network.

Nutrient sensing and Tor

Treatment of yeast cells with rapamycin triggers events that mimic the effects of nutrient starvation, including inhibition of ribosome biogenesis and protein translation, and inducing autophagy and G_0 entry (reviewed in [15]). These and other observations support the model that the Tor pathway responds to nutrient cues to regulate cell growth.

Several lines of evidence link amino acid sensing and Tor signaling to membranes of the vesicular trafficking system in *S. cerevisiae* (reviewed in [16]). First, Tor1 has been proposed to sense glutamine [17]. Second, Tor proteins and TORC components localize to internal membranes, including the vacuole, which is the major cellular amino acid reservoir [8,13, 18]. Third, TORC1 and the vacuolar EGO/Gse complex, which in response to amino acids regulates sorting of the general amino acid permease Gap1, orchestrate microautophagy [19, 20]. Fourth, a role for the class C Vps complex, which functions in vesicle trafficking between

endosomes and the vacuole, has been proposed in mediating intracellular amino acid homeostasis for efficient Tor signaling [21].

The link between Tor and amino acid sensing is not limited to *S. cerevisiae* and has also been documented in *S. pombe* where *tor1+* regulates amino acid uptake in a rapamycin-sensitive fashion controlled by Tsc/Rheb signaling [22,23]. Interestingly, homologs of the TORC1 upstream regulators Tsc1/Tsc2 and Rheb are also conserved in *S. pombe*, *C. albicans* and *C. neoformans* (Table 1). These findings suggest that amino acid sensing is a conserved regulatory mechanism among several pathogenic fungi and further understanding will lead to new discoveries in Tor regulation that can be exploited to control the pathogenesis associated with these particular organisms.

TORC1 effectors of transcription and translation

The transcriptional response to inhibition of TORC1 with rapamycin revealed global roles in positively regulating ribosome biogenesis, while blocking the expression of nitrogen catabolite regulated (NCR), retrograde response (RTG), and stress responsive (STRE) genes [24–28].

The Tap42-Sit4 PP2A-like phosphatase mediates both TORC1 inhibition of transcription and activation of translation and cell growth [25,29]. The prevailing model is that, under ample nutrient conditions, TORC1 phosphorylates Tap42, thereby favoring Tap42-Sit4 complex formation [30]. Nutrient deprivation or rapamycin treatment results in dissociation of this complex and targeting of Sit4 towards specific substrates. An alternative model posits that inactive Tap42-Sit4 complex is tethered to membranes via TORC1, and rapamyicn treatment or nitrogen starvation releases activated Tap42-Sit4 complex into the cytosol [31]. However, this model is not supported by TORC1 characterization studies and awaits further confirmation. The NCR genes, regulated by the transactivators Gln3 and Gat1 and the repressor Ure2, are among the best-studied examples of TORC1 transcriptional regulation. TORC1 activity prevents Sit4-mediated dephosphorylation of Gln3 and Gat1 and thereby blocks nuclear translocation [25]. However, recent findings indicate this pathway is even more complex. Gln3 nuclear localization in response to nitrogen source quality requires Golgi to endosome trafficking, and regulation of Gat1 is not strongly Ure2- or Sit4-dependent [32–34]. In general, control of nuclear translocation has emerged as a common mechanism by which Tor regulates gene expression.

In addition, Tap42-Sit4 and Sit4 interactions with its associated proteins, Sap185 and Sap190, regulate the phosphorylation levels of Gcn2 and eIF2α to control the rate of translation and in particular Gcn4 translation [35,36].

Both the TORC1 and the cAMP-PKA pathways govern ribosome biogenesis in response to nutrients. This process entails the coordinated expression of ribosomal protein, Ribi, rRNA, and tRNA genes, and therefore involves the activity of Pol I, Pol II, and Pol III, respectively [37]. Recent studies have identified Sch9 kinase as an important TORC1 effector of ribosome biogenesis [38,39]. Moreover, Sch9 partially mediates TORC1 effects on the Rim15 kinase and control over G0 entry [40].

TORC2 effectors of actin polarization and stress responses

A requirement for TORC2 in actin polarization (via control of the Rho1/Pkc1/MAPK cell integrity pathway) was first revealed by genetic studies indicating that TORC2 activates Rom2, the guanine nucleotide-exchange factor for Rho1 [41]. Recently, the AGC kinase Ypk2 and the PH domain proteins Slm1 and Slm2, all of which drive actin polarization, were shown to be direct TORC2 substrates [42–44]. How these TORC2 effector branches coordinately regulate cell integrity and actin polarization remains to be determined. In addition, the activities

of both Ypk2 and Slm1,2 are influenced by phytosphingolipids and required to regulate ceramide synthesis, a process important in stress response [43]. Interestingly, TORC2 mutants show reduced ceramide syntheses and this defect and the inability of *slm1* and *slm2* cells to cope with oxidative and heat stresses, are both alleviated by calcineurin defects [45–47]. Moreover, upon stress conditions Slm1 and Slm2 are dephosphorylated by calcineurin, and this event is required to activate stress responses [46–48].

This illustrates that Ypk2 and the Slm proteins integrate nutrient (nitrogen) and lipid signals and TORC2-Slm and calcineurin signaling antagonistically govern stress survival (Figure 1).

Rapamycin effectors in human fungal pathogens

Currently, systemic mycoses are treated with an armamentarium of antifungal drugs consisting of nucleic acid inhibitors (5-flucytosine), polyenes (amphotericin B and nystatin), ergosterol biosynthesis inhibitors (azoles) and echinocandins (caspofungin, micafungin) (reviewed in [49]). A separate group of antifungal compounds are the immunophilin-targeting drugs CsA, FK506 (tacrolimus), and rapamycin (sirolimus), which due to their immunosuppressive activity have been less appreciated as potential antifungal agents [50]. Recently, there is renewed interest in the antifungal activity of rapamycin fostered by the development of less immunosuppressive rapamycin analogs and findings that lipid-formulated rapamycin, amphotericin B, and 5-flucytosine act synergistically *in vitro* [50,51].

A single Tor homolog (Tor1) has been identified in the human fungal pathogens *C. neoformans* and *C. albicans* and the fungicidal activity of rapamycin in these two species is exerted via conserved FKBP12-rapamycin complexes that bind Tor1 and thereby inhibit its activity [52,53].

Interestingly, at sublethal concentrations, rapamycin blocks filamentous differentiation in *S. cerevisiae*, *C. albicans*, and *C. neoformans* [54,55] (Figure 2). In *C. albicans* filamentous growth is essential for virulence and consequently is a potential target for antifungal therapy [56–58]. In this pathogen, evidence-linking Tor1 to filamentous growth continues to mount with several Tor1 signaling components being associated with this developmental transition. A clear example is the link between nitrogen availability and filamentous growth in *C. albicans* [59]. As discussed above, in *S. cerevisiae* Tor1 signaling mediates the cellular response to nitrogen limitation via the Sit4 protein phosphatase and the GATA transactivators Gln3 and Gat1, which regulate the expression of nitrogen utilization genes, including the ammonium permease Mep2 [25–28]. In *C. albicans*, *mep2/mep2* and *gln3/gln3* loss of function mutations block filamentous growth under limiting nitrogen conditions and *gln3/gln3* mutants are avirulent in murine models of disseminated disease [59–61]. Additionally, the protein phosphatase Sit4 also plays an important role during filamentous growth and virulence of *C. albicans* [29,62].

It is increasingly evident that the Tor1 nutrient sensing pathway regulates important virulence traits in *C. albicans*. Furthermore, components of this pathway including TORC1 and TORC2, upstream regulators (Tsc1,2 and Rheb) and effectors (Tap42, Sit4, Sch9, and Ypk2) appear to be conserved among several fungal organisms, including pathogenic fungi (Table 1). This conservation will ultimately allow the use of model and pathogenic fungi for further characterization of Tor signaling and identification of fungal-specific Tor effectors that can be harnessed as potential targets for antifungal therapy.

Calcineurin

The calcineurin inhibitors FK506 and CsA were initially isolated as potent immunosuppressive drugs [63,64] and subsequently became cornerstones of therapy in solid organ and bone marrow

transplantation. More recent evidence has suggested a role for these drugs, and non-immunosuppressive analogs, as novel antifungal therapeutics [50,65]. Calcineurin is conserved from yeasts to humans and is crucial for mediating cellular stress responses. Functional calcineurin consists of two subunits, a catalytic A and a regulatory B subunit, both of which are essential for function [66]. When Ca^{2+} fluxes into the cytosol from either intracellular stores or extracellular sources, calcineurin is bound by Ca^{2+}-calmodulin causing a conformational change that relieves repression of the catalytic site by an autoinhibitory domain [66]. FK506 and CsA form intracellular complexes with FKBP12 and cyclophilin, respectively [67], and these complexes then bind to and block calcineurin function [68–72](Figure 1). FK506-FKBP12 and CsA-cyclophilinA also inhibit calcineurin in pathogenic fungal species including *C. neoformans, C. albicans*, and *Aspergillus fumigatus* [70,73–79].

In pathogenic fungi, calcineurin plays a crucial role in virulence. In *C. neoformans* calcineurin mutants are attenuated for virulence in animal models of infection due to their inability to grow at body temperature (37°C) [75,80]. Similarly, *C. albicans* calcineurin mutants are attenuated in a murine systemic infection model [81–83]; in this case calcineurin mutants are not temperature sensitive, but serum and cation sensitive and thus unable to survive calcium stress imposed by serum [84]. Interestingly, the role of calcineurin in *C. albicans* virulence appears to be host niche specific, as strains lacking calcineurin are attenuated for virulence in systemic and ocular infection models [81–83,85], but fully virulent in murine pulmonary, vaginal, and oropharyngeal models [86](Reedy, Filler, and Heitman unpublished data). In the pulmonary pathogen *A. fumigatus*, calcineurin is required for morphogenesis. Strains lacking calcineurin form short, blunted filaments and are thus significantly attenuated for virulence through a third distinct mechanism of action [87].

The attenuated virulence of fungal calcineurin mutants suggests that inhibition of calcineurin alone could have therapeutic potential; moreover, calcineurin inhibitors can also be utilized in combination therapy with current antifungal agents. In *in vitro* studies with *C. albicans, A. fumigatus, C. neoformans*, and the dermatophyte *Trichophyton mentagrophytes* calcineurin inhibitors convert normally fungistatic azoles, as well as other clinically available antifungals, into fungicidal compounds. This synergistic action extends to azole resistant fungal strains [73,77–79,88–91]. An *in vivo* proof-of principle study demonstrated that a CsA-fluconazole combination was more effective than either drug alone at treating candidal endocarditis infection in rats [92,93]. Subsequent studies document improved survival and disease resolution with combination rather than monotherapy in the treatment of *C. albicans* murine keratitis [85], catheter biofilms [94], and treatment of *T. mentagrophytes* model skin infections [90]. The clinical use of calcineurin inhibitors is limited by their immunosuppressive activity, however non-immunosuppressive analogs (FK506: L-685,818 from Merck and CsA: 211–810 and 209–825 from Novartis) are available that still inhibit fungal calcineurin and spare host calcineurin [73,74,88]. Research aimed at identifying additional components of the calcineurin signaling that could bypass the immunosuppressive activity of calcineurin inhibition is currently underway [65,95–100].

Hsp90

Recent studies reveal that Hsp90, a component of a chaperone complex induced by heat stress, governs the trajectory of drug resistance in fungi [96]. Using genetically engineered yeast strains in which Hsp90 expression can be reduced, or small molecule inhibitors of Hsp90 (geldanamycin), Hsp90 was shown to be required for both the rapid emergence of azole drug resistance, and for its maintenance. Hsp90-dependent drug resistance involves alterations in the ergosterol biosynthetic pathway targeted by azole drugs, whereas azole resistance conferred by over-expression of pumps that extrude drugs is Hsp90-independent. Potent drug synergism was observed between Hsp90 inhibitors and azoles in *C. albicans*, and with candins in *A.*

fumigatus [96]. These findings parallel previous studies in which calcineurin inhibitors exhibited synergistic antifungal activity with azoles against *C. albicans* [76,83]. A model has been advanced suggesting calcineurin might therefore be a direct client protein of Hsp90 [101], and both genetic and protein interaction data suggests the two proteins physically and functionally interact in *S. cerevisiae* [98,102].

An attractive feature is that several calcineurin inhibitors are already FDA approved for clinical use and phase II and III clinical trials are ongoing for geldanamycin and its analogs (17-AAG , 17-DMAG) for a variety of oncological indications based on their chemotherapeutic potential [101]. Given limited success of current antifungal regimens for many systemic and topical fungal infections, the emergence of drug resistance, and increasing numbers of susceptible patients, combinatorial drug approaches hold considerable appeal. These combinations potentiate activity of azoles or candins, in some cases rendering them fungicidal rather than merely fungistatic, extend their therapeutic range, and concomitantly block emergence of some classes of drug resistant mutants. It will be a challenge to combine drugs inhibiting targets highly conserved between fungi and humans, and which in some cases have immunosuppressive or toxic side effects. This is particularly relevant for the Hsp90 inhibitors geldenamycin and radicicol that target Hsp90′s highly conserved ATP binding pocket to block ATP-dependent chaperone activity [103]. Nevertheless, even minor structural differences in conserved drug binding pockets can be exploited to develop target specific inhibitors. A champion example is the Cox2 specific inhibitors that exploit a single amino acid difference in the active site of Cox1 and Cox2 [104–106]. Additionally, the Hsp90 chaperone complex includes not only Hsp90 but also many additional co-factors, which might be targets for fungal specific inhibitors. Fungal specific calcineurin inhibitor analogs have also been identified [74], and the wealth of structural and enzymatic data for these targets (FKBP12, cyclophilin A, calcineurin, and Hsp90) renders these pathways attractive from a medicinal chemistry perspective [107]. The challenge ahead is to translate these *in vitro* findings to studies in heterologous host and animal models as a prelude to clinical testing as novel antimicrobial approaches in humans.

Conclusions and outlook

Small bioactive compounds produced by soil microorganisms have potent and specific activities against conserved cellular pathways providing molecular tools for dissection of cellular functions. The immunosuppressive drugs cyclosporin A, FK506, rapamycin, and geldanamycin are all microbial products that inhibit targets conserved from unicellular yeasts and pathogenic fungi, such as *S. cerevisiae, S. pombe, C. albicans* and *C. neoformans* to complex organisms including humans. The molecular targets of these drugs (cyclophilin A, FKBP12, calcineurin, Tor, and Hsp90) function in conserved signaling cascades that couple environmental stimuli to cell growth and proliferation (from yeasts to humans). Thus, studies of natural product action in model genetic systems are contributing to our understanding of therapeutic action in humans. Moreover, these agents have broad spectrum, potent antimicrobial activities, both alone and in combination with established antifungal drugs including the azoles and candins, and therefore represent novel, lead strategies for antifungal therapeutic development.

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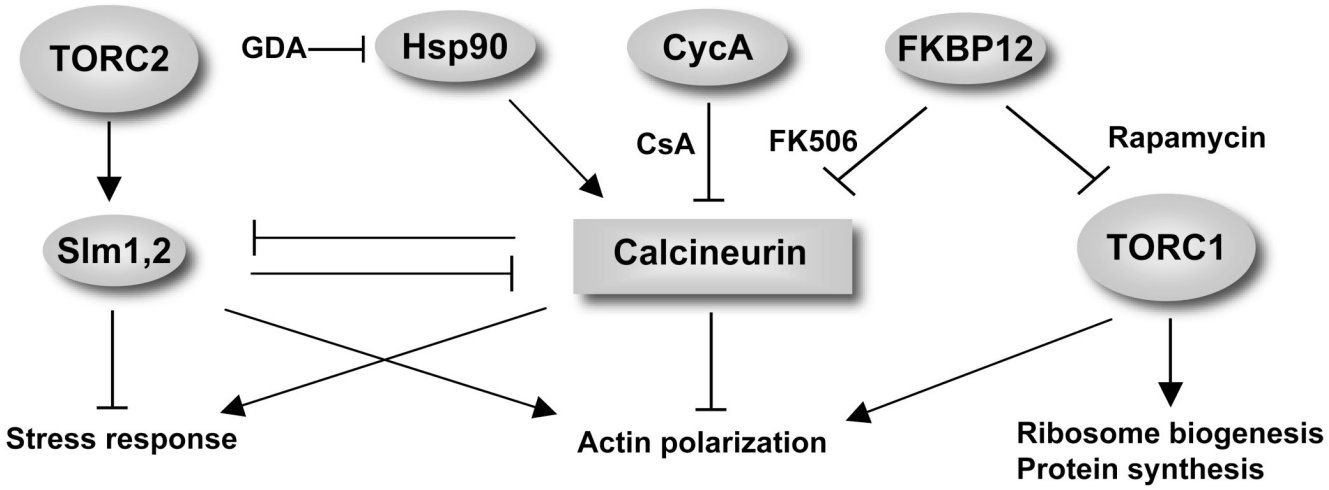

Figure 1. The antifungal drugs cyclosporin A, FK506, rapamycin and geldanamycin target conserved signaling pathways with inter-related functions in regulating responses to nutrients and stress

CsA and FK506 form stable complexes with the prolyl isomerases cyclophilin A (CycA) and FKBP12 respectively, which subsequently bind to and inhibit calcineurin function. The Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin is a complex, composed of a regulatory and two catalytic subunits hypothesized to be stabilized (or maintained in a signaling competent form) by Hsp90, and this effect can be blocked by Hsp90 inhibitors, including geldanamycin (GD). Calcineurin plays important roles in responding to stress-induced Ca^{2+} signals and acts by controlling transcription of stress responsive genes while counteracting actin cytoskeleton polarization. Stress activates calcineurin to dephosphorylate the Slm PH domain proteins, thereby antagonizing TORC2-Slm signaling. Under optimal growth conditions these calcineurin functions are opposed by the rapamycin insensitive TORC2 kinase via direct phosphorylation of Slm1 and Slm2. In complex with FKBP12, rapamycin binds TORC1 and thereby blocks its functions in ribosome biogenesis, protein synthesis and actin polarization.

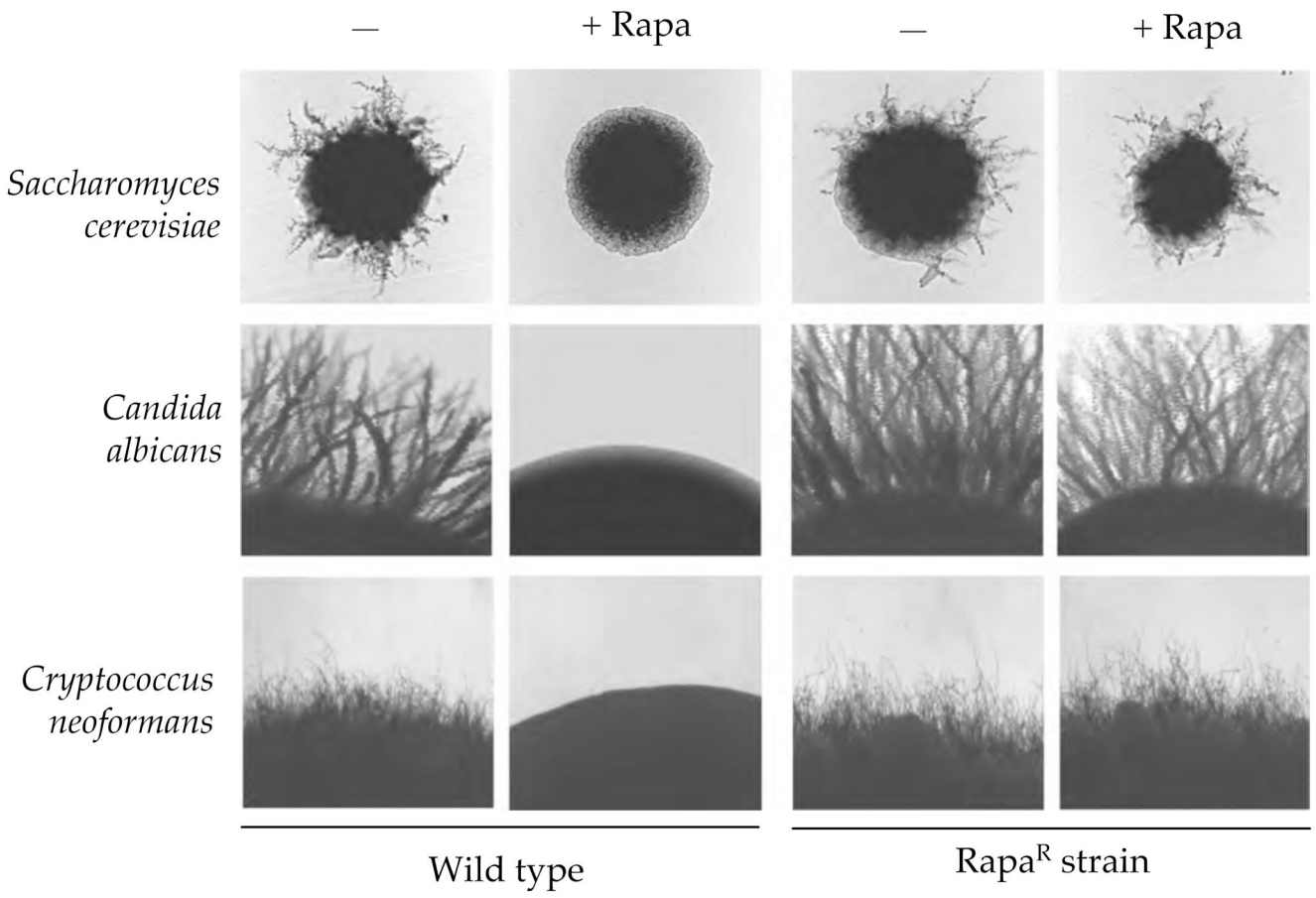

Figure 2. Rapamycin inhibits hyphal growth of model and pathogenic fungi

Hyphal growth of model and pathogenic fungi is inhibited by rapamycin. *S. cerevisiae* wild type (MLY61**a**/ α) and rapamycin resistant strain *TOR1-4/TOR1*[54] were grown for 3 days at 30°C on SLAD medium with or without 10 nM rapamycin. *C. albicans* wild type (strain SC5314) and *TOR1-1/TOR1* [53] strains were grown on Spider medium with or without 20 nM rapamycin for seven days at 37°C. *C. neoformans* teleomorphic forms (*Filobasidiella neoformans*) were generated from JEC20 (*MAT* **a**) and JEC21 (*MATα*) matings and JEC20 and JEC21 *TOR1-1* [52] matings on V8 media (pH 7). Matings were performed in the presence or absence of 150 nM rapamycin at room temperature for 3 days.

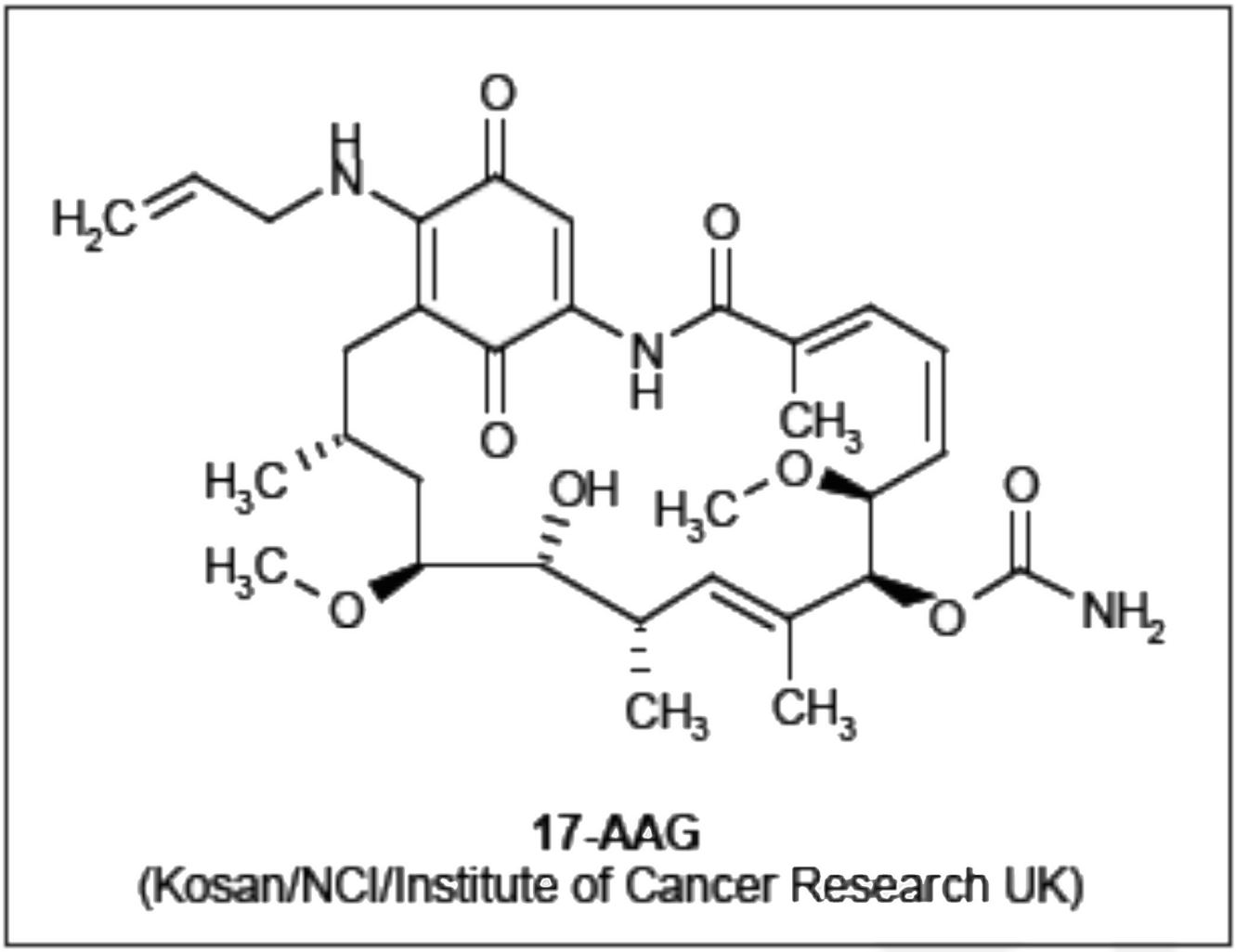

Figure 3. The structure of 17-AAG

Table 1

Tor pathway signaling components and putative homologs in model and pathogenic fungi

Tor signaling components	*S. cerevisiae* homologs	*S. pombe* homologs	*C. albicans* homologs	*C. neoformans* var. *neoformans* homologs	*C. neoformans* var. *grubii* homologs
Upstream regulators					
Tsc1	-	*tsc1*$^{+}$	orf19.4110	-	-
Tsc2	-	*tsc2*$^{+}$	orf19.1798	CNC05620	CNAG_02953.1
Rheb	*RHB1* [1]	*rhb1*$^{+}$	orf19.5994 (*RHB1*)	CNB03660 (*RHEB*)	CNAG_03876.1
TORC1					
Tor1	*TOR1*	*tor2*$^{+}$	orf19.2290 (*TOR1*)	CNF03740 (*TOR1*)	CNAG_06642.1
Tor2	*TOR2*	-	-	-	-
Kog1	*KOG1*	*mip1*$^{+}$	orf19.418 (*KOG1*)	CND01940	CNAG_01063.1
Tco89	*TCO89*	*tco89*$^{+}$	orf19.761 (*TCO89*)	-	-
Lst8	*LST8*	*wat1*$^{+}$	orf19.3862	CNM01130	CNAG_06107.1
	-	*toc1*$^{+}$	-	-	-
	YKL033W [3]	*tti1*$^{+}$ [2]	orf19.3062	CNB00940	CNAG_06709.1
	TEL2> [3]	*tel2*$^{+}$ [2]	orf19.7101	CNI02310	CNAG_04318.1
	CKA1 [3]	*orb5*$^{+}$ [2]	orf19.3530 (*CKA2*)	CNF02800 (*CK2*)	CNAG_05694.1
TORC2					
Tor2	*TOR2*	*tor1*$^{+}$	-	-	-
Lst8	*LST8*	*wat1*$^{+}$	orf19.3862	CNM01130	CNAG_06107.1
Avo1	*AVO1*	*sin1*$^{+}$	orf19.5221	CNJ01240	CNAG_04693.1
Avo2	*AVO2*	-	orf19.215	-	CNAG_02727.1
Avo3	*AVO3/TSC11*	*ste20*$^{+}$	orf19.728 (*TSC11*)	CNM01660 (*STE16*)	CNAG_06165.1
Bit61	*BIT61*	*bit61*$^{+}$	-	-	-
Bit2	*BIT2*	-	-	-	-
	YKL033W [3]	*tti1*$^{+}$ [2]	orf19.3062	CNB00940	CNAG_06709.1
	TEL2 [3]	*tel2*$^{+}$ [2]	orf19.7101	CNI02310	CNAG_04318.1
	CKA1 [3]	*orb5*$^{+}$ [2]	orf19.3530 (*CKA2*)	CNF02800 (*CK2*)	CNAG_05694.1
Downstream effector targets					
Sit4	*SIT4*	*ppe1*$^{+}$	orf19.5200 (*SIT4*)	CND05500	CNAG_01436.1
Tap42	*TAP42*	SPCC63.05	orf19.4626 (*SCH9*)	CNA03170	CNAG_00335.1
Sch9	*SCH9*	*sck1*$^{+}$	orf19.829	CNN00360 (*SCH9*)	CNAG_06301.1 (*SCH9*)

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Tor signaling components	***S. cerevisiae* homologs**	***S. pombe* homologs**	***C. albicans* homologs**	***C. neoformans* var. *neoformans* homologs**	***C. neoformans* var. *grubii* homologs**
Ypk2	*YPK2*	*gad8*$^{+}$	orf19.399	CNJ01100	CNAG_04678.1

[1]Uncoupled from *S. cerevisiae* TORC1 signaling.

[2]TORC1 and TORC2 interacting proteins in *S. pombe*

[3]Unknown if interacting with *S.cerevisiae* TORC1 and TORC2 complexes.

Tor pathway signaling homologs in pathogenic fungi were identified by comparing previously characterized *S. cerevisiae* and *S. pombe* components through reciprocal best-hit BLASTp searches.