Ras-Mediated Signal Transduction and Virulence in Human Pathogenic Fungi

Jarrod R. Fortwendel*

Department of Microbiology and Immunology, University of South Alabama, Mobile AL, USA

Abstract

Signal transduction pathways regulating growth and stress responses are areas of significant study in the effort to delineate pathogenic mechanisms of fungi. In-depth knowledge of signal transduction events deepens our understanding of how a fungal pathogen is able to sense changes in the environment and respond accordingly by modulation of gene expression and re-organization of cellular activities to optimize fitness. Members of the Ras protein family are important regulators of growth and differentiation in eukaryotic organisms, and have been the focus of numerous studies exploring fungal pathogenesis. Here, the current data regarding Ras signal transduction are reviewed for three major pathogenic fungi: Cryptococcus neoformans, Candida albicans and Aspergillus fumigatus. Particular emphasis is placed on Ras-protein interactions during control of morphogenesis, stress response and virulence.

Keywords: Ras; GTPase; Fungal morphogenesis; Fungal virulence

Introduction

In recent years, a multitude of signal transduction pathways have been identified as regulators of morphogenesis in fungi. Due to their control over cell shape and response to environmental change, many of these pathways have also been associated with virulence attributes of the pathogenic fungi. Of the major signaling modules that fit this paradigm, Ras pathways have received considerable attention. The Ras super-family is composed of membrane-associated GTPase proteins serving as major signal transduction elements in eukaryotic cells [1]. Ras proteins are considered to be “molecular switches” existing in active and inactive states, bound to either Guanosine Triphosphate (GTP) or Guanosine Diphosphate (GDP), respectively. Ras is activated by interaction with Guanosine Nucleotide Exchange Factors (GEFs) and inactivated by interaction with GTPase Activator Proteins (GAPs). In mammalian cells, interaction of Ras proteins with specific GAPs, GEFs, and downstream effectors is, in part, controlled through sub-cellular localization and compartmentalization of Ras to distinct membrane signaling platforms [2]. Differential sub-cellular localization of mammalian Ras proteins is accomplished through post-translational farnesylation of the “CAAX” motif (C=cysteine, A=aliphatic amino acid, X=any amino acid) that serves as a recognition sequence for farnesyltransferase enzymes [3]. Once prenylated at the CAAX box cysteine residue, Ras is further processed by a Ras Converting Enzyme (RCE) on the endoplasmic reticulum. This maturation step catalyzes the proteolytic cleavage of the “–AAX” residues from the CAAX motif [4]. The carboxy terminus is then carboxymethylated by action of an isoprenylcysteine carboxymethyltransferase [5,6]. In mammalian cells, mature Ras proteins then follow one of two paths to the plasma membrane; 1) Ras homologs with a conserved cysteine residue in the hypervariable domain are palmitoylated by a palmitoyltransferase and trafficked through the secretory system [7-10], or 2) non-palmitoylated Ras homologs can traffic to the plasma membrane through a non-classical pathway and become associated with the plasma membrane via a poly-basic domain just upstream of the CAAX motif [11]. Ras palmitoylation is reversible in mammalian systems and plasma membrane localized Ras has been shown to cycle back to the endomembrane system through the activity of a thioesterase located at the plasma membrane [12,13]. As subjects of both temporal and spatial control of activity, the Ras GTPases function as binary switches that, despite their apparent simplicity, control activity of multiple pathways within the mammalian cell.

Due to their central role in cancer biology, mammalian Ras proteins have been studied to a great extent and much is now known of the molecular mechanisms regulating Ras-mediated malignancy. The pivotal role of Ras in cancer formation and progression is, in large part, due to their multifunctional nature as regulators of cellular development and differentiation. The existence of functional Ras homologs in fungi was first realized in the model yeast organism, Saccharomyces cerevisiae, where two Ras proteins were identified: Ras1p and Ras2p. These two Ras homologs are thought to have evolved from ancient genome duplication [14]. As such, Ras1p and Ras2p are highly homologous at the amino acid level and appear to play overlapping roles in S. cerevisiae developmental processes. Ras2p is expressed at significantly higher levels than Ras1p, a theme present in other fungi containing two Ras homologs [15]. Ras2p was initially found to be necessary for growth on nonfermentable carbon sources, entry into the cell cycle during germination, and for completion of mitosis when deleted along with Ras1p [16,17]. Importantly, a conserved role for Ras in polarized growth processes among yeast organisms has also been observed. In response to either nitrogen starvation or excess glucose, diploid S. cerevisiae strains undergo a “filamentous growth” process whereby the yeast cells elongate and begin to bud in a unipolar fashion to form long strands [18,19]. S. cerevisiae haploid cells also convert to a polarized phenotype, called haploid invasive growth, after prolonged incubation on rich media [20]. These strands are not true hyphae, but they are considered a model for the sustained polarized

*Corresponding author: Jarrod R. Fortwendel, Department of Microbiology and Immunology, 5851 USA Drive North, Medical Sciences Building 2102, University of South Alabama, AL 36688, USA, Tel: (251) 460-6681; Fax (251) 460-7931; E-mail: jfortwendel@jaguar1.usouthal.edu

Received May 23, 2012; Accepted July 03, 2012; Published July 05, 2012

Citation: Fortwendel JR (2012) Ras-Mediated Signal Transduction and Virulence in Human Pathogenic Fungi. Fungal Genomics Biol 2:105. doi:10.4172/2165-8056.1000105

Copyright: © 2012 Fortwendel JR. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
growth seen in filamentous organisms. In S. cerevisiae, deletion of Ras leads to defects in the induction of filamentous growth under both conditions. Expression of a constitutively active form of Ras2p also causes cytoskeletal defects, evident by actin mislocalization, and induces the filamentous growth phenotype [21]. Like their human homologs, Ras2p localizes to the plasma membrane of S. cerevisiae. This specific localization is important for Ras signal transduction, as mislocalization of a constitutively activated Ras2p abrogates activated Ras2p phenotypes [22]. In the fusion yeast, Schizosaccharomyces pombe, the sole Ras homolog, Ras1p, controls aspects of actin and microtubule assembly [23]. Therefore, Ras1p plays a major role in regulating cell morphology and size. S. pombe ras1 null cells are sterile, display abnormal cell shape and lack the ability to undergo polarized growth [24].

Yeast Ras pathway components are primarily composed of Cdc42 and mitogen-activated protein kinase (MAPK) pathways in S. cerevisiae and S. pombe, as well as the cyclic AMP (cAMP) / Protein Kinase A (PKA) pathway in S. cerevisiae. In S. cerevisiae, Ras1p is activated by the GEF, Cdc25p, and is negatively regulated by the GAPs, Ira1p and Ira2p [25,26]. Active Ras2p proteins bind to and activate adenylyl cyclase to produce cAMP, in turn activating the PKA pathway [27,28]. The S. cerevisiae PKA pathway has been implicated in numerous cellular activities and is overall known to be a negative regulator of the general stress response. Therefore, constitutive activation of Ras2p leads to the suppression of stress responses in S. cerevisiae causing increased heat shock and nitrogen starvation sensitivity [29]. However, the Ras2 null mutant displays a temperature sensitive actin polarization defect that cannot be suppressed by over-expression of PKA pathway components [29]. These findings suggest a PKA-independent, Ras-mediated actin control. To this end, Ras2p also regulates a MAPK pathway controlling the polarized morphogenesis observed in both diploid and haploid cells under stress. Here, Ras2p signals through Cdc42p to the Ste20 protein kinase [15,30]. Another mediator of Ras2p signal transduction is the GEF-like protein, Lte1p. The Lte1p protein is a regulator of mitotic exit, coupling mitosis to polarized morphogenesis in S. cerevisiae [31]. Initially, the role of Lte1p was believed to be as a GEF for the mitotic exit network protein, Tem1p, but has since been reported to act as a direct inhibitor of Ras2p activity in areas of cell division [31]. These findings are very intriguing, as GEF proteins are normally considered to be positive regulators of Ras activity.

In contrast to S. cerevisiae, Ras2p function, S. pombe Ras1p is not necessary for activation of the PKA pathway [24]. Instead, Ras1p regulates primarily a mating MAPK pathway including the kinases Byst2p, Byr1p, and Spk1p [32,33]. Ras1p also interacts with Scd1p, a putative GEF for Cdc42p [34]. Through Scd1p, Ras1p controls cytoskeletal change and cell shape likely by modulation of Cdc42p activity levels [23]. In S. pombe, Ras1p activity is positively regulated by two known GEFs, Ste6p and Efc25p, and as in S. cerevisiae, localization of the Ras1p is important for its regulation. For example, Ras1p that is restricted to endo-membranes interacts with Efc25p and signals through the Cdc42-mediated pathway to control cell morphology [35]. In contrast, Ras1p restricted to the plasma membrane is controlled by Ste6p and signals through the MAP kinase pathway to mediate the mating response [35]. Through identification of Ras-controlled molecular mechanisms, the yeast model systems laid the early groundwork required for investigation of Ras function in growth, response to stress and virulence of pathogenic fungi.

Roles of Ras Proteins in Fungal Morphogenesis, Stress Response and Virulence

Cryptococcus neoformans

Cryptococcus neoformans is a pleomorphic yeast and is a model organism for studying signal transduction in fungal growth and virulence. Infection with C. neoformans encapsulated yeasts primarily manifests in immunocompromised patients, although another species, C. gattii, is known to infect immunocompetent individuals and was recently associated with an outbreak in the pacific northwest [36]. In response to specific environmental signals, C. neoformans can undergo two forms of yeast-to-hyphal transition. For mating, strains of opposite mating types will form filamentous structures under nutrient deprivation [37]. In addition, strains of the MATa mating type can undergo a process termed ‘haploid fruiting’, wherein filaments are formed during the production of basidiospores [38]. Signal transduction events regulating these processes have been the focus of intense study in fungal development [39].

The Ras1 gene of C. neoformans was originally identified by homology to known Ras proteins and was found to be a major regulator of high temperature growth, differentiation and virulence [40,41]. The Ras1 protein is a homolog of the proto-typical Ras proteins from mammalian cells, including H-ras, which has domains required for binding of GTP / GDP, association with GAP and GEF regulatory proteins, interaction with downstream effector proteins, and domains for post-translational modification with prenyl groups (CAAX box) and palmitoyl moieties (dual cysteine motif). Deletion of C. neoformans Ras1 causes poor growth at 37°C and complete inhibition of growth at 39°C [40]. However, shorter periods of growth at high temperature showed that the ras1 mutant cells are not inviable, but only growth arrested, after short incubations at high temperatures. Growth is recovered in the ras1 mutant upon switching to incubation at 30°C [40]. Inhibition of growth at 37°C is associated with the inability of the ras1 deletion mutant to properly polarize actin. The ras1 mutant displays properly localized yet depolarized actin in large, unbudded cells at 37°C [42]. These findings suggested a role for C. neoformans Ras1 in maintaining proper cell morphology under stress, likely through regulation of actin dynamics required for proper cellular morphology and bud formation. Since growth at high temperatures is required for C. neoformans virulence, the ras1 mutant is also less virulent in a rabbit model of cryptococcosis [40]. However, Ras1 deletion does not result in differences in capsule size or melanin production [40]. As these are major C. neoformans virulence factors, the lack of virulence in the ras1 mutant is attributed mainly to decreased growth at 37°C. Ras1 was later shown to be important for serum-induced capsule growth in vitro however, as many factors affect capsule induction in C. neoformans, this is likely not the major mechanism through which Ras1 signaling impacts virulence [43,44]. Although support of cellular growth under heat stress is considered the major role of Ras1 during infection in mammalian models, the ras1 mutant also displays decreased virulence at low temperature in the non-mammalian models Caenorhabditis elegans, Drosophila melanogaster, and Galleria mellonella [45-47]. These findings suggest that, at least for these non-mammalian models, Ras1 may play roles in C. neoformans virulence that go beyond the control of growth under temperature stress.

Much like S. pombe Ras1, the C. neoformans Ras1 gene also plays important roles in mating and haploid fruiting, two processes requiring yeast-to-hyphal differentiation [24,40,42,48]. C. neoformans can be induced to mate when opposite mating type strains are cocultured...
under nutrient starvation [49]. Under these conditions, yeast cells undergo a sustained polarized growth allowing opposite mating type filaments to fuse and form basidia. When the ras1 mutant was cultured in mating conditions with a competent mating partner, no hyphal projections are observed and no recombinant basidiospores are formed [40]. These findings indicated that Ras1 is essential for mating in C. neoformans. Subsequent mechanistic studies to address how Ras1 impacts mating and hyphal growth showed that the ras1 mutant is defective in both pheromone production and in response to a competent mating partner [40,50]. Further supporting a role in mating, Ras1 plays a critical function in transcriptional control of pheromone response genes, including: MFA1, encoding alpha mating pheromone; CPR1, encoding pheromone receptor, a G-protein coupled receptor; and GPB1, encoding the Gβ protein subunit of the pheromone receptor [50,51-53]. Similar to the sexual differentiation that occurs in the presence of a mating partner, certain C. neoformans strains can also undergo sexual differentiation, called haploid fruiting, when cultured under nitrogen starvation conditions [38]. This differentiation again results in a yeast-to-hyphal transition, forming hyphae that are similar to, but distinct from, mating filaments. Deletion of Ras1 causes the inability of C. neoformans to form hyphae on filament agar, whereas expression of a constitutively active Ras1 (Ras1Q67L) in the H99 genetic background leads to robust haploid fruiting [40,50]. In addition, hyphae formed by the Ras1Q67L mutant adhered to and invaded the agar to a greater extent than the wild type or ras1 mutant strains [40]. Together, these data identified roles for Ras1 in C. neoformans cellular morphology, specifically yeast cell shape and polarized morphogenesis during yeast-to-filament transitions. The C. neoformans genome also encodes a second Ras homolog, Ras2, which is expressed at very low levels compared to the Ras1 gene [42]. Deletion of Ras2 causes no notable defects, but deletion of both Ras genes causes defects more severe than deletion of either alone [42]. In addition, Ras2 over-expression can partially suppress the ras1 mutant growth and mating defects [42]. These findings suggested that, although Ras1 and Ras2 have both shared and a distinct role in growth, Ras1 is the predominant Ras protein controlling growth and virulence of C. neoformans.

Early attempts to identify Ras pathway signaling components revealed roles for cAMP and pheromone MAPK-pathway components in mating, but not haploid fruiting or growth at 37°C. Addition of exogenous cAMP or over-expression of GPB1 or MFA1 are each able to fully or partially suppress the ras1 mating defect, whereas none of these conditions can rescue the ras1 high temperature growth defect [40,50]. None of these conditions can suppress the ras1 defect in haploid fruiting [50]. These data informed a model which places C. neoformans Ras1 upstream of the pheromone response pathway for control of mating, but did not identify Ras1 signaling components important for high temperature growth and, therefore, virulence. The initial clue towards uncovering Ras1 pathways regulating high temperature growth and morphology in a mating pathway-independent manner came from a multicopy suppressor screen that identified Rac1, a Rho-family protein, as a suppressor of the ras1 high temperature growth defect [54]. Interestingly, Rac1 over-expression is also able to suppress the ras1 mating defect [54]. Deletion of RAC1 causes deficient filament production on filamentation agar and under mating conditions, and this hyphal deficiency is not associated with reduced pheromone production, ineffective cellular fusion, or basidium formation and sporulation [54]. Hyphae that are produced from RAC1 mutant mating cell fusion display aberrant morphology without disrupted actin localization [54]. Together, these results suggested that Ras1 sends signals downstream to a second GTPase protein, Rac1, to control aspects of high temperature growth and polarized morphogenesis. Ras subfamily proteins have been shown to operate in GTPase signal transduction cascades to regulate gene expression and modulate cytoskeletal events for drastic morphogenic changes in a variety of organisms, including fungi. These cascades commonly involve Rho-family proteins like Rac and Cdc42. In addition to Rac1, C. neoformans contains two Cdc42 paralogs; CDC42 [previously DCH2] and CDC420 [previously CDC42] [55,56]. Several lines of evidence have recently emerged demonstrating the importance for Ras-mediated Cdc42 function in high temperature growth and cellular morphogenesis of C. neoformans. First, Cdc42, a putative GEF for Cdc42, physically interacts with Ras1 in a yeast-two-hybrid assay in a GTP-dependent manner [56]. This finding indicates that the Ras1-Cdc42 interaction occurs preferentially when Ras is in an active state, suggesting a functional interaction. Deletion of Cdc42 causes decreased growth at 39°C, an actin polarization defect that mimics the ras1 mutant at high temperatures, and loss of pathogenicity [56], Ras1 has been shown to signal through Cdc42 as deletion of CDC42 does not further exacerbate ras1 phenotypes and over-expression of Ras1 in the cdc42 mutant does not abrogate the cdc42 high temperature growth defect [56]. These epistasis experiments place Cdc42 downstream of Ras1 in a common signaling pathway. In addition, over-expression of either Cdc42 parologue can at least partially suppress the ras1 growth defect, while deletion of Cdc42, alone or in combination with Cdc420, causes phenotypes similar to the ras1 mutant, including: decreased growth at 37°C [cdc42A, cdc42B, cdc42D], increased sensitivity to latrunculin B [cdc42A, cdc42B, cdc42D], defective actin polarization [cdc42A cdc42D], and loss of virulence [cdc42A] [55]. Taken together, these results support the hypothesis that the Rho-family proteins, Rac1, Cdc42, and Cdc420, function downstream of Ras1 to coordinate morphogenesis, high temperature growth and virulence in C. neoformans. What proteins function further downstream of these Ras1 effectors to mediate growth and virulence? Among as yet unidentified effectors, downstream signals are known to involve: 1) the protein-activated kinase homologs, Ste20 and Pak1 [54-56]; 2) septin proteins important for actin organization during cytokinesis [55,57]; and 3) Wsp1, a WASP protein important for the promotion of actin assembly [58,59].

Fungal Ras proteins, like their human counterparts, contain a C-terminal CAAX motif for farnesyltransferase recognition and many also contain predicted palmitoylation motifs. For example, Ras proteins of S. cerevisiae, S. pombe and C. albicans contain a single cysteine, located just up-stream of the CAAX motif, together called “CAAX”, as a predicted palmitoylation site [60]. In contrast, C. neoformans and the mould organisms, like A. fumigatus, contain a dual-palmitoylation motif with tandem cysteines [60]. Among the human pathogenic fungi, studies with C. neoformans were the first to report the importance of properly localized Ras protein for activation of pathways required for growth and virulence. When intact, lipidation of the C-terminal domains aids the Ras1 protein in associating mainly with the plasma membrane [60]. Mutation of the prenylation residue, residing in the highly conserved CAAX-box, mislocalizes Ras1 to the cytosol where it is unable to complement the morphogenesis and mating defects associated with the ras1 mutant [60]. In contrast, the dual palmitoylation residues present just upstream of the C. neoformans Ras1 CAAX box are differentially important for Ras1 function in morphology, virulence, and mating. Whereas mutation of one palmitoylation residue has no observable effect on Ras function, mutation of both residues results in a Ras1 protein that is non-functional for growth at 39°C but is competent for development of mating filaments [60]. As expected
from its inability to suppress the ras1 high temperature growth defect, the palmitoylation-deficient Ras1 protein is unable to support full virulence in a murine model of cryptococcosis [60]. Although the effect of mislocalization is profound, the exact mechanism behind the loss of Ras function remains unclear.

Candida albicans

Ras subfamily proteins also play an essential role in morphogenesis and virulence in the yeast pathogen, Candida albicans. C. albicans is a human commensal yeast that can be associated with numerous forms of disease ranging from superficial to systemic infections, based largely on the immune status of the host. Unlike many yeasts, C. albicans has the unique ability to grow as budding cells, as strands of elongated yeast cells (pseudohyphae), and as “true” hyphae, containing parallel sidewalls and septa [61]. This variation in morphology is considered a virulence attribute, as yeast that cannot undergo the yeast-to-hyphal transition display reduced pathogenicity [62,63]. Multiple signal transduction pathways have been identified as regulators of the C. albicans yeast-to-hyphal transition and the Ras pathway is a major contributor.

The C. albicans Ras gene, Ras1, was cloned via its ability to suppress the viability defect of the ras1/ras2 S. cerevisiae mutant [64,65]. However, unlike S. cerevisiae, the C. albicans genome only contains one proto-typical Ras homolog [64,65]. The predicted C. albicans RAS1 gene product, Ras1p, contains conserved cysteine residues for prenylation and palmitoylation, making Ras1p more similar to S. cerevisiae Ras1p/Ras2p and C. neofor mans Ras1, than to the C. neoformans RAS1 gene product. Deletion of Ras1 is not lethal in C. albicans, but the mutant exhibits slower growth and does not respond to serum by formation of hyphae or germ tubes [64,65]. In addition to loss of serum-induction, Ras1 deletion causes the inability to form hyphae under multiple conditions that induce a yeast-to-hyphal transition in C. albicans [65]. In contrast, expression of an activated form of Ras1p encourages the morphological transition of C. albicans from yeast to hyphae. Early studies showed that a mutant expressing a dominant active ras1G13V mutation produces hyphal projections in a shorter time period than the wild type, when grown on solid agar under hyphal-inducing conditions [64]. Later studies revealed that constitutively active Ras1p induces constitutive hyphal growth, even under non-hyphal-inducing conditions [65]. Similar to S. cerevisiae, the activated Ras1p strain is also more sensitive to heat shock and displays decreased accumulation of glycogen [64]. In accordance with the S. cerevisiae, S. pombe and C. neofor mans data, these early studies supported a role for the Ras pathway in morphological transitions to polarized growth. Since Ras1 controls the dimorphic switch from yeast to hyphae, and the hyphal form of C. albicans is associated with pathogenicity, the Ras1 null strain was further hypothesized to control virulence. This hypothesis was supported by studies that revealed increased survival of mice infected with the ras1A mutant in an intravenous mouse model of candidiasis [65]. Ras1 null cells isolated from the kidneys displayed short deformed yeast while wild type and complemented strains were fully formed hyphae [65]. In addition, in vitro culture of the homozygous ras1A mutant with murine primary macrophages revealed the inability of Ras1 null cells to form hyphae and destroy macrophages after ingestion [65]. These data indicate that, like C. neoformans Ras1, Ras1p regulates hyphal development and virulence in C. albicans. Mating in C. albicans requires a morphological switch from small, white yeast cells to a larger, more elongated opaque phenotype [66,67]. Although the opaque phenotype is unstable at 37°C in vitro, physiological levels of CO2 in human tissue are able to stabilize the opaque phenotype at body temperature, arguing that mating may occur in the human host [68]. Recent studies have identified N-acetylglucosamine (GlcNAc), a carbohydrate produced by human gastrointestinal tract bacteria, as an inducer of the white-to-opaque phenotype switch in Candida species [69,70]. However, white yeast cells bearing a Ras1 homozygous null mutation display greatly decreased rates of switching to the opaque phenotype, indicating that Ras1p is a major regulator of this morphological change [69]. Interestingly, GlcNAc is also an activator of Ras1p-dependent hyphal growth in C. albicans [71]. These reports highlight the versatility of the Ras1p pathway during response to environmental signals inducing either sexual differentation or polarity.

In contrast to C. neoformans Ras1 function, many of the phenotypic outcomes of Ras signaling in C. albicans are mediated by the cAMP-activated PKA pathway. This connection was originally made in S. cerevisiae and subsequently found to be conserved in C. albicans. Deletion of CDC35, the sole adenylate cyclase of C. albicans, leads to loss of detectable cAMP levels, slow growth and the inability to undergo the yeast-to-hyphal morphological transition [72]. In a strain lacking the both CDC35 alleles, constitutive activation of Ras1p is unable to induce the yeast-to-hyphal transition, placing Ras1p upstream of Cdc35p and the PKA pathway [72]. In addition, deletion of EFG1, a major transcription factor downstream of the PKA pathway, blocks hyphal induction mediated by constitutively activated Ras1p [65]. The PKA protein complex, composed of two active subunits isoforms [Tpk1p and Tpk2p] and one regulatory subunit isoform [Bcy1p], is known to control response to stress and is a major regulator of C. albicans virulence [73]. Therefore, as a major regulator of cAMP production, Ras1p is a predominant mediator of morphogenesis and stress response in C. albicans via control of PKA activity. Interestingly, the Ras-cAMP-PKA pathway also controls the timing of Programmed Cell Death [PCD] in C. albicans. In response to environmental stressors, C. albicans cells can enter a pattern of PCD that bears some similarity to apoptotic patterns of mammalian cells [74]. Constitutive activation of Ras1p speeds up the transition to death, whereas a Ras1Δ strain is delayed in this transition [74]. Stimulatory or inhibitory mutations in adenylate cyclase and PKA have similar effects, suggesting that suppression of the general stress response pathway through over-activation of the Ras-PKA pathway accelerates PCD in C. albicans [74]. A connection between Ras signaling and PCD has not been reported in other human pathogenic fungi, though apoptotic-like PCD processes have been explored in C. neoformans and A. fumigatus [75-77].

C. albicans was recently reported to contain a second Ras homolog, RAS2. However, RAS2 is an atypical Ras homolog lacking the majority of the conserved G box elements found in proto-typical Ras proteins [78]. Despite the differences, RAS2p was found to be an active GTPase and may play an antagonistic role in cAMP production regulated by RAS1p [78]. No further studies have revealed potential interaction or crosstalk between RAS1p- and Ras2p-controlled pathways.

Similar to the other yeasts described above, RAS1p also signals through a Cdc42-dependent pathway to control morphogenetic changes in response to environmental signals. Cdc42p, and its exchange factor, Cdc24p, are essential for vegetative growth in C. albicans due to a dual role in regulation of filamentation and mitosis [79,80]. However, using mutants previously described in S. cerevisiae that separate Cdc42's roles in these two processes, one study has generated filamentation-specific mutations that do not affect Cdc42p's role in completion of mitosis [80]. These mutant strains were found to have reduced expression of Efg1p transcription factor-dependent genes involved in the yeast-to-hyphal transition. Some of these
mutants also displayed reduced ability to damage endothelial cells, indicating the importance for Cdc42-mediated morphogenesis in C. albicans virulence [80]. Evidence of Ras-mediated control over Cdc42p signaling in C. albicans was shown when over-expression of HST7, a Cdc42-regulated MEK kinase, or CPR1, a Cdc42 pathway transcription factor, were able to partially suppress a ras1Δ filamentation defect [65]. In addition, deletion of either HST7 or CPR1 is able to block filamentation induced by expression of a constitutively active Ras1p [65]. These studies place Ras1p upstream of the Cdc42 pathway, similar to previous studies on Ras signaling in S. cerevisiae. One study has shown that Ras1p also directs actin patch and cable formation to regulate endocytosis and secretion during hyphal growth [81]. These processes are mediated through the formin, Bni1p, and the WASP homolog, Wall1p, in C. albicans likely through Cdc42p and have helped to inform a general model of how fungal organisms utilize Ras/Cdc42 GTase signal transduction to support polarized morphogenesis [81]. Unlike S. cerevisiae, C. albicans contains a Ras homolog, Rac1p, and an accompanying GEF, Dck1p, that control filamentation in embedded agar [82,83]. Although a direct connection between Rac1p and Ras1p signaling has not been explored, embedded agar filamentation is a process that also requires Ras1p signaling. Rac1p and Dck1p, along with another protein, Lmo1p, have been shown to signal through both filamentation and cell wall integrity MAPK pathways to link these two important processes during growth [84]. However, Rac1 and Cdc42 appear to contribute different roles to morphogenesis in C. albicans, as RAC1 over-expression cannot suppress cdc42Δ related defects [82]. An interesting question remains regarding the possibility that Ras1p may signal upstream of both Cdc42 and Rac1 pathways to coordinate proper morphogenesis in C. albicans during invasive infections.

Spatial regulation of Ras signaling has been recently studied in C. albicans as well. As expected, GFP-Ras1p localizes to the plasma membrane of both yeast and hyphal cells [85]. Similar to S. cerevisiae, C. albicans Ras1p contains a CAAX box for prenylation and only a single conserved cysteine (C287) for palmitoylation. Mutation of C287 decreases Ras1p abundance and causes mislocalization of GFP-Ras1p to endo-membrane structures, whereas mutation of the CAAX box cysteine (C288) causes cytosolic localization [85]. The Ras1p-C288S mutant is unable to support filamentous growth when cultured under hyphal inducing conditions, suggesting that prenylation of Ras1p is absolutely required for function in C. albicans [85]. Although the Ras1p-C287S mutant grows as wild type under most conditions, this mutant is also unable to form hyphal under conditions requiring activation of the Ras pathway [85]. However, constitutive activation of the Ras1p-C287S mutant suppresses this hyphal defect without affecting Ras1p localization or membrane dynamics [85]. Together, these data argue that, at least for C. albicans, Ras1p localization to the plasma membrane may be more important for activation than for interaction with specific effectors.

**Aspergillus fumigatus**

Growth of *A. fumigatus*, like most filamentous fungi, involves progression through sequential steps of development. When cultured in the presence of a carbon and nitrogen source, growth begins with the process of conidial germination. This initial step is characterized by a period of isotropic swelling of conidia, initiation of mitosis and the establishment of polarized growth [86]. Continued growth requires the formation of interconnected hyphal networks through the maintenance of polarized extension and the formation of new growth axes. The *A. fumigatus* assexual developmental program concludes with the formation of conidiophores bearing conidial chains. In the immunocompromised host, *A. fumigatus* hyphae invade tissue and disseminate to distant sites via the bloodstream [87]. Therefore, the completion of proper morphogenetic processes is tightly linked to the ability to establish and maintain invasive disease.

In *A. fumigatus*, the major Ras homolog, RasA, plays critical roles in initiation of germination, polarized morphogenesis, cell wall integrity, and virulence. *A. fumigatus* mutants with decreased (dominant negative, DNrasA) or absent (ΔrasA) RasA activity display a lag in germination initiation, whereas mutants with constitutively activated RasA break dormancy in the absence of a carbon or nitrogen source [88-90]. This precocious germination caused by increased RasA activity is associated with an exaggerated isotropic growth phase and increased nuclear content [89,90]. Following germination, the ΔrasA mutant develops severe defects in hyphal morphogenesis characterized by slow growing hyphae incapable of maintaining polarity [88]. As proper polarized hyphae are a prerequisite for the establishment and maintenance of invasive Aspergillosis (IA), the ΔrasA mutant is hypovirulent in a mouse model of IA [91]. Interestingly, increased RasA activity also produces decreased radial outgrowth [89,92]. However, unlike the ΔrasA mutant, hyphae produced under constitutive RasA activity develop hyper-vascularized sub-apical compartments that spontaneously lyse [89]. These data support the importance of temporal regulation of RasA activity throughout polarized morphogenesis, as both increased and decreased activity levels are inhibitory for hyphal outgrowth [88,89]. This hypothesis is further supported by earlier studies with *Aspergillus nidulans* revealing that expression of dominant active or dominant negative rasA alleles can halt fungal growth during various stages of development [93,94]. Although *A. fumigatus* is the only mould for which a rasA deletion has been obtained, Ras-mediated growth and development in other human pathogenic moulds, *Penicillium marneffei* (a dimorphic pathogen) and *Mucor racemosus*, has been studied through dominant active and dominant negative mutational analysis [95,96]. In both of these fungi, proper regulation of RasA signaling is required for growth and morphogenesis. Similar to *C. neoformans*, the *A. fumigatus* genome contains a second Ras homolog, rasB, that does not contain the conserved palmitoylation motif seen in the proto-typical Ras homologs [90]. Cellular expression of rasB is lower than rasA, and deletion of rasB leads to decreased growth, increased hyphal branching and a moderate reduction in virulence [90,97,98]. Although differences in carboxy-terminal localization signals imply different cellular localizations of RasA and RasB, they may play overlapping roles in hyphal development and virulence [88]. Taken together, the current literature supports a conserved role for the RasA signaling pathway in polarized growth and morphogenesis of filamentous fungi. Since polarized morphogenesis is required for fulminant invasive disease, the RasA pathway is hypothesized to be an important virulence determinant for many filamentous pathogens.

Roles for the Ras pathway in fungal cell wall formation have been reported for *C. albicans* where the ras1Δ mutant displays resistance to cell wall perturbation by calcofluor white and has increased Hog1p phosphorylation [98,99]. However, a surprising role for Ras pathway signaling in support of fungal cell wall integrity was first directly identified in *A. fumigatus* [88]. The fungal cell wall plays an essential role in protection against external stress and the proper morphogenesis of hyphae [100]. Because of this, inhibiting synthesis of cell wall components and disrupting the already assembled cell wall provide attractive antifungal strategies against *A. fumigatus* infections. When treated with the cell wall inhibitors Congo red or Nipkomycin Z, growth of the *A. fumigatus* ΔrasA mutant is severely inhibited, forming large swollen cells with no polarized growth axis [88,92]. Aberrant
phenotypes produced in the ΔrasA mutant by treatment with each stressor can be partially remediated by the addition of sorbitol to the growth medium, suggesting a cell wall defect [88]. The ΔrasA mutant also displays increased sensitivity to other cell wall stressors, including: caffeine, used to test cell wall integrity in yeast; SDS, a detergent that disrupts the cell membrane; and Fungin, a polyene that disrupts ion exchange through the cell membrane [88]. In addition to rasA deletion, constitutive activation of RasA leads to hypersensitivity to cell wall stress produced by caspofungin, a cell wall antifungal compound that inhibits β-glucan synthesis [92]. When considered with the morphogenetic analyses described previously, these data argue that temporal control of RasA activity is required for proper coordination of polarized growth and cell wall formation. Although A. fumigatus is the only mold organism for which Ras-mediated cell wall integrity has been identified, the C. neoformans Ras1 protein has recently been implicated in cell wall stress responses. Through a transcriptome analysis of Ras1-controlled genes, C. neoformans Ras1 was found to mediate expression of genes important for cell wall and membrane biogenesis [101]. Further analysis revealed a Ras1 mutant hypersensitivities that was even more pronounced than the HOG1 deletion mutant [101]. The C. neoformans ras1 null mutant was also found to be hypersensitive to fluorooxonil and SDS, indicating the osmosensitivity is due to an underlying cell wall defect [101]. Taken together, these findings suggest that control of cell wall integrity and or formation may be a common role for Ras pathways among fungi.

In contrast to the wealth of information generated for Ras signaling in C. neoformans and C. albicans, no studies have yet verified Ras pathway components in A. fumigatus and their potential roles in the phenotypes described above. However, it is reasonable to hypothesize that some combination of the highly conserved Cdc42, Rac, and PKA homologs of A. fumigatus are central to RasA signal transduction. Of these major downstream Ras effectors, the PKA holoenzyme and the single Rac homolog have been previously studied in A. fumigatus. The PKA enzyme complex consists of two catalytic subunits, encoded by one of two isoforms [pKaC or pKaN], and two regulatory subunits, encoded by the single pKAr gene. PKA signaling in A. fumigatus regulates germination, metabolism, pigment production and virulence [102-106]. Although PKA is a major downstream effector of Ras signaling in yeast, exogenous cAMP cannot complement the ΔrasA defective growth phenotypes in A. fumigatus [88]. These results may indicate that, like C. neoformans Ras1, RasA signaling in A. fumigatus better fits a model where Ras is largely independent of the cAMP/PKA pathway. This model is also supported by work in A. nidulans showing that Ras and PKA signaling impact germination through distinct pathways [107]. Because RasA deletion causes aberrations of hyphal formation suggestive of cytoskeletal abnormalities, it is likely that the Rac and Cdc42 pathways are important downstream components. The single Rac homolog of A. fumigatus, RacA, has been shown to regulate hyphal morphology during vegetative growth [108]. Interestingly, the ΔracA mutant was also found to produce decreased levels of reactive oxygen species (ROS), a known regulator of apical dominance in Aspergillus hyphae [109], mimicking inhibitors of NADPH oxidase [108]. The contributions of the Cdc42 homolog, ModA, has not yet been addressed in A. fumigatus growth and virulence, but one study has explored the roles of both the Rac and Cdc42 homologs in the model fungus, A. nidulans. This study found that the A. nidulans ModA and RacA proteins share an essential role in growth, with ModA serving as the major GTPase controlling morphogenesis [110]. Although hyphal tip organization was unaffected in ΔmodA, epistasis experiments suggest that ModA may promote the activation of the formin, SepA, to impact hyphal morphology [110]. A later study found that both ModA and RacA play roles in the localized production of ROS at the hyphal tips, providing another mechanism through which these GTPase cascades regulate hyphal morphogenesis [109]. Although not yet studied, it is interesting to speculate that RasA may be a shared upstream activator of ModA and RacA to control formin-mediated modulation of the actin cytoskeleton, as well as localized generation of ROS. Although much of the data from the study of individual signaling pathways within A. fumigatus and A. nidulans can be extrapolated to RasA function, more work needs to be accomplished to gain a clear understanding of the role RasA plays in activation of these putative effectors during A. fumigatus invasive growth.

As with the yeast pathogens discussed above, the spatial regulation of RasA is important for most RasA-pathway functions during A. fumigatus vegetative growth and pathogenesis. Expression of a GFP-RasA fusion in a ΔrasA mutant fully complements the growth deficiencies associated with loss of RasA [91]. The GTP-RasA protein localizes to the plasma membrane, but shows no enrichment at sites of polarity [91]. Similar to C. neoformans, the RasA carboxy-terminus contains a dual cytosine motif for palmitoylation-mediated association with the plasma membrane. Although mutation of either cytosine alone causes no observable defects in hyphal growth, ablation of both conserved cysteines leads to a largely non-functional RasA mutant. The palmitoylation-deficient RasA protein is mislocalized to internal membranes and the mutant strain exhibits polarized growth defects coupled with decreased cell wall integrity [91]. These findings are reminiscent of the ΔrasA mutant. In addition, virulence of the palmitoylation-deficient mutant is reduced similar to the ΔrasA mutant [91]. However, the palmitoylation deficient mutant does produce a hyphal growth rate that is greater than the ΔrasA mutant, suggesting some level of functionality is retained by the mislocalized protein [91]. This partial functionality could be the result of inefficient activation of the palmitoylation-deficient RasA mutant due to inability to interact with the regulatory GAP and GEF proteins, or because of decreased interaction with specific downstream effectors that can only be engaged from the plasma membrane. The regulation of Ras localization and its role in activation of growth and virulence pathways is an area requiring further study in pathogenic fungi.

A Future for Ras in Pathogenic Fungi: Ras Pathways as Targets for Antifungal Therapy

In summary, the current data support a model where fungal Ras proteins play conserved roles in polarized growth and virulence, at least in part, through modulation of the GTPase signaling partners Cdc42 and Rac, leading to gene expression, localized ROS production, and modulation of the actin cytoskeleton. In addition, Ras-mediated growth and virulence through PKA stress signaling appears to play a larger role in C. albicans than in C. neoformans or A. fumigatus. The Ras pathway data collected thus far have aided in the development of greatly improved models for fungal growth, differentiation and virulence of pathogenic fungi. It is clear that, although the precise mechanisms are not fully understood, spatiotemporal control of Ras pathway activation is key to achieving the appropriate signals for growth and, therefore, virulence. Can we take advantage of this information for therapeutic benefit? Are there aspects of Ras pathways that may serve as useful antifungal targets? The fact that inhibition or ablation of Ras activity decreases virulence in the pathogenic fungi discussed above argues that this signaling pathway may represent a rich landscape of potential targets. Although any novel Ras pathway inhibitor may make an excellent stand-alone antifungal therapy, an obvious area of
exploration would be combination therapy of Ras inhibition coupled with antifungal agents targeting the cell wall and/or membrane or with agents targeting parallel signaling pathways controlling morphogenesis. Such combinations have the potential to cause complete inhibition of fungal growth or even cell death. For example, additive effects are seen when loss of Ras activity is coupled with cell wall inhibition in A. fumigatus and C. neoformans [88,91,92,101], or with Amphotericin B treatment in C. neoformans [101], or with calcineurin inhibition in A. fumigatus and C. neoformans [92,111].

To approach the inhibition of Ras protein signaling, the most readily apparent targets lie in the enzymes governing Ras protein maturation and localization. Inhibition of Ras localization has been an area of major interest for anti-cancer therapies for some time. Individual targets include the inhibition of Ras protein farnesyltransferases, palmitoyltransferases, CAAX proteases and isoprenylcysteine carboxymethyltransferases [112-115]. Although many of the farnesyltransferase inhibitors developed as cancer therapies have failed clinical trials for multiple reasons [112], the post-translational prenylation of Ras is still one area of interest for novel antifungal therapies. For example, early Ras pathway studies in C. neoformans revealed an inhibitory effect of farnesyltransferase inhibitors [FTI’s] on fungal growth [111]. In addition, a recent in depth study of farnesyltransferase protein structure in C. neoformans has provided valuable insight into the potency of FTI’s against this fungus and highlighted possibilities for rational design of new FTI’s to inhibit fungal Ras localization [116]. Although a mechanistic understanding of palmitoylation has only recently been described and novel inhibitors of palmitoylation are in their infancy, the mutational analyses performed thus far argue that inhibition of Ras palmitoylation may be an interesting avenue for future antifungal therapies. Recent studies in A. fumigatus have shown that the palmitoylation inhibitor, 2-bromopalmitate, causes mislocalization of RasA from the plasma membrane and inhibits hyphal growth in a dose-dependent manner [91]. In addition, although the exact mechanism is not understood, exogenously conjugated linoleic acid appears to inhibit the yeast-to-hyphae transition through mislocalization of the Ras1p protein in C. albicans [117]. Whatever form it may take, inhibition of Ras protein maturation is an area deserving future attention in the pathogenic fungi.

Ras-effector interactions are also areas requiring further exploration. Both upstream activators and downstream effectors of Ras signal transduction could be areas of potential antifungal targets. Through the studies described above, we have collected a large amount of information on the downstream effectors employed by Ras to generate proper fungal morphology for growth and virulence. Although there are no reports of the direct inhibition of Ras-effector interactions in pathogenic fungi, a collection of studies in C. albicans has shown that molecules linked to cargo peptides can be targeted to CRIB-domains of Cdc42 effector proteins, inhibiting their interaction in vitro [118]. In addition, the inhibition of Cdc42-effector interactions via the CRIB-domain could be specifically targeted to fungal proteins with minimal effects on their human counterparts [118]. These studies make a strong case that the inhibition of signal transduction may be a very promising future for antifungal therapies. A similar methodology could be employed against Ras pathway interactions if further study can highlight fungal-specific aspects of Ras signal transduction. Compared to what we now know of Ras signal transduction to downstream effectors in pathogenic fungi, we know very little about how Ras activity is regulated by GAPs and GEFs, and how exactly this regulation takes place in time and space within the cell. In mammalian cells, Ras activity is activated by GEF proteins that are recruited to the membrane via an activated receptor tyrosine kinase [119]. Fungal genomes do not encode identifiable receptor tyrosine kinase homologs, therefore, regulation of the GEFs controlling Ras activation is less clear. Studies in model fungi have revealed that the S. pombe Ras homolog, Ras1p, is differentially regulated by two GEFs; one that controls morphogenesis and one that is transcriptionally up-regulated in response to pheromone, driving Ras pathway signaling towards mating control [120]. Early studies in yeast suggested that Ras GAPs may be involved in the response to intracellular acidification induced by glucose, implying at least one method through which fungal GAPs may control Ras activity in response to nutritional changes [121,122]. Deletion of the A. nidulans Ras GAP, GapA, causes many phenotypes that mimic the activated RasA phenotype of A. fumigatus, showing conservation of Ras GAP function between species [89,90,123]. Although these findings support conserved control mechanisms for fungal Ras proteins, we still do not have a strong understanding of how Ras protein interactions may contribute to the regulation of Ras activity in the setting of the host tissue.

For any approach to be fungal-specific, we will need a better understanding of how Ras structural biology and Ras pathway interactions differs from that of humans. This will undoubtedly strengthen our ability to specifically target fungal Ras-pathways, with minimal effect on the human host. In addition, a better understanding of the spatiotemporal control of Ras activity during cellular morphogenesis, stress response, and virulence will provide invaluable information with which to build even better models of fungal growth.

Acknowledgements

JRF is supported by NIH/NIAMD Research Scholar Development Award 1 K22 AI89786-01A1.

References

1. Garcia-Ranea JA, Valencia A (1998) Distribution and functional diversification of the ras superfamily in Saccharomyces cerevisiae. FEBS Lett 434: 219-225.
2. Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG (2007) Post-translational modifications and regulation of the Ras superfamily of GTPases as anticancer targets. Nat Rev Drug Discov 6: 541-555.
3. Choy E, Chiu VK, Silletti J, Feoktistov M, Morimoto T, et al. (1999) Endomembrane trafficking of Ras: the CAAX motif targets proteins to the ER and Golgi. Cell 98: 69-80.
4. Schmidt WK, Tam A, Fujimura-Kamada K, Michaelis S (1998) Endoplasmic reticulum membrane localization of Cdc24p and Ste24p, yeast proteases involved in carboxy-terminal CAAX protein processing and amino-terminal a-factor cleavage. Proc Natl Acad Sci USA 95: 11175-11180.
5. Hrycyna CA, Sapperstein SK, Clarke S, Michaelis S (1991) The Saccharomyces cerevisiae STE14 gene encodes a mthyltransferase that mediates C-terminal methylation of a-factor and RAS proteins. EMBO J 10: 1699-1709.
6. Romano JD, Schmidt WK, Michaelis S (1998) The Saccharomyces cerevisiae prenyltransferase carboxyl methyltransferase Ste14p is in the endoplasmic reticulum membrane. Mol Biol Cell 9: 2231-2247.
7. Bartels DJ, Mitchell DA, Dong X, Deschenes RJ (1999) Erf2, a novel gene product that affects the localization and palmitoylation of Ras2 in Saccharomyces cerevisiae. Mol Cell Biol 19: 6775-6787.
8. Hancock JF, Magee AI, Childs JE, Marshall CJ (1989) All ras proteins are palmitoylated but only some are palmitoylated. Cell 57: 1167-1177.
9. Rocks O, Gerauer M, Vartak N, Koch S, Huang ZP, et al. (2010) The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. Cell 141: 458-471.
10. Roy S, Plowan S, Robbiat B, Prior IA, Muncke C, et al. (2005) Individual palmitoyl residues serve distinct roles in H-ras Trafficking, microlocalization, and signalling. Mol Cell Biol 25: 6722-6733.
11. Hancock JF, Paterson H, Marshall CJ (1990) A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21ras to the plasma membrane. Cell 63: 133-139.

12. Camp LA, Hofmann SL (1993) Purification and properties of a palmitoyl-protein thioesterase that cleaves palmitate from H-Ras. J Biol Chem 268: 22566-22574.

13. Goodwin JS, Drake KR, Rogers C, Wright L, Lippincott-Schwartz J, et al. (2005) Depalmitoylated Ras traffics to and from the Golgi complex via a nonvesicular pathway. J Cell Biol 170: 261-272.

14. Wolfe KH, Shields DC (1997) Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387: 708-713.

15. Mösch HU, Kübler E, Krauppmann S, Fink GR, Braus GH (1999) Crossstalk between the Ras2p-controlled mitogen-activated protein kinase and cAMP pathways during invasive growth of Saccharomyces cerevisiae. Mol Biol Cell 10: 1325-1335.

16. Jiang Y, Davis C, Bretscher A (2001) Ras Regulates the polarity of the yeast actin cytoskeleton through the stress response pathway. Mol Biol Cell 12: 1541-1555.

17. Masuda T, Kariya K, Shinkai M, Okada T, Kataoka T (1995) Protein kinase Byr2 is a target of Ras1 in the fission yeast Saccharomyces pombe. J Biol Chem 270: 1979-1982.

18. Wang Y, Xu HP, Riggs M, Rodgers L, Wigler M (1991) byr2, a Schizosaccharomyces pombe gene encoding a protein kinase capable of partial suppression of the ras1 mutant phenotype. Mol Cell Biol 11: 3554-3563.

19. Chen CR, Li YC, Chen J, Hou MC, Papadaki P, et al. (1999) Moe1, a conserved protein in Schizosaccharomyces pombe, interacts with a Ras effector, Scd1, to affect proper spindle formation. PNAS 96: 517-522.

20. Onken B, Wiener H, Philips MR, Chang EC (2006) Compartmentalized signaling of Ras in fission yeast. Proc Natl Acad Sci U S A 103: 9045-9050.

21. Kidd SE, Hagen F, Tscharke RL, Huyhn M, Bartlett KH, et al. (2004) A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). PNAS 101: 17258-17263.

22. Kwon-Chung KJ, and Popkin TJ (1976) Ultrastructure of septal complex in Filobasidiella neoformans (Cryptococcus neoformans). J Bacteriol 126: 524-528.

23. Wickes BL, Mayorga ME, Edman U, Edman JC (1996) Dimorphism and haploid flowering in Cryptococcus neoformans: association with the alpha-mating type. Proc Natl Acad Sci U S A 93: 7327-7331.

24. Lengeler KB, Davidson RC, D’souza C, Harashima T, Shen WC, et al. (2000) Signal Transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64: 746-785.

25. Alspaugh JA, Cavallo LM, Perfect JR, Heitman J (2000) Ras1 regulates filamentation, mating and growth at high temperature of Cryptococcus neoformans. Mol Microbiol 36: 352-365.

26. Tanaka K, Nambu H, Katoh Y, Kai M, Hidaoka Y (1999) Molecular cloning of homologs of RAS and RHO1 genes from Cryptococcus neoformans. Yeast 15: 1133-1139.

27. Waugh MS, Nichols CB, DeCesare CM, Cox GM, Heitman J, et al. (2002) Ras1 and Ras2 contribute shared and unique roles in physiology and virulence of Cryptococcus neoformans. Microbiology 148: 191-201.

28. Haynes BC, Skowrya ML, Spencer SJ, Gish SR, Williams M, et al. (2011) Toward an Integrated Model of Capsule Regulation in Cryptococcus neoformans. PLoS Pathog 7: e1002411.

29. Zaragoza O, Fries BC, Casadevall A (2003) Induction of Capsule Growth in Cryptococcus neoformans by mammalian serum and CO2. Infect Immun 71: 6152-6164.

30. Apidianakis Y, Rahme LG, Heitman J, Ausubel FM, Calderwood SB, et al. (2004) Challenge of Drosophila melanogaster with Cryptococcus neoformans and Role of the innate immune response. Eukaryot Cell 3: 413-419.

31. Mylonakis E, Ausubel FM, Perfect JR, Heitman J, Calderwood SB (2002) Killing of Caenorhabditis elegans by Cryptococcus neoformans as a model of yeast pathogenesis. Proc Natl Acad Sci U S A 99: 15675-15680.

32. Mylonakis E, Moreno R, El Khoury JB, Idnurm A, Heitman J, et al. (2005) Galleria melonella as a model system to study Cryptococcus neoformans pathogenesis. Infect Immun 73: 3842-3850.

33. Nadin-Davis SA, Nasim A, Beach D (1986) Involvement of ras in sexual differentiation but not in growth control in fission yeast. EMBO J 5: 2963-2971.

34. Kwon-Chung KJ (1976) Morphogenesis of Filobasidiella neoformans, the sexual state of Cryptococcus neoformans. Mycologia 68: 821-833.

35. Waugh MS, Vailla MA, Heitman J, Andrew Alspaugh JA (2003) Ras1 controls pheromone expression and response during mating in Cryptococcus neoformans. Fungal Genet Biol 38: 110-121.

36. Chung S, Karos M, Chang YC, Lukasz J, Wikes BL, et al. (2002) Molecular analysis of CPRalpha, a MATalpha-specific pheromone receptor gene of Cryptococcus neoformans. Eukaryot Cell 1: 432-439.

37. Davidson RC, Moore TDE, Odom AR, Heitman J (2000) Characterization of the MFA1 homolog of the human fungal pathogen cryptococcus neoformans. Mol Microbiol 38: 1017-1026.

38. Wang P, Perfect JR, Heitman J (2000) The G-Protein beta subunit GPB1 is required for mating and haploid flowering in Cryptococcus neoformans. Mol Cell Biol 20: 352-362.

39. Vailla MA, Nichols CB, Fernandes L, Cramer KL, Alspaugh JA (2005) A Rac homolog functions downstream of Ras1 to control hyphal differentiation and high-temperature growth in the pathogenic fungus Cryptococcus neoformans. Eukaryot Cell 4: 1066-1078.
55. Ballou ER, Nichols CB, Miglia KJ, Kozubowski L, Alspaugh JA (2010) Two CDC42 paralogues modulate Cryptococcus neoformans thermotolerance and morphogenesis under host physiological conditions. Mol Microbiol 75: 763-780.

56. Nichols CB, Perfect ZH, Alspaugh JA (2007) A Ras1-Cdc24 signal transduction pathway mediates thermotolerance in the fungal pathogen Cryptococcus neoformans. Mol Microbiol 63:1116-1130.

57. Kozubowski L, Heltman J (2010) Septins enforce morphogenetic events during sexual reproduction and contribute to virulence of Cryptococcus neoformans. Mol Microbiol 75: 658-675.

58. Shen G, Whittington A, Wang P (2011) Wsp1, a GBD/CRIB Domain-Containing WASP homolog, is required for growth, morphogenesis, and virulence of Cryptococcus neoformans. Eukaryot Cell 10: 521-529.

59. Shen G, Zhou E, Alspaugh JA, Wang P (2012) Wsp1 is downstream of Cin1 and regulates vesicle transport and actin cytoskeleton as an effector of Cdc42 and Rac1 in Cryptococcus neoformans. Eukaryot Cell 11: 471-481.

60. Nichols CB, Ferreyra J, Ballou ER, Alspaugh JA (2009) Subcellular localization directs signaling specificity of the Cryptococcus neoformans Ras1 protein. Eukaryot Cell 8: 181-189.

61. Sudbery PE (2011) Growth of Candida albicans hyphae. Nat Rev Micro 9: 737-748.

62. Cutler JE (1991) Putative virulence factors of Candida albicans. Ann Rev Microbiol 45: 187-218.

63. Leberer E, Ziegelbauer K, Schmidt A, Harcus D, Dignard D, et al. (1997) Virulence and hyphal formation of Candida albicans require the Ste20p-like protein kinase CaClp4. Curr Biol 7: 539-546.

64. Feng Q, Summers E, Guo B, Fink G (1999) Ras signaling is required for serum-induced hyphal differentiation in Candida albicans. J Bacteriol 181: 6339-6346.

65. Leberer E, Harcus D, Dignard D, Johnson L, Ushinsky S, et al. (2001) Ras links cellular morphogenesis to virulence by regulation of the MAP kinase and cAMP signalling pathways in the pathogenic fungus Candida albicans. Mol Microbiol 42: 673-687.

66. Lohse MB, Johnson AD (2009) White–opaque switching in Candida albicans. Curr Opin Microbiol 12:650-654.

67. Miller MG, Johnson AD (2002) White-Opaque switching in Candida albicans is controlled by mating-type Locus homeodomain proteins and allows efficient mating. Cell 110: 293-302.

68. Huang G, Srikantha T, Sahni N, Yi S, Soll DR (2009) CO2 regulates white-to-opaque switching in Candida albicans. Mol Microbiol 75: 862-875.

69. Huang G, Yi S, Sahni N, Daniels KJ, Srikantha T, et al. (2010) N-Acetylglucosamine induces white to opaque switching, a mating prerequisite ahead of print.

70. Xie J, Du H, Guan G, Tong Y, Kourkoumpetis TK, et al. (2012) N-Acetylglucosamine induces White-to-Opaque Switching and Mating in Candida tropicalis, providing new insights into adaptation and fungal sexual evolution. Eukaryot Cell 11: 773-782.

71. Cho T, Hamnatake H, Kaminishi H, Hagihara Y, Watanabe K (1992) The relationship between cyclic adenosine 3′,5′-monophosphate and morphology in exponential phase Candida albicans. J Med Vet Mycol 30: 35-42.

72. Rocha CRC, Schröppel K, Harcus D, Marci C, Dignard D, et al. (2001) Signaling through adenyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus Candida albicans. Mol Cell Biol 12: 3631-3643.

73. Hogan DA, Sundstrom P (2009) The Ras/cAMP/PKA signaling pathway and virulence in Candida albicans. J Gen Virol 90: 1104-1112.

74. Phillips AJ, Crowe JD, Ramsdale M (2006) Ras pathway signaling accelerates programmed cell death in the pathogenic fungus Candida albicans. Proc Natl Acad Sci USA 103: 726-731.

75. Ikeda R, Sawamura K (2008) Bacterial and H2O2 stress-induced apoptosis-like events in Cryptococcus neoformans. Res Microbiol 159: 628-634.

76. Ramsdale M (2008) Programmed cell death in pathogenic fungi. Biochim Biophys Acta 1738:1369-1380.

77. Riches DL, Miley MD, Bhabhra R, Robson GD, Rhodes JC, et al. (2007) The Aspergillus fumigatus metacaspases CasA and CasB facilitate growth under conditions of endoplasmic reticulum stress. Mol Microbiol 63: 591-604.

78. Zhu Y, Fang HM, Wang YM, Zeng GS, Zheng XD, et al. (2009) Ras1 and Ras2 play antagonistic roles in regulating cellular cAMP level, stationary-phase entry and stress response in Candida albicans. Mol Microbiol 74: 862-875.

79. Bassilana M, Blyth J, Arkowitz RA (2003) Cdc24, the GDP-GTP exchange factor for Cdc42, is required for invasive hyphal growth of Candida albicans. Eukaryot Cell 2: 9-18.

80. VandenBerg AL, Ibrahim AS, Edwards JE Jr, Toennes KA, Johnson DI (2004) Cdc42p-GTPase regulates the budded-to-hyphal-form transition and expression of hypha-specific transcripts in Candida albicans. Eukaryot Cell 3: 724-734.

81. Martin R, Walther A, Wendland J (2005) Ras1-induced hyphal development in Candida albicans requires the formin Bir1. Eukaryot Cell 4: 1712-1724.

82. Bassilana M, Arkowitz RA (2006) Rac1 and Cdc42 have different roles in Candida albicans development. Eukaryot Cell 5: 321-329.

83. Hope H, Bogliolo S, Arkowitz RA, Bassilana M (2008) Activation of Rac1 by the guanine nucleotide exchange factor Dock1 is required for invasive filamentous growth in the pathogen Candida albicans, Mol Biol Cell 19: 3638-3651.

84. Hope H, Schmauch C, Arkowitz RA, Bassilana M (2010) The Candida albicans ELMO homologue functions together with Rac1 and Dock1, upstream of the MAP Kinase Cek1, in invasive filamentous growth. Mol Microbiol 76: 1572-1590.

85. Piispanen AE, Bonnefoi O, Carden S, Deaveu A, Bassilana M, et al. (2011) Roles of Ras1 membrane localization during Candida albicans hyphal growth and farnesol response. Eukaryot Cell 10: 1473-1484.

86. Momany M, Taylor I (2000) Landmarks in the early duplication cycles of Aspergillus fumigatus and Aspergillus nidulans: polarity, germ tube emergence and septation. Microbiology 146: 3279-3284.

87. Ben-Ami R, Lewis RE, Kontoyiannis DP (2010) Enemy of the (immunosuppressed) state: an update on the pathogenesis of Aspergillus fumigatus infection. Br J Haematol 150: 406-417.

88. Fortwendel JR, Fuller KK, Stephens TJ, Bacon WC, Askew DS, et al. (2008) Aspergillus fumigatus RasA regulates asexual development and cell wall integrity. Eukaryot Cell 7: 1530-1539.

89. Fortwendel JR, Juvvadi PR, Rogg LE, and Steinbach WJ (2011) Regulatable Ras activity is critical for proper establishment and maintenance of polarity in Aspergillus fumigatus. Eukaryot Cell 10: 611-615.

90. Fortwendel JR, Panepinto JC, Seltz AE, Askew DS, Rhodes JC (2004) Aspergillus fumigatus rasA and rasB regulate the timing and morphology of asexual development. Fung Genet Biol 41: 129-139.

91. Fortwendel JR, Juvvadi PR, Rogg LE, Askew Y, Burns KA, et al. (2012) Plasma membrane localization is required for RasA-mediated polarized morphogenesis and virulence of Aspergillus fumigatus. Eukaryot Cell: [Epub ahead of print].

92. Fortwendel JR, Juvvadi PR, Pinchai N, Perfect BZ, Alspaugh JA, et al. (2009) Differential Effects of Inhibiting Chitin and 1,3-β-D-Glucan Synthesis in Aspergillus fumigatus. Antimicrob Agents Chemother 53: 476-482.

93. Osherov N, May G (2000) Comitnd Germination in Aspergillus nidulans requires RAS Signaling and protein synthesis. Genetics 155:647-656.

94. Som T, Kolaparthi VS (1994) Developmental decisions in Aspergillus nidulans are modulated by Ras activity. Mol Cell Biol 14: 5333-5348.

95. Boyce KJ, Hynes MJ, Andrianopoulos A (2005) The Ras and Rho GTPases require Ras activity in Aspergillus nidulans: polarity, germ tube emergence and septation. Microbiology 146: 3279-3284.

96. Deveau A, Piispanen AE, Jackson AA, Hogan DA (2010) Farnesol Induces Hydrogen Peroxide Resistance in Candida albicans Yeast by Inhibiting the Ras-Cyclic AMP Signaling Pathway. Eukaryot Cell 9: 569-577.

97. Harcus D, Nantel A, Marci C, Rigby T, Whiteway M (2004)
Profiling of Cyclic AMP Signaling in Candida albicans. Mol Biol Cell 15: 4490-4499.

100. Latgé JP (2007) The cell wall: a carbohydrate armour for the fungal cell. Mol Microbiol 66: 279-290.

101. Maeng S, Ko YJ, Kim GB, Jung KW, Floyd A, et al. (2010) Comparative Transcriptome Analysis Reveals Novel Roles of the Ras and Cyclic AMP Signaling Pathways in Environmental Stress Response and Antifungal Drug Sensitivity in Cryptococcus neoformans. Eukaryot Cell 9: 360-378.

102. Fuller KK, Richie DL, Feng X, Krishnan K, Stephens TJ, et al. (2011) Divergent Protein Kinase A isozymes co-ordinately regulate conidial germination, carbohydrate metabolism and virulence in Aspergillus fumigatus. Mol Microbiol 75: 1045-1056.

103. Fuller KK, Zhao W, Askew DS, and Rhodes JC (2009) Deletion of the Protein Kinase A Regulatory Subunit Leads to Deregulation of Mitochondrial Activation and Nuclear Duplication in Aspergillus fumigatus. Eukaryot Cell 8: 271-277.

104. Grosse C, Heiniekamp T, Kniemeyer O, Gehrke A, Brakhage AA (2008) Protein Kinase A Regulates Growth, Sporulation, and Pigment Formation in Aspergillus fumigatus. Appl Environ Microbiol 74: 4923-4933.

105. Liebmann B, Müller M, Braun A, Brakhage AA (2004) The Cyclic AMP-Dependent Protein Kinase A Network Regulates Development and Virulence in Aspergillus fumigatus. Infect Immun 72: 5193-5203.

106. Zhao W, Panepinto JC, Fortwendel JR, Fox L, Oliver BG, et al. (2006) Deletion of the Regulatory Subunit of Protein Kinase A in Aspergillus fumigatus Alters Morphology, Sensitivity to Oxidative Damage, and Virulence. Infect Immun 74: 4865-4874.

107. Fillinger S, Chaverroche M-K, Shimizu K, Keller N, D’Enfert C (2002) cAMP and ras signalling independently control spore germination in the filamentous fungus Aspergillus nidulans. Mol Microbiol 44: 1001-1016.

108. Li H, Barker BM, Grahl N, Puttkamonkul S, Bell JD, et al. (2011) The Small GTPase RasA Mediates Intracellular Reactive Oxygen Species Production, Polarized Growth, and Virulence in the Human Fungal Pathogen Aspergillus fumigatus. Eukaryot Cell 10: 174-186.

109. Semighini CP, Harris SD (2008) Regulation of apical dominance in Aspergillus nidulans hyphae by reactive oxygen species. Genetics 179: 1919-1932.

110. Virag A, Lee MP, Si H, Harris SD (2007) Regulation of hyphal morphogenesis by cdol2 and rac1 homologues in Aspergillus nidulans. Mol Microbiol 66: 1579-1586.

111. Vallim LA, Fernandes L, Alspaugh JA (2004) The RAM1 gene encoding a protein-farnesytransferase β-subunit homologue is essential in Cryptococcus neoformans. Microbiology 150: 1025-1035.

112. Appels NM, Beijnen JH, Schellens JHM (2005) Development of Farnesyl Transferase Inhibitors: A Review. Oncologist 10: 565-578.

113. Downward J (2003) Targeting Ras signalling pathways in cancer therapy. Nat Rev Cancer 3: 11-22.

114. Manandhar SP, Hildebrandt ER, Schmidt WK (2007) Small-Molecule Inhibitors of the Rho1p CaaX Protease. J Biol Chem 282: 983-993.

115. Winter-Vann AM, Kamen BA, Bergo MO, Young SG, Meilyk S, et al. (2003) Targeting Ras signaling through inhibition of carboxyl methylation: An unexpected property of methotrexate. Proc Natl Acad Sci USA 100: 6529-6534.

116. Hast MA, Nichols CB, Armstrong SM, Kelly SM, Hellinga HW, et al. (2011) Structures of Cryptococcus neoformans Protein Farnesyltransferase Reveal Strategies for Developing Inhibitors That Target Fungal Pathogens. J Biol Chem 286: 35149-35162.

117. Shareck J, Nantel A, Belhumeur P (2011) Conjugated Linoleic Acid Inhibits Hyphal Growth in Candida albicans by Modulating Ras1p Cellular Levels and Downregulating TEC1 Expression. Eukaryot Cell 10: 565-577.

118. Su Z, Li H, Li Y, Ni F (2007) Inhibition of the Pathogenically Related Morphologic Transition in Candida albicans by Disrupting Cdc42 Binding to Its Effectors. Chem Biol 14: 1273-1282.

119. Margolis B, Skolnik EY (1984) Activation of Ras by receptor tyrosine kinases. J Am Soc Nephrol 5: 1288-1299.

120. Papadaki P, Pizov Z, Onken B, Chang EC (2002) Two Ras Pathways in Fission Yeast Are Differentially Regulated by Two Ras Guanine Nucleotide Exchange Factors. Mol Cell Biol 22: 4598-4606.

121. Colombo S, Ma P, Cauwenberg L, Winderlick J, Crauwels M, et al. (1998) Involvement of distinct G-proteins, Gpa2 and Ras, in glucose- and intracellular acidification-induced cAMP signalling in the yeast Saccharomyces cerevisiae. EMBO J 17: 3326-3341.

122. Colombo S, Ronchetti D, Thvelevim J, Winderlick J, Martegani E (2004) Activation State of the Ras2 Protein and Glucose-induced Signaling in Saccharomyces cerevisiae. J Biol Chem 279: 46715-46722.

123. Harispe L, Portela C, Saccocchio C, Peñalva MA, Gorfinkel L (2008) Ras GTPase-Activating Protein Regulation of Actin Cytoskeleton and Hyphal Polarity in Aspergillus nidulans. Eukaryot Cell 7: 141-153.