

Lipid Signaling in Pathogenic Fungi

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Key Words

farnesol, prostaglandin, *Cryptococcus*, sphingolipid, *Aspergillus*, *Candida*

Abstract

Lipid signaling in pathogenic fungi has been studied to determine the role of these pathways in fungal biology and human infections. Owing to their unique nature, they may represent targets for future antifungal treatments. Farnesol signaling was characterized as a quorum-sensing molecule, with exposure inhibiting filamentation. Research has shown involvement in both the Ras1-adenylate cyclase and MAP kinase pathways. In species of *Aspergillus*, farnesol exposure induces apoptosis-like changes and alterations in ergosterol synthesis. Eicosanoid production has been characterized in several pathogenic fungi, utilizing host lipids in some cases. The role in virulence is not known yet, but it may involve modulation of host lipids. Sphingolipid signaling pathways seem to center around the production of diacylglycerol in the formation of inositol phosphorylceramide. Diacylglycerol activates both melanin production through laccase and transcription of antiphagocytic protein, both of which are involved in virulence.

Contents

INTRODUCTION	120
FARNESOL SIGNALING	120
EICOSANOID SIGNALING	124
SPHINGOLIPID SIGNALING	125
CONCLUSIONS	127

INTRODUCTION

Pathogenic fungi such as *Candida*, *Aspergillus*, and *Cryptococcus* continue to be a significant medical issue worldwide. The search for more effective and specific therapies for these infections continues to this day. Among the pathways and systems examined for potential new drug targets, lipid signaling has emerged as an attractive and interesting field. The lipid signaling pathways involving farnesol (FA), eicosanoids, and sphingolipids in these fungi have been the best characterized to date.

FARNESOL SIGNALING

One major player in lipid signaling pathways of pathogenic fungi is FA, a 15-carbon oxygenated lipid made up of isoprene moieties. FA has the distinction of being the first quorum-sensing molecule identified in eukaryotes. Hornby et al. (26) reported that a lipid-soluble molecule released into the media was responsible for inoculum size-dependent growth changes in *Candida albicans*, characterized by a reduction in yeast-to-mycelia transitions. Further evaluations of FA-related phenotypes revealed that both biofilm formation and hyphal development are suppressed in *C. albicans*. This phenomenon may not be limited to *C. albicans*, as there is evidence that FA reduces hyphal formation in *C. dubliniensis* (23) and alters gene expression and cell polarization in *C. parapsilosis* (55). FA secretion and biofilm formation have been documented for eight *Candida* species under a variety of conditions (66), although *C. albicans* seems to have the highest FA secretion.

FA-mediated hyphal blockage was evaluated under a variety of different conditions, including several defined media and serum-containing conditions (43). *C. albicans* grown in all defined media tested showed similar FA-related reduction in germ tube formation, with about 1 μ M of FA needed to show a 50% decrease. FA susceptibility was greatly reduced in the presence of serum (albumin), implying that the dynamics of FA signaling in relation to pathophysiology may be more complex than in vitro studies suggest. Mosel et al. (43) also showed that FA had little effect on existing germ tubes, suggesting the main role of FA is prevention of formation initiation. In addition to the effects on hyphal formation, FA is partially protective against oxidative stress (67), but not in a manner that induces classical antioxidant genes such as catalase or superoxide dismutases.

A potential link between FA production and pathogenicity was alluded to by Navarathna et al. (45), who both increased FA production and enhanced virulence of *C. albicans* in mouse models of infection when the fungus was pretreated with low concentrations of azoles. Azoles are a class of antifungal drugs that inhibits the synthesis of ergosterol, a major component of fungal membranes. This azole-induced production of FA had been seen previously when it was treated with a variety of different azoles (27). Farnesyl pyrophosphate (FPP) is a common precursor to the synthesis of both FA and ergosterol, the major sterol component of fungal membranes. Because azole drugs disrupt ergosterol synthesis after the formation of FPP, treatment with azoles leads to an accumulation of FPP that then leads to an increased production of FA.

Since this observation, several aspects of virulence have been examined with respect to FA. Navarathna et al. (46) created a mutant strain of *C. albicans* with reduced endogenous FA production and showed that this strain had reduced virulence in mouse models compared with wild-type and reconstituted strains. Additionally, exogenous FA administration increased murine mortality (46) and altered host cytokine production (46, 47, 58)

Farnesol (FA):

isoprenoid lipid involved in quorum sensing in *Candida* and apoptosis in *Aspergillus*

Eicosanoids: a class of lipids derived from fatty acids like arachidonic acid that are involved in inflammation and immunomodulation in humans

Sphingolipids: a class of lipids including species with N-linked acylation of a sphingosine backbone and several variations of head groups

during infection. Shchepin et al. (58) looked at synthetic compounds comparable to FA in quorum-sensing attributes in relation to mouse models of *C. albicans* infection. They showed that although the synthetic compounds varied in their function as virulence factors, neither of the compounds tested protected mice from infection. This could imply a strict structural requirement of these molecules for function. Taken together, these data further support the link between FA and *Candida* pathogenesis and highlight the need for future investigations into the exploitation of this molecular process for therapies.

The various effects of FA on *Candida* are clear, but what signaling pathways underlie these phenotypes? The answer to this question may be the key to understanding the complex role of FA in the life cycle and pathogenesis of this fungus. Some clues to these processes come from microarray analyses. Cao et al. (6) looked at gene expression in *C. albicans* biofilms inhibited by FA exposure. They found that along with genes involved in processes such as drug resistance and heat shock, the expression of several genes associated with hyphal formation was altered when exposed to FA. Genes of interest in this group include *TUP1* (dTMP uptake), *PDE2* (phosphodiesterase), and *CRK1* (Cdc2-related kinase). *Tup1* is a transcriptional repressor that is upregulated in the presence of FA, whereas *CRK1* and *PDE2* are downregulated. *TUP1* downregulation is associated with hyperfilamentation, while *PDE2* and *CRK1* have been hypothesized to positively effect filamentation. Taken together, FA affects the expression of these genes in a manner consistent with the effect of FA treatment on hyphal formation. Enjalbert et al. (14) studied effects of FA on gene expression profiles in strains of *C. albicans* that had resumed growth after stationary phase upon transfer to fresh media. In these cases, FA delayed this rebound of growth and changed the expression of genes that are normally affected (positively or negatively) during hyphal formation. Among the affected genes are cyclin genes *HGCI*, *CLN3*, and *PCL2* and histone genes. For example, *HGCI* is

necessary for hyphal development (69) and the microarray data show that FA exposure downregulates *HGCI*. Another group examined the expression profile of *C. albicans* genes in the early yeast-to-hyphae transition period when grown in nonenriched media (8). They found that many genes affected by FA are also affected by high cell density, which would be expected from a quorum-sensing molecule. In general, they found that under these conditions expression profiles were similar to those seen in studies of gene expression *C. albicans* following phagocytosis (38).

Although important to the molecular biology of signaling, gene expression alone cannot determine the specific roles of factors in the cellular processes underlying FA signaling. Further examination of some of the genes/proteins indicated in the microarray studies has yielded interesting results. *Tup1* acts globally to repress hyphal formation (3). *Tup1* interacts with other proteins such as *Tcc1* (30) that function as corepressors after complexing with DNA binding proteins such as *Nrg1* (5) and *Rfg1* (29, 33), which are homologous to *Saccharomyces cerevisiae* proteins *Nrg1p* and *Rox1p*, respectively. When *TUP1* is deleted, the strain shows constitutive filamentous growth with an inability to grow as yeast (3). Furthermore, activation of *Tup1* and related factors results in a downregulation of genes that are involved in filament formation (4, 44). *Nrg1* is a repressor of filament growth, and deletions of *NRG1* have phenotypes similar to *TUP1* deletions (5). *nrg1*^{-/-} mutants show no virulence in mouse models of candidiasis. Another interesting aspect of the study examines hyphal genes that are affected by *Nrg1*, with *Tup1*-controlled genes *ECE1* and *HWPI* (4, 64) constitutively expressed in the *nrg*^{-/-} mutant. *RFGI* deletion showed a phenotype similar to the *nrg*^{-/-}, and the double mutant showed colonies with a more pronounced wrinkled appearance than did the singular deletions. Examinations of the link between FA, *Tup1*, and these related DNA binding proteins have shown promising results. Kebaara et al. (31) recently established this link by examining the response of various knockout mutants to

Hyphae: long branching filamentous cells in fungi and the major type of vegetative growth

AC: adenylate cyclase

MAP: mitogen-activated protein

FA. The *tup1*^{-/-} and *nrg*^{-/-} mutants do not exhibit a morphological response to FA, whereas the *rfg1*^{-/-} mutant does respond to FA. As seen before, treatment with FA increases *TUPI* expression with a concomitant decrease in hyphae-specific genes such as *HWPI* and *RBT1*. Interestingly, both *tup1*^{-/-} and *nrg1*^{-/-} mutants overproduced FA up to 19 times more than wild type did. The implications of this finding suggest a possible feedback mechanism between *Tup1*, *Nrg1*, and the production of FA.

Other pathways seem to be involved in FA-related changes in the morphology of *C. albicans*. Ras1, a GTPase, activates the enzyme adenylate cyclase (AC), which produces the common second messenger cyclic-AMP (cAMP). cAMP activates protein kinase A (PKA), which phosphorylates many downstream factors. One of these is a transcription factor called Efg1, which induces the morphological transition to hyphae (18, 35). Davis-Hanna et al. (12) established that this Ras1-AC-PKA-Efg1 pathway is inhibited by FA and rescued from that inhibition in an Efg1-dependent manner upon addition of a cAMP analog, dibutyryl-cAMP.

There is evidence that FA influences mitogen-activated protein (MAP) kinase cascades through reduced expression of genes such as *HST7* (a MAP kinase kinase) and *CPHI* (a transcription factor involved in hyphal formation) upon treatment, and the reduced cascade activity was measured indirectly by showing general amino acid permease 1 (GAP1) (56). Because Ras1 is a common factor of the MAP kinase pathway and the PKA-Efg1 pathway, it is possible that this is the molecule that FA acts upon. A summary of factors in farnesol signaling in *C. albicans* can be found in **Figure 1**.

Another kinase gene involved in FA response and quorum sensing in *C. albicans* is *CHK1*, which encodes a histidine kinase. The deletion of *CHK1* led to a strain (*chk1*^{-/-}) that was unresponsive to FA treatment even at higher doses, whereas deletion of other histidine kinases such as *Shn1p* and *Nik1p* produced strains that responded to FA normally (34). This is possibly connected to previous findings in which *CHK1*

interacts with MAP kinase, one of its downstream regulators, or another yet unidentified mediator. *Chk1* does not appear to be involved in quorum-related antifungal resistance (52).

The focus of FA signaling thus far has been on *C. albicans*, but other pathogenic fungi have shown responses to FA as well. Recently, biofilm formation in *Pneumocystis carinii* has been described and is inhibited by exposure to FA in this pathogenic fungus as well (11). Of note, some *Aspergillus* species have shown various effects upon FA treatment. FA does not affect the hyphal morphology of *A. nidulans*, but it does inhibit conidiation of *A. niger* (37). Along with this conidiation inhibition, FA treatment decreases the intracellular levels of cAMP, which is produced by AC. Treatment with inhibitors of AC induces a phenotype similar to that induced by FA, which suggests that cAMP may be involved in the mechanism of reduced conidiation. *A. nidulans* does show an interesting phenotype upon FA treatment, which is consistent with morphological changes associated with apoptosis (57). This phenotype includes DNA fragmentation and nuclear condensation. Recent studies have further examined this phenotype and found a decrease in mRNA transcripts for proteins involved in transcription, translation, ergosterol biosynthesis, and ribosomal biogenesis along with an increase in transcripts for mitochondrial proteins. These studies found that treatment with FA induces autophagy and mitochondrial fragmentation. Ergosterol biosynthesis in *A. nidulans* seems to be affected, as evidenced by a punctate distribution of filipin staining (fluorescent compound that binds ergosterol) as opposed to the uniform distribution of untreated fungi. Because the studies of ergosterol polarization have involved sphingolipid-rich lipid rafts in *C. albicans* (41), it would be worthwhile to further explore the links between FA exposure and characteristics of lipid raft domains. Of interest to signaling studies is the observation that the protein kinase C (PKC)-deficient strain of *A. nidulans* (*calc2*) showed resistance to FA treatment. Some studies have shown links between PKC and autophagy (51), though not in *Aspergillus*. Could

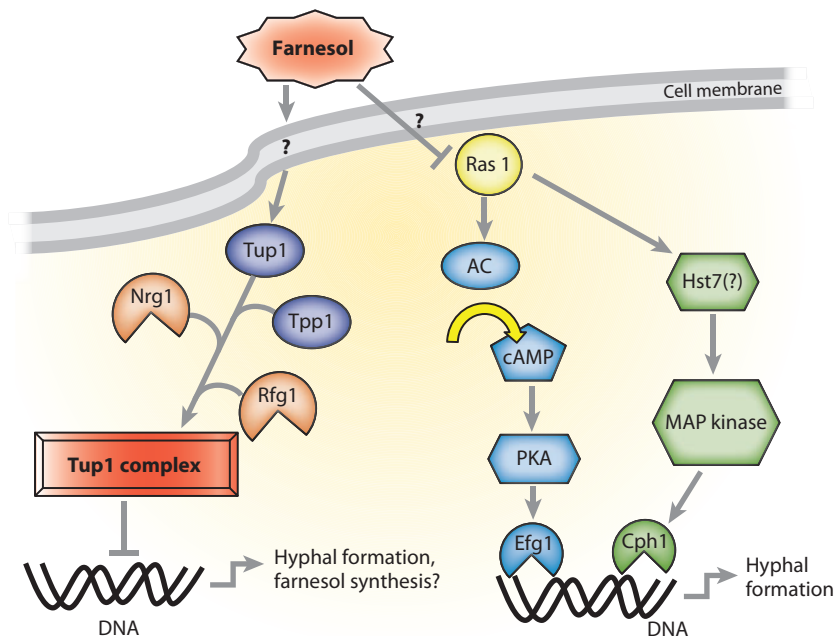


Figure 1

Known and hypothetical components of farnesol signaling in *Candida albicans*. Upon treatment with farnesol, Tup1 global transcriptional repressor associates with Tpp1, Nrg1, and Rfg1 to form a DNA binding complex. This complex inhibits gene expression of proteins involved in hyphal formation and may play a role in farnesol feedback pathways. Additionally, farnesol affects components of AC and MAP kinase signaling pathways. Farnesol may act through a common component, Ras, and inhibit those cascades that eventually lead to transcription of factors in hyphal formation. Abbreviations: AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; MAP, mitogen-activated protein; PKA, protein kinase A.

these FA-related observations be explained by a common cause involving PKC?

One notable aspect of the research described above relating to *Aspergillus* species and FA is that *Aspergillus* species do not seem to produce detectable amounts of FA. This could mean that the phenotypes described are laboratory artifacts and that these phenomena do not occur in nature. More likely is the idea that FA, in addition to quorum sensing, is produced by *Candida* to compete with other organisms such as *Aspergillus*. This idea adds a different dimension to the FA studies, implying a complex system of regulating not only the growth of *Candida* itself but also its competition with other fungi and microbes for resources. The FA-mediated interaction between *Candida* and *Aspergillus* is not the only documented case of different microorganisms interacting through FA. *Pseudomonas*

aeruginosa is a gram-negative bacteria that often coexists with *Candida* species in clinical situations such as cystic fibrosis. FA inhibits swarming motility in *P. aeruginosa* (42) and reduces the production of pyocyanin, a known virulence factor (10).

In summary, many signaling genes and factors influenced by FA lead to the morphological changes observed. Although it can seem daunting, important common factors that appear in these studies suggest an overall theme of signaling and phenotype. The Ras1-cAMP connection appears to be common not only in *Candida*, but also in several pathways implicated in the FA response, including *Aspergillus*. In the past few years, significant advancements have furthered our understanding of this signaling molecule. Future research will revolve around the mechanism by which FA activates these pathways,

PGE₂: prostaglandin E₂

COX: cyclooxygenase

alternative pathways involved in these morphological changes, the role of FA in complex microbial interactions, and whether FA can be exploited therapeutically.

EICOSANOID SIGNALING

Although a major player in fungal lipid signaling, FA is not the only lipid molecule that affects signaling pathways in fungi. Eicosanoids are one such family of lipids that plays roles in a number of pathogenic fungi. Eicosanoids are 20-carbon-long oxygenated lipids derived from fatty acids such as arachidonic acid. Included in the family of eicosanoids are groups of lipids, such as prostaglandins and leukotrienes, that have various effects related to immune function and inflammation in humans.

Noverr et al. (49) first reported the occurrence of prostaglandins and leukotrienes in culture supernatants of *Cryptococcus neoformans* and *C. albicans*. Synthesis of the prostaglandins appeared to be from both de novo pathways and salvage of precursors (such as arachidonic acid) from the media. However, Wright et al. (68) confirmed that there is no arachidonic acid produced endogenously by *Cryptococcus*. *C. albicans* takes up arachidonic acid (13). Other pathogenic fungi synthesize these eicosanoids. Noverr et al. (50) showed that several different strains and species of pathogenic fungi produce both leukotrienes and prostaglandins in culture. This production was dramatically increased when arachidonic acid was added to culture media. Future studies confirmed that the prostaglandins purified from *C. neoformans* (15) and *C. albicans* (17) were of the prostaglandin E₂ (PGE₂) subtype. Once purified, these prostaglandins exhibited effects on mammalian cells (including cytokine modulation) similar to effects exhibited by mammalian PGE₂. Inhibitors of cyclooxygenase (COX), a class of enzymes that synthesize prostaglandins from arachidonic acid, have varied effects on these fungi. *Paracoccidioides brasiliensis* shows reduced production of PGE₂ when treated with COX inhibitors (2). In *C. albicans*, synthesis of PGE₂ is stopped by COX and lipoxygenase inhibitors

that are nonspecific with respect to the isoenzyme targeted (17). However, compounds that specifically inhibit the COX2 isoenzyme do not affect PGE₂ production. Alem et al. (1) found a difference between PGE₂ production by suspended *Candida* and *Candida* associated with a biofilm, with the latter producing more. Both of these conditions showed a block in PGE₂ synthesis upon treatment with nonspecific COX inhibitors. Because *C. albicans* has no homolog to COX enzymes, alternative synthesis strategies were examined. Two such enzymes, the fatty acid desaturase *Ole2* and the copper oxidase *Fet3*, were implicated when deletion of these enzymes produced strains with greatly reduced PGE₂ synthesis. Given that deletion of either enzyme did not completely abolish PGE₂ production, it seems that other synthesis pathways may exist. Taken together, the synthesis of PGE₂ in *C. albicans* might occur through an enzyme with some general structural similarity but little homology to the known COX enzymes. As for the effect of COX inhibitors on *C. neoformans*, the answer is not as clear. Early reports (49) showed that COX inhibitor treatment decreased the amount of PGE₂ in cultures; however, this seemed to result from a decrease in the number of viable cells.

More recent studies (15) have shown that COX inhibitor treatment does not affect cryptococcal production of PGE₂. Like *C. albicans*, *C. neoformans* has no homolog to known COX enzymes. These two observations suggest that an entirely different mechanism of PGE₂ synthesis occurs in these fungi. Erb-Downward et al. (16) followed up on these ideas and found that several polyphenolic chemicals that typically inhibit lipoxygenase activity inhibit production of PGE₂. Laccase, the enzyme in *Cryptococcus* responsible for melanin synthesis, binds phenolic substances and therefore was examined for a role in eicosanoid production. Deletion or inhibition of laccase resulted in a lack of PGE₂ production, but laccase alone was unable to synthesize PGE₂ from either arachidonic acid or prostaglandin H₂. Interestingly, laccase converted prostaglandin G₂ to PGE₂. The multicopper oxidase *Fet3* is homologous

to laccase (17). These findings indicate that we are just beginning to understand prostaglandin production in *Cryptococcus*.

Little is known about the direct effect of PGE₂ on these fungi, though Levitin et al. (36) reported several alterations in the transcription profile of *C. albicans* upon exogenous exposure. Another class of lipids derived from omega-3-polyunsaturated fatty acids like arachidonic acid is the resolvins. In human cells, these compounds are anti-inflammatory and affect migration of neutrophils in later stages of inflammation. Recently, Haas-Stapleton et al. (22) described the synthesis of fungal resolvins in *C. albicans*. The synthesis of these resolvins was inhibited by lipoxygenase inhibitors, and production of these lipids by *C. albicans* affected neutrophil chemotaxis, phagocytosis, and intracellular killing at different concentrations. This finding is more evidence for the role of eicosanoid-derived lipids in fungal modulation of the host immune system.

Phospholipases may also play a role in the eicosanoid signaling story. Phospholipases cleave fatty acid moieties from larger lipid molecules. Freeing arachidonic acid and other eicosanoids precursors from these molecules is the first step toward prostaglandin synthesis. *Cryptococcus* phospholipase (*PLB1*) is cell-wall-bound secreted, and deletion mutants deficient in this gene show cell wall defects (60) and reduced virulence in murine models of cryptococcosis (9). Noverr et al. (48) showed that this strain was deficient in production of certain prostaglandins and leukotrienes when given phospholipid precursors but not when given arachidonic acid directly. Owing to their observations that alveolar macrophages showed growth inhibition of the *plb1* strain upon phagocytosis, they hypothesized that *Plb1* is required for growth inside macrophages. Wright et al. (68) found that the prostaglandins synthesized during the macrophage-*Cryptococcus* interaction were derived from host (macrophage) arachidonic acid. Treatments, such as tipranavir, that reduced production of phospholipases in *Cryptococcus* have shown therapeutic effects on models of cryptococcosis (7). In summary, phos-

pholipases may play an important role in prostaglandin metabolism in pathogenic fungi and should be further evaluated for their role in virulence and host-fungi interactions.

As mentioned, *Candida* and *Cryptococcus* are not the only pathogenic fungi to produce eicosanoids. Genes identified in *Aspergillus nidulans* and *A. fumigatus* that are similar to COX genes also encode fatty acid oxygenases (63). The strains produced when these genes (*ppoA*, *ppoB*, and *ppoC*) were deleted showed deficits in PGE₂ production. Further, a triple deletion mutant lacking all three of the *PPO* genes was resistant to oxidative stress and hypervirulent in a murine model of pulmonary aspergillosis. The authors hypothesize that the host immune system is further activated by the fungal prostaglandins in wild-type *Aspergillus* and that the triple deletion mutant is hypervirulent due to a lack of this activation.

Eicosanoid production and signaling in pathogenic fungi has made interesting advances in recent years. Studies presented here have shown that these oxylipins are produced in many fungi that are pathogenic to humans, and that their production may be involved in the pathogenesis of these fungal infections. Future studies focused on signaling mechanisms in these fungi upon autocrine/paracrine exposure to self-produced eicosanoids would be interesting, especially studies examining more complex effects of these prostaglandins on other commensal microbes. Another aspect of the research that focuses on fungi-host interactions is the idea that the fungi are hijacking host lipids for their own processes. (This phenomenon is not unheard of; see for example research on *Chlamydia trachomatis* in Reference 65). The exact role that these prostaglandins play in pathogen virulence and host response would be crucial for the development of therapies that target these pathways.

SPHINGOLIPID SIGNALING

A class of lipids gaining new prominence in both mammalian and fungal research is the sphingolipid family. In mammals, these lipids,

IPC: inositol phosphorylceramide

DAG: diacylglycerol

Pkc1: protein kinase C1

App1: antiphagocytic protein 1

which contain a sphingoid backbone often bound to an acyl chain by an *N*-amide linkage, have shown numerous effects in processes such as apoptosis, stress responses, and cell proliferation. In recent years, the role of sphingolipids in pathogenic fungi, in terms of signaling, growth, and virulence, has become a rapidly growing field. Because many of these enzymes and products are structurally distinct from their mammalian counterparts, they have the potential for therapeutic targeting with minimal effects on the host. Advanced mathematical models have been created and experimentally tested using the sphingolipid biosynthetic pathway of *C. neoformans* and its relation to pathogenesis (19).

One such enzyme that is unique to the fungal sphingolipid synthesis pathway is inositol phosphorylceramide (IPC) synthase. IPC synthase is responsible for the removal of an inositol-phosphate group from phosphatidylinositol and the transfer of that group to the terminal hydroxy group of phytoceramide. This reaction forms IPC and diacylglycerol (DAG) as a byproduct. Examinations of this enzyme in virulence of *C. neoformans* have shown that IPC synthase downregulation causes defects in melanin production (a known virulence factor in *C. neoformans*) and reduced growth inside alveolar macrophages. Both of these phenotypes may be responsible for the reduced virulence of strains with impaired IPC synthase (39, 40). The enzyme involved in the reverse process, inositol phosphosphingolipid-phospholipase C (Isc1), is also involved in virulence. Isc1 removes the phosphorylinositol moiety from IPC, forming phytoceramide. When this enzyme is deleted, the resulting strain (Δ *isc1*) is hypercapsulated and hypovirulent in immunocompromised mouse models of cryptococcosis and seems to be an obligate extracellular pathogen (59). The role of Isc1 likely revolves around macrophage interactions, as macrophage depletion results in the dissemination of the Δ *isc1* strain to the central nervous system.

The production of DAG by IPC synthase is a major component of this enzyme's role in virulence. DAG seems to regulate cryptococcal

virulence factors in two ways. First, DAG is a component in the melanin synthesis pathway. DAG activates PKC in mammalian cells through binding to the C1 domain of PKC (28). Heung et al. (25) found that this activation occurs in *C. neoformans* as well, with DAG produced from IPC synthase reactions binding to the C1 domain of the cryptococcal PKC (Pkc1) (24) and causing an increase in kinase activity. Deletion of the specific C1 domain stops the DAG-dependent activation of Pkc1. This disruption of Pkc1 activity causes alterations in the cell wall, preventing the enzyme laccase (melanin-producing enzyme) from properly associating. It was shown here that this is the mechanism by which IPC synthase regulates melanin synthesis. Aside from the effects on laccase, the involvement of Pkc1 in cell wall integrity is another aspect of this pathway. Cell wall integrity is crucial for fungal growth and virulence, often becoming the target of antifungal therapies. Gerik et al. (21) deleted 10 genes in the Pkc1 signaling pathway and found that phosphatase Ppg1 and kinases Bck1 and Mkk2 are all required for proper cell wall integrity. Along with these defects were predictable impairments in melanin production and capsule synthesis, as well as protection from oxidative and nitrosative stresses (20).

The second way that DAG production by IPC synthase regulates virulence factors is through the production of antiphagocytic protein (App1). App1 is not only involved in protection from phagocytosis, but also binds complement receptors CR2 and CR3 (61). The importance of App1 in virulence is clear from the phenotype of the deletion strain Δ *app1*, which is hypervirulent in complement-deficient mice but hypovirulent in mice with T-cell deficiencies (39). IPC synthase was indirectly involved in the transcription of *APP1*. DAG produced by IPC synthase activates the activating transcription factor 2 (Atf2), which binds to an ATF consensus sequence in the promoter region of *APP1* (62). Upon binding, Atf2 up-regulates the expression of App1, confirming another pathway by which DAG from IPC synthase regulates virulence of *C. neoformans*.

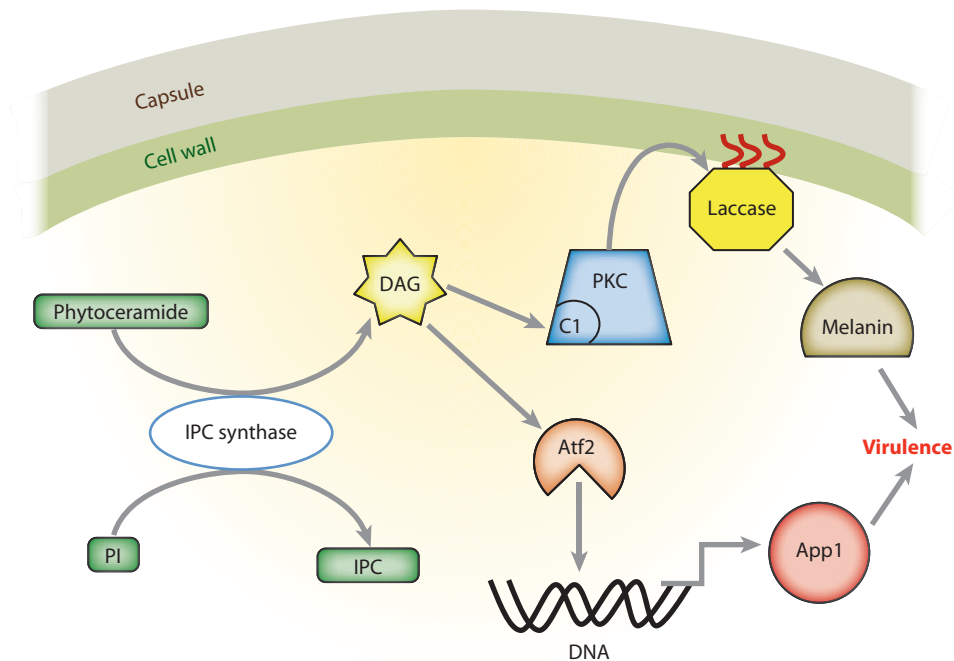


Figure 2

Sphingolipid signaling in *Cryptococcus neoformans*. IPC synthase in *C. neoformans* produces DAG as a byproduct of IPC synthesis. DAG binds to the C1 domain of protein kinase C, which is important for cell wall integrity. This integrity is crucial for localization of laccase, the enzyme responsible for melanin synthesis. In addition, DAG also activates the transcription factor Atf2, which leads to transcription of App1. Both App1 and melanin are virulence factors in *C. neoformans*. Abbreviations: IPC, inositol phosphorylceramide; PI, phosphatidylinositol; App1, antiphagocytic protein 1; Pkc1, protein kinase C1 (with C1 domain); DAG, diacylglycerol.

Figure 2 summarizes the IPC regulation of virulence factors in *C. neoformans*.

The future of sphingolipid pathway involvement in fungal disease is promising. Fluctuations in the pathway can be predicted with sophisticated mathematical models (19) and applied to pathogenesis. The IPC synthase-related pathways dominate the field of lipid signaling in pathogenic fungi to date, but other components of the fungal sphingolipid pathways are related to virulence. The deletion of the gene that encodes glucosylceramide synthase (*GCS1*) in *C. neoformans* yields a strain ($\Delta gcs1$) that is avirulent in inhalational mouse models of infection (54). This strain disseminates in a manner similar to $\Delta isc1$ when macrophages are depleted (32). Though the mechanism of this attenuated virulence is under

examination (53), it is possible that lipid signaling plays a role in this phenotype as well. In *C. albicans*, enzymes known as $\Delta 8$ -desaturases are responsible for the unique introduction of a double bond at the eighth carbon of the backbone of the fungal ceramide species. The deletion of this desaturase enzyme resulted in decreased hyphal growth and morphological alterations. Many enzymes in the sphingolipid pathway are still being characterized and deleted and may provide many more links between these lipids and virulence.

CONCLUSIONS

Lipid signaling in pathogenic fungi is a constantly growing field. New factors and fungal species are being studied constantly and the

understanding of existing pathways is always improving. Aside from giving new insights into the biology of these important human pathogens, these pathways may represent a new

class of antifungal treatments, if properly targeted. The discoveries presented here will likely be the foundation of many new advances in the years to come.

SUMMARY POINTS

1. FA, a fungal quorum-sensing molecule, inhibits hyphal formation. FA exposure appears to alter gene expression in *Candida* and involve Ras, MAP kinase, and histidine kinase signaling pathways.
2. In *Aspergillus*, FA treatment induces apoptosis and alters production of membrane lipids such as ergosterol.
3. Eicosanoid production occurs in many pathogenic fungi. The prostaglandins are produced using host lipids and may be synthesized to manipulate the host immune system.
4. Sphingolipid signaling affects transcription of antiphagocytic proteins and the production of melanin in *Cryptococcus* through the production of DAG by IPC synthase.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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57. Showed farnesol induces apoptosis in *Aspergillus*.

62. Showed link between DAG and App1 expression through Atf2.



Contents

Frontispiece	
<i>Lars G. Ljungdahl</i>	xii
A Life with Acetogens, Thermophiles, and Cellulolytic Anaerobes	
<i>Lars G. Ljungdahl</i>	1
Regulation of Translation Initiation by RNA Binding Proteins	
<i>Paul Babitzke, Carol S. Baker, and Tony Romeo</i>	27
Chemotaxis-Like Regulatory Systems: Unique Roles in Diverse Bacteria	
<i>John R. Kirby</i>	45
Aminoacyl-tRNA Synthesis and Translational Quality Control	
<i>Jiqiang Ling, Noah Reynolds, and Michael Ibba</i>	61
Resurrected Pandemic Influenza Viruses	
<i>Terrence M. Tumpey and Jessica A. Belser</i>	79
Interspecies Chemical Communication in Bacterial Development	
<i>Paul D. Straight and Roberto Kolter</i>	99
Lipid Signaling in Pathogenic Fungi	
<i>Ryan Rhome and Maurizio Del Poeta</i>	119
Biological Insights from Structures of Two-Component Proteins	
<i>Rong Gao and Ann M. Stock</i>	133
Role of GTPases in Bacterial Ribosome Assembly	
<i>Robert A. Britton</i>	155
Gene Transfer and Diversification of Microbial Eukaryotes	
<i>Jan O. Andersson</i>	177
Malaria Parasite Development in the Mosquito and Infection of the Mammalian Host	
<i>Ahmed S.I. Aly, Ashley M. Vaughan, and Stefan H.I. Kappe</i>	195
How Sweet it is! Cell Wall Biogenesis and Polysaccharide Capsule Formation in <i>Cryptococcus neoformans</i>	
<i>Tamara Lea Doering</i>	223

Mitochondrial Evolution and Functions in Malaria Parasites <i>Akbil B. Vaidya and Michael W. Mather</i>	249
Probiotic and Gut Lactobacilli and Bifidobacteria: Molecular Approaches to Study Diversity and Activity <i>Michiel Kleerebezem and Elaine E. Vaughan</i>	269
Global Emergence of <i>Batrachochytrium dendrobatidis</i> and Amphibian Chytridiomycosis in Space, Time, and Host <i>Matthew C. Fisher, Trenton W.J. Garner, and Susan F. Walker</i>	291
Anaerobic Oxidation of Methane: Progress with an Unknown Process <i>Katrin Knittel and Antje Boetius</i>	311
The <i>Trypanosoma brucei</i> Flagellum: Moving Parasites in New Directions <i>Katherine S. Ralston, Zakayi P. Kabututu, Jason H. Melehani, Michael Oberholzer, and Kent L. Hill</i>	335
Plants, Mycorrhizal Fungi, and Bacteria: A Network of Interactions <i>Paola Bonfante and Iulia-Andra Anca</i>	363
Evolutionary Roles of Upstream Open Reading Frames in Mediating Gene Regulation in Fungi <i>Heather M. Hood, Daniel E. Neafsey, James Galagan, and Matthew S. Sachs</i>	385
Single-Cell Ecophysiology of Microbes as Revealed by Raman Microspectroscopy or Secondary Ion Mass Spectrometry Imaging <i>Michael Wagner</i>	411
Microbiology of the Atmosphere-Rock Interface: How Biological Interactions and Physical Stresses Modulate a Sophisticated Microbial Ecosystem <i>Anna A. Gorbushina and William J. Broughton</i>	431
What Sets <i>Bacillus anthracis</i> Apart from Other <i>Bacillus</i> Species? <i>Anne-Brit Kolst, Nicolas J. Tourasse, and Ole Andreas Økstad</i>	451
The Expanding World of Methylotrophic Metabolism <i>Ludmila Chistoserdova, Marina G. Kalyuzhnaya, and Mary E. Lidstrom</i>	477
Genomics, Genetics, and Cell Biology of Magnetosome Formation <i>Christian Jogler and Dirk Schüler</i>	501
Predatory Lifestyle of <i>Bdellovibrio bacteriovorus</i> <i>Renee Elizabeth Sockett</i>	523
Plant-Growth-Promoting Rhizobacteria <i>Ben Lugtenberg and Faina Kamilova</i>	541

<i>Photobabds</i> and a Host of Hosts Nick R. Waterfield, Todd Ciche, and David Clarke	557
Management of Oxidative Stress in <i>Bacillus</i> Peter Zuber	575
Sociobiology of the Myxobacteria Gregory J. Velicer and Michiel Vos	599

Index

Cumulative Index of Contributing Authors, Volumes 59–63	625
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Errata

An online log of corrections to *Annual Review of Microbiology* articles may be found at <http://micro.annualreviews.org/>