Virulence-Associated Enzymes of *Cryptococcus neoformans*

Fausto Almeida,a,b Julie M. Wolf,a Arturo Casadevall,a,c

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York, USAa; Department of Cellular and Molecular Biology, Ribeirão Preto Medical School, University of Sao Paulo, Ribeirão Preto, Sao Paulo, Brazilb; Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USAc

Enzymes play key roles in fungal pathogenesis. Manipulation of enzyme expression or activity can significantly alter the infection process, and enzyme expression profiles can be a hallmark of disease. Hence, enzymes are worthy targets for better understanding pathogenesis and identifying new options for combating fungal infections. Advances in genomics, proteomics, transcriptomics, and mass spectrometry have enabled the identification and characterization of new fungal enzymes. This review focuses on recent developments in the virulence-associated enzymes from *Cryptococcus neoformans*. The enzymatic suite of *C. neoformans* has evolved for environmental survival, but several of these enzymes play a dual role in colonizing the mammalian host. We also discuss new therapeutic and diagnostic strategies that could be based on the underlying enzymology.

The facultative intracellular fungal pathogen *Cryptococcus neoformans* is the causative agent of cryptococcosis, a disease that primarily affects individuals with impaired immunity, such as those with advanced HIV infection (1, 2). *C. neoformans* is a ubiquitous environmental fungus associated with both pigeon guano and eucalyptus trees, and its environmental niche ranges from the tropical to the temperate (3). *C. neoformans* infection is acquired from the environment via inhalation, after which it forms a local infection in the lungs. This infection may be cleared, may be contained as a granuloma, or may disseminate from this initial site, leading to pneumonia and/or meningocencephalitis, the latter being uniformly fatal if untreated. Despite the availability of antifungal therapy, more than 650,000 people die each year from *C. neoformans* infection (1, 2, 4). The principal virulence factors of *C. neoformans* are a polysaccharide capsule, melanin production (5, 6), the ability to grow at body temperature (7), and the secretion of extracellular enzymes (7). These virulence factors confer a selective advantage to *C. neoformans* for both residing in the environment and in a mammalian host. Tightly controlled regulation leads to expression of enzymes required for fungal survival and host damage once inside its mammalian host (8).

Many enzymes contribute to the composite cryptococcal virulence phenotype. Dissection of the pathogenic role of these enzymes will enhance our understanding of cryptococcal pathogenic mechanisms and facilitate directed inhibitor development and/or vaccine discovery. We have included a table summarizing basic information regarding global *C. neoformans* enzymology (Table 1) and a schematic displaying localization of most of the highlighted enzymes discussed (Fig. 1). In this review, we discuss in detail the most important virulence-associated enzymes (Table 2), as well as additional target enzymes with potential for rational antifungal drug design (Table 3). We examine this information in the context of infection and analyze candidate target enzymes for drug inhibition and vaccine discovery.

**POLYSACCHARIDE CAPSULE**

*C. neoformans* is the only fungal pathogen with a polysaccharide capsule, an outermost polysaccharide structure located just outside the cell wall. The two major polysaccharide capsule constituents are glucuronoxylomannan (GXM) and glucuroxylomanogalactan (GXMGal) (9–11). GXM is the major component of *C. neoformans*, a compound of α-1,3-linked mannose residues with xylosyl and glucuronol side groups (12), whereas GXMGal is made of α-1,6-linked galactose residues with xylose, mannose, and glucuronic acid (13). The capsule also contains nonpolysaccharide components, such as mannoprotein (MP) (10, 14, 15), although these MP components may represent transient components destined for cellular export.

The role of capsule in environmental growth is unknown, although speculations have been made that the capsule protects the fungus from desiccation or acts as a food source (16). During mammalian infection, the capsule participates in resisting phagocytosis and modulating the immune response (17–21). Not only protective against phagocytosis in both mammalian and lepidopteran hosts (22, 23), the capsule also protects the fungus after ingestion by serving as a free radical sink that can shield the cell from oxidative bursts (24). Hence, while the capsule is not part of the enzymatic microbial arsenal, the machinery responsible for capsule synthesis and assembly does directly contribute to cryptococcal virulence. The primary structures of GXM and GXMGal subunits have been defined, but the mechanisms of subunit assembly into >106-Da branched structures have not (25, 26). The degree of branching and conformation of polysaccharides imply an elaborate assembly and regulatory enzymatic machinery (27).

The subunits of GXM and GXMGal are large glycans that require several glycosyltransferases for synthesis. Both xylosyltransferase and glucuronolyltransferase activities are involved in capsular polysaccharide biosynthesis (28–31). A xylosyltransferase, Cxt1, was the first glycosyltransferase identified with a defined role in capsule synthesis (31). It is a large transmembrane protein with β-1,2-xylosyltransferase activity (31), and deletion of the corresponding gene (CXT1) decreased capsular β-1,2-xylene linkages and fungal growth in the lung in a mouse model of infection (30).

Several acapsular mutants were obtained through identifica-
| Enzyme                                | Function(s)                                                                 | EC no. | Reference(s) |
|---------------------------------------|-----------------------------------------------------------------------------|--------|--------------|
| **Localized on capsule and/or cell wall** |                                                                             |        |              |
| 1,3-β-Glucan synthase                 | Involved in β-glucan synthesis                                              | 2.4.1.34 | 135         |
| Acid phosphatase                      | Involved in fungal cell adhesion to host tissues, localized in lysosomes, and related to virulence (Table 2) | 3.1.3.2 | 106, 136, 137 |
| Cas1 glycosyltransferase              | Participates in O-acetylation                                              | 2.4.1.X | 138         |
| Chitin deacetylase                    | Involved in chitin metabolism                                              | 3.5.1.41 | 139         |
| Chitin synthase                       | Involved in chitin synthesis                                              | 2.4.1.16 | 140         |
| Chitinase                             | Involved in chitin degradation                                             | 3.2.1.14 | 141         |
| Creatinine deaminase                  | Involved in arginine and proline metabolism                                | 3.5.4.21 | 142         |
| Esterase lipase                       | Catalyzes hydrolysis of fatty acids                                       | 3.1.1.3 | 136         |
| GDP-mannose pyrophosphorylase         | Involved in GDP-mannose synthesis                                          | 2.7.7.13 | 143         |
| Glucan 1,3-β-glucosidase              | Involved in glucan synthesis                                              | 3.2.1.58 | 16          |
| Glucan 1,4-α-glucosidase              | Involved in glucan synthesis                                              | 3.2.1.3 | 16          |
| Gmt1 GDP-mannose                      | Transport of GDP-mannose                                                  | 2.7.7.22 | 144         |
| Lactonohydrolase                      | Deficient strains show larger capsule size and facilitated immune evasion  | 3.1.1.15 | 37          |
| N-Acetylgalactosaminoglycan deacetylase | Involved in polysaccharide metabolism                                     | 3.1.1.58 | 145         |
| Phosphoaminase                        | Involved in amino acid synthesis                                          |         | 136         |
| Phosphomannomutase                    | Involved in GDP-mannose synthesis                                          | 5.4.2.8 | 143         |
| Phosphomannomannase isomerase         | Involved in GDP-mannose synthesis                                          | 5.3.1.8 | 143         |
| Uph1 ATPase                           | Required for vesicle acidification                                        |         | 146         |
| Utx1 decarboxylase                    | Converts UDP-glucuronic acid to UDP-xylitol                                 |         | 147         |
| α-1,3-Glucanase                       | Involved in glucan synthesis                                              | 3.2.1.59 | 16          |
| α-Amylase                             | Hydrolyzes alpha bonds of several polysaccharides and involved in cell wall building | 3.2.1.1 | 148         |
| α-Glucosidase                         | Breaks down disaccharides to glucose and starch and involved in cell wall building | 3.2.1.20 | 136         |
| α-Mannosidase                         | Involved in cell building through mannosate metabolism                     | 3.2.1.24 | 136         |
| α-Mannosyltransferase                 | Involved in polysaccharide metabolism                                      | 2.4.1.132 | 38, 149     |
| β-Endoglucanase                       | Involved in cell wall formation                                            | 3.2.1.4 | 148         |
| β-Glucosidase                         | Involved in cell wall formation                                            | 3.2.1.21 | 136         |
| β-Glucuronidase                       | Involved in cell wall formation, catalyzing breakdown of complex carbohydrates | 3.2.1.31 | 136         |
| **Secreted/released**                 |                                                                             |        |              |
| Acyltransferase                       | Involved in food acquisition                                               | 3.1.1.3 | 92          |
| Alkaline phosphatase                  | Involved in regulation of signaling cascades and several protein structure and localized in endoplasmic reticulum | 3.1.3.1 | 150         |
| Aspartyl protease                     | Involved in food acquisition                                               | 3.4.23.X | 111         |
| Cellulase                             | Involved in polysaccharide degradation                                    | 3.2.1.4 | 151         |
| DNase                                 | DNA degradation and related to virulence (Table 2)                         | 3.1.21.1 | 79          |
| Metalloprotease                       | Catalyzes mechanism that involves a metal and related to virulence (Table 2) | 3.4.24.77 | 113, 152    |
| Phospholipase B                       | Similar to phospholipase C function, degrades cell membrane components, supports fungal attachment to host cells, localized on cell wall, and related to virulence (Table 2) | 3.1.1.5 | 91, 92      |
| Phospholipase C                       | Degrades cell membrane components, supports fungal attachment to host cells, and related to virulence (Table 2) | 3.1.4.11 | 93          |
| Protease                             | Performs proteolysis interfering with host defense response                 | 3.4.21.53 | 107, 108    |
| S2P endopeptidase                     | Performs proteolysis                                                      | 3.4.24.85 | 153         |
| Serine peptidase                      | Performs proteolysis, coordinating several physiological functions         | 3.4.21.X | 152         |
| Superoxide dismutase                  | Catalyzes dismutation of toxic superoxide, converting superoxide to hydrogen peroxide and oxygen and related to virulence (Table 2) | 1.15.1.1 | 83-85       |
| **Localized intracellularly**         |                                                                             |        |              |
| 2-Methylcitrate synthase              | Converts acyl groups into alkyl groups on transfer                         | 2.3.3.5 | 154         |
| 3-β-Hydroxysteroid 3-dehydrogenase   | Oxidizes a substrate by reduction reaction that transfers 1 or more hydrides to electron acceptor | 1.1.1.270 | 155         |
| 6-Phosphogluconate dehydrogenase      | Involved in production of ribulose                                         | 1.1.1.44 | 156, 157    |
| Acetate kinase                        | Catalyzes formation of acetyl-CoA                                          | 2.7.2.1 | 158         |
| Aconitase                             | Catalyzes isomerization of citrate to isocitrate and involved in response to nitrosative stress | 4.2.1.3 | 159         |

(Continued on following page)
TABLE 1 (Continued)

| Enzyme                              | Function(s)                                 | EC no. | Reference(s) |
|-------------------------------------|---------------------------------------------|--------|--------------|
| Adenylyl cyclase Cac1               | Converts ATP to cAMP                        | 4.6.1.1 | 160          |
| Alternative oxidase                 | Part of electron transport chain in mitochondria | 1.10.3.11 | 161          |
| Aminopeptidase                      | Catalyzes cleavage of amino acids from amino terminus of protein | 3.4.11.21 | 137          |
| C-9-methyltransferase               | Involved in glycosphingolipid pathway       | 2.1.1.129 | 127          |
| Can2 carbonic anhydrase             | Responds directly to intracellular carbon oxide | 4.2.1.3 | 162, 163    |
| Casein kinase 1                     | Dephosphorylation of Hog1 under stress conditions | 2.7.11.1 | 164          |
| Catalase                            | Protects cells from oxidative damage by reactive oxygen species | 1.11.1.6 | 137, 150    |
| Cytochrome c peroxidase             | Takes reduced equivalents from cytochrome c and reduces hydrogen peroxide to water | 1.11.1.15 | 165          |
| Deacetylase                         | Removes acetyl groups from lysine in proteins and is localized in cell wall | 3.5.1.108 | 166          |
| Dolichyl-diphosphoglycerol-glucosyltransferase | Participates in N-glycan biosynthesis       | 2.4.99.18 | 167          |
| Ferrochelatase                      | Catalyzes final step in heme biosynthesis from highly photoreactive porphyrins | 4.99.1.1 | 168          |
| Flippase                            | Participates in phospholipid translocation between membrane sides and localized in cell wall | 3.6.3.1 | 169, 170    |
| Glucose-6-phosphate dehydrogenase   | Is in pentose phosphate pathway, maintaining the level of coenzyme NADPH | 1.1.1.49 | 171          |
| Glucose-phosphate isomerase         | Catalyzes conversion of glucose-6-phosphate into fructose-6-phosphate | 5.3.1.9 | 172          |
| Glucosylceramide synthase           | Involved in glycosylceramide synthesis, localized in cell wall, and related to virulence (Table 2) | 2.4.1.80 | 127, 128    |
| Glucuronyltransferase               | Involved in biosynthetic pathway of O-acetylated mannann | 2.4.1.17 | 28           |
| Glutathione peroxidase              | Protects cells from oxidative damage         | 1.11.1.9 | 173          |
| Glyoxal oxidase                     | Copper metalloenzyme that catalyzes oxidation of aldehydes to corresponding carbonyl compounds, coupled to reduction of dioxygen to H2O2 | 1.2.1.23 | 148          |
| Homoisoalactate dehydrogenase       | Participates in lysine biosynthesis          | 1.1.1.87 | 115          |
| Homoserine kinase                   | Participates in glycerol, serine, and threonine metabolism | 2.7.1.39 | 174          |
| Homoserine O-acetyltransferase      | Participates in methionine and sulfur metabolism | 2.3.1.31 | 175          |
| Hyaluronate synthase                | Involved in production of glycosaminoglycan at cell surface | 2.4.1.212 | 176          |
| Imidazolase glyceral-phosphate dehydratase | Participates in histidine biosynthesis          | 4.2.1.19 | 177          |
| IMP dehydrogenase                   | Participates in GTP biosynthesis            | 1.1.1.205 | 178          |
| Inositol phosphotransferase 1       | Involved in glycosphingolipid pathway        | 2.7.1.X | 127          |
| Inositol-phosphorylderase synthase   | Involved in glycosphingolipid pathway        | 2.7.1.X | 179          |
| Ire1 kinase                         | Involved in cellular response to unfolded proteins | 2.7.11.1 | 180          |
| Isocitrate lyase                    | Catalyzes cleavage of isocitrate to succinate and glyoxylate | 4.1.3.1 | 181          |
| Laccase                             | Polyphenol oxidase and copper-containing oxidase enzyme, localized in cell wall, and related to virulence (Table 2) | 1.10.3.2 | 45, 46, 50  |
| Malate dehydrogenase                | Catalyzes oxidation of malate to oxalacetate | 1.1.1.37 | 182          |
| Mannitol-1-phosphate 5-dehydrogenase| Participates in fructose and mannose metabolism | 1.1.1.17 | 183, 184    |
| Mannose-1-phosphate guanylyltransferase (GDP) | Participates in fructose and mannose metabolism | 2.7.7.22 | 144          |
| Mannosyl phosphorylinositol ceramide synthase | Involved in glycosphingolipid pathway | 2.4.X.X | 127          |
| Mannosyltransferase                 | Participates in O-mannosylation of proteins and involved in cell wall integrity and morphogenesis | 2.4.1.109 | 185          |
| Myristoyl-CoA protein N-myristoyltransferase | Catalyzes transfer of myristate from CoA to proteins | 2.3.1.97 | 116          |
| Pde1 phosphodiesterase              | Modulates cAMP                               | 3.1.4.1 | 186          |
| Phosphoglucomutase                  | Participates in interconversion of glucose 1-phosphate and glucose 6-phosphate | 5.4.2.2 | 172          |
| Protein farnesyltransferase         | Participates in formation of farnesyl protein and diphosphate | 2.5.1.58 | 187          |
| Rho1 GTPase                         | Involved in MAPK cascade                     | 3.6.5.2 | 188          |
| RNase III                           | Binds and cleaves double-stranded RNA        | 3.1.26.3 | 189          |
| Saccharopine dehydrogenase          | Participates in lysine metabolism            | 1.3.1.10 | 190          |
| Sphingolipid methyltransferase 1    | Participates in methylation of glucosylceramide | 2.1.1.1 | 191          |
| Sterol 14α-demethylase               | Involved in sterol metabolism               | 1.4.13.7 | 192          |
| Sterol 24-C-methyltransferase       | Involved in sterol metabolism               | 1.5.1.1 | 193          |
| Thiol peroxidase                    | Reduces peroxides and inhibits hydrogen peroxide response | 1.11.1.7 | 194          |

(Continued on following page)
tion of rough colonies. This type of screen identified four genes required for capsule formation: CAP10, CAP59, CAP60, and CAP64. Although these genes are not essential, their mutation does confer defects in growth and in mouse models of infection (17, 32–35). Cells from these mutant strains lacked or produced extremely reduced capsule, but these mutations did not correlate with enzymatic deficiency in UDP-glucose dehydrogenase, UDP-glucuronate decarboxylase, UDP-glucuronol:acceptor transferase, UDP-xyllosyl:acceptor transferase, or lipid-linked oligosaccharide biosynthetic pathways. CAP10 is a putative x-
losyltransferase gene, and cap10Δ mutants show a pleiotropic phenotype, which includes enlarged cell size, smaller extracellular vesicles, and affected expression of some virulence factors \((36)\). CAP10 therefore is required for both capsule formation and other aspects of fungal virulence.

Capsular lactonohydrolase also affects multiple capsule-related phenotypes \((37)\). A strain lacking lactonohydrolase \((lhc1Δ)\) produced capsules with a larger size and altered branching, density, and solvation compared to the parental strain. These capsular structure alterations increased virulence in murine infection \((37)\). Taken together, these results suggest that lactone may be involved in cross-linking of the capsule.

α-1,3-Mannosyltransferase (encoded by CMT1) synthesizes the mannose backbone of GXM and thus plays a crucial role in capsule synthesis. However, α-1,3-mannosyltransferase activity is more involved in in capsule biosynthesis than in the serotype D \(C. neoformans\) \((38, 39)\). Serotypes A and D represent two of the four \(C. neoformans\) serotypes: \(C. neoformans\) var. \(neoformans\) (serotypes A and D) and \(C. neoformans\) var. \(gattii\) (serotypes B and C), which can be distinguished according to their growth differences on diagnostic media \((40)\). The strain-specific capsule synthesis differences, such as the role of CMT1, show the importance of studying multiple strain backgrounds.

Much remains to be learned about the enzymatic machinery involved in capsule synthesis, including enzyme localization and kinetics. Detailed studies of capsule structure and the enzymatic machinery involved are critical for a better understanding of the function of the capsule production and regulation.

**MELANIN SYNTHESIS**

Melanin formation protects \(C. neoformans\) from oxidative damage as well as from both heat and cold \((41, 42)\). Melanin is synthesized on 2,3- or 3,4-diphenol substrates by a phenoloxidase and accumulates in the \(C. neoformans\) cell wall \((43, 44)\). The melanin-synthesizing enzyme has two classical laccase characteristics: a glycosylated copper-containing protein with the ability to oxidize diphenolic substrates and the ability to produce decarboxy dopamine \((45, 46)\). \(C. neoformans\) melanin synthesis occurs only in the presence of exogenous dihydroxyphenols, since no known \(C. neoformans\) endogenous substrate exists. Several diphenols can serve as the substrates for pigment synthesis by \(C. neoformans\) laccase \((47)\), such as the substrates consisting of para- and orthodiphenols, monophenols, l-dopa, and esculin, indicating that the enzyme has broad specificity and the ability to generate pigments from different compounds \((47–53)\). Iron increases laccase activity, but hydrogen peroxide has no effect on enzymatic activity, despite the antioxidant properties of melanin \((54)\).

The genes \(LAC1\) and \(LAC2\) encode two laccases, but a single deletion in \(LAC1\) is able to prevent melanin production \((55–58)\). Lac1 localizes in the cell wall, while Lac2 is cytoplasmic, but Lac2 can localize to the cell wall in the absence of Lac1 \((55)\). \(lac1Δ\) mutants are easily identified as white colonies when cultivated on catecholamine-containing media \((59)\). The lac1Δ mutant shows decreased virulence in survival studies with rabbit infection \((59)\), corroborating the important role in the fungal virulence \((5, 46)\). In addition to its cell wall localization, laccase is packaged into extracellular vesicles, a nontraditional mechanism of secretion, and can therefore mediate damage away from the laccase-producing fungal cell \((Fig. 1)\).

Melanin is considered a powerful antioxidant, since it may protect cryptococcal cells against oxygen- and nitrogen-derived oxidants of the type made by host effector cells \((5, 60–62)\). In addition to its capacity to absorb free radical fluxes, melanin can also contribute to acquired resistance against the antifungals.

**TABLE 2 Enzymes related to the virulence in Cryptococcus neoformans**

| Enzyme                  | Comment(s)                                                                 | Reference(s) |
|-------------------------|-----------------------------------------------------------------------------|--------------|
| Acid phosphatase        | Deficient strains show affected virulence in mouse and \(Galleria mellonella\) models of infection | 106          |
| DNase                   | Acts in degrading host DNA and supplies \(C. neoformans\) with nucleotides    | 79           |
| Glucosylceramide synthase | Required for virulence in murine model of infection                        | 127, 128     |
| Laccase                 | Deficient strains showed decreased virulence in survival studies with rabbit and mouse models of infection | 59          |
| Mannosyltransferase     | Required for virulence in murine model of infection                        | 185          |
| Metalloprotease         | Deficient strains unable to cross endothelium in \(in vitro\) model of human blood-brain barrier and is required for invasion of central nervous system | 113          |
| Phospholipase B         | Required in invasion of host tissue and dissemination in murine model       | 95           |
| Phospholipase C         | Shown to be important for several virulence phenotypes                     | 101, 102     |
| Superoxide dismutase    | Attenuated growth of deficient strains within macrophages                   | 89           |
| Urease                  | Deficient strains less virulent than wild-type strain in mouse model of infection and is involved in fungal escape from lung to cross blood-brain barrier | 76           |
| Xylosylphosphotransferase | Deficient strains manifest reduced growth in lung tissue in mouse model of infection | 30           |

**TABLE 3 Possible target enzymes for rational antifungal drug design**

| Enzyme(s)                        | Comment(s)                          | Reference(s) |
|----------------------------------|--------------------------------------|--------------|
| 14α-Demethylase                  | A critical enzyme in sterol assembly | 119          |
| Glucosylceramide synthase        | Glucosylceramide plays critical role in pathogenicity of \(C. neoformans\) | 127, 128     |
| Laccase                          | Melanization aids virulence          | 60, 63, 64, 65|
| Myristoyltransferase             | Myristoylation inhibition is fatal for \(C. neoformans\) | 116, 117     |
| Phosphoribosylaminomimazole carboxylase | Mutants that cannot synthesize adenine have reduced virulence | 114          |
| Pyrophosphorylase and cytosine-specific permease | Enzymes are basis of \(C. neoformans\) flucytosine resistance | 201, 202     |
| Sterol synthesis enzymes         | Sterol synthesis enzyme mutants show resistance to flucanazole and amphotericin | 122-124      |
amphotericin B and caspofungin, since nonmelanized cryptococcal cells are more susceptible than melanized cells to amphotericin B and caspofungin. Moreover, killing assays demonstrated that addition of melanin particles to amphotericin B or caspofungin significantly reduces their toxicities against \textit{C. neoformans} (63–65). Thus, melanin and laccase are considered promising targets for drugs against \textit{C. neoformans} infection.

**EXTRACELLULAR ENZYMES**

As nature’s “recyclers,” environmental fungi secrete a number of degradative enzymes to breakdown macromolecules and obtain nutrients in the environment (7, 66–69). \textit{C. neoformans} is no exception and releases a number of lipases, proteases, and DNAses. However, during the infection process, the same degradative enzymes contribute to virulence by destroying tissues, promoting fungal survival, and interfering with effective immune responses.

Urease is almost universally expressed by \textit{C. neoformans} isolates. In the environment, \textit{C. neoformans} is often isolated from avian excreta (70, 71). To survive and grow on this medium, the fungus must metabolize creatinine, xanthines, and uric acid. High urea activity may benefit the fungus under these conditions (72–74), as the enzyme catalyzes the hydrolysis of urea to ammonia and carbamate. Urease is considered a major cryptococcal virulence factor (75). A urease knockout (\textit{UREI}) strain of \textit{C. neoformans} was significantly less virulent than the wild-type strain in a mouse model of infection (76). Urease plays a role in fungal escape from the lung to cross the blood-brain barrier but is not required for fungal growth once inside the brain (76). Urease production varies among clinical isolates; however, the vast majority (99.6%) demonstrate some level of urease activity (74, 77, 78). Nevertheless, occasional urease-negative variants have been isolated in clinical isolates (77), suggesting that this enzyme can be dispensable, provided that there are compensatory virulence mechanisms.

Extracellular DNase is produced by \textit{C. neoformans} in high quantities (79). This DNase may degrade host DNA secreted by neutrophils as part of the innate immune response (80) and additionally may supply \textit{C. neoformans} with nucleotides. A survey of several yeast species, including \textit{C. neoformans}, suggests a correlation between urease activity and extracellular DNase production (79). DNase activity is stronger in clinical strains than in environmental strains, further suggesting DNase may play a role as a virulence factor (81).

Superoxide dismutases (SODs) convert superoxide to hydrogen peroxide and oxygen (82). Two SODs have been described in \textit{C. neoformans} (83–88). SOD contributes to virulence of \textit{C. neoformans} by facilitating growth within macrophages (89), through a mechanism that is likely to involve protection of the fungus against superoxide generated by host immune response (2). In this regard, melanin and SOD may stimulate complementary defenses for the \textit{C. neoformans} cells’ protection against oxidative damage. SOD production is regulated by temperature, with increases in expression at 37°C compared to 25°C. Thus, increased SOD production at body temperatures may protect the fungus against oxidizing agents produced from host effector cells (90).

Phospholipases degrade cell membrane phospholipids in an enzyme–dependent mechanism. \textit{C. neoformans} extracellular supernatants contain phospholipase B, phospholipase C, lysophospholipase, and acyltransferase (91–93), and phospholipase activity supports fungal attachment to host cells (94). Phospholipase B promotes fungal invasion of host tissue (95) and hydrolyzes phospholipids in lung surfactant and the plasma membrane (92, 96). Moreover, it contributes to fungal survival by maintaining cell wall integrity (97) and provides nutrients that can be used as sole carbon sources by \textit{C. neoformans} during the infection (98, 99). As described above, it has also been localized to the cell wall (97), and its transport to the plasma membrane and cell wall is \textit{N}-glycan dependent (100). Phospholipase C is crucial for several virulence phenotypes (melanin production, growth at 37°C, phospholipase B secretion, and antifungal drug resistance) and is also involved in homeostasis regulation, cell separation following cytokinesis, and cell wall integrity (101, 102).

Phosphatases remove a phosphate group from their substrates and play important roles in regulating protein structure and signaling cascades (103, 104). A secreted acid phosphatase is involved in fungal cell adhesion to host tissues, suggesting an important role in establishing infection (105). Acid phosphatase is encoded by the gene \textit{APH1} in \textit{C. neoformans}. In both wax worm and murine models of cryptococcosis, \textit{aph1Δ} strain-infected animals survived longer than those in the wild-type-infected model (106), demonstrating the importance of this enzyme during infection.

Proteases break down proteins and are considered important virulence factors, contributing to tissue invasion, colonization, and alteration of the host defense response. Protease activity in \textit{C. neoformans} cultures has been reported by several investigators (107–111). Proteases play important roles in host cell penetration and virulence of \textit{C. neoformans} (112). Recently, a metalloprotease was identified by proteomic analyses of the extracellular proteins from \textit{C. neoformans} and found to be required for invasion of the central nervous system in murine infection of \textit{C. neoformans} (113). Moreover, the metalloprotease knockout (\textit{mpr1Δ}) strain was unable to cross the endothelium in an \textit{in vitro} model of the human blood-brain barrier (113).

**DRUG DESIGN AND RESISTANCE**

Definition of enzymatic pathways can provide crucial targets for antimicrobial drug design. One way to identify targets is to identify unique metabolic requirements for cryptococcal growth and/or virulence. An example of this is the \textit{C. neoformans} phosphoribosylaminomimidazole carboxylase gene (\textit{ADE2}). Mutants with mutations in this gene lack an enzyme required for adenine synthesis and thus have reduced virulence compared to the wild-type strain (114). This observation suggests potential for rational drug design utilizing differences in adenine synthesis pathways between host and pathogen (as first suggested in reference 7). Several candidate enzymes in \textit{C. neoformans} have been studied regarding fungal amino acid synthesis (e.g., homocitrate synthase, homoisocitrate dehydrogenase, α-aminoadipate reductase, saccharopine reductase, and saccharopine dehydrogenase) (115). However, comparisons between \textit{C. neoformans} var. \textit{neoformans} and \textit{C. neoformans} var. \textit{gattii} have shown that candidate targets do not necessarily translate across \textit{Cryptococcus} species. Saccharopine reductase, an enzyme involved in lysine synthesis, was not detected in \textit{C. neoformans} var. \textit{gattii} but was detected in \textit{C. neoformans} var. \textit{neoformans}. This \textit{C. neoformans} var. \textit{gattii} strain was able to grow even in the absence of lysine (115), indicating that further research to identify enzymes essential across all \textit{Cryptococcus} species is required.

Another essential process for \textit{C. neoformans} is protein myristoylation. \textit{C. neoformans} myristoyltransferase catalyzes the transfer of myristate from coenzyme A (CoA) to the amino-terminal
glycine residue of a subset of cellular proteins, and this enzyme is essential for C. neoformans viability (116, 117). N-Myristoyl proteins and myristoylation inhibition by the myristic acid analog 4-octatetradecanoic acid are crucial for this organism (118). Thus, therapies directed at myristoylation may also be a possible target for rational antifungal drug design.

In some cases, an antifungal target is well defined, but multiple enzymes involved in target synthesis provide several inhibitory strategies. Sterols and their synthetic pathways are major antifungal targets in many fungi, but resistance leads to difficulties in patient treatment. Fluconazole-resistant strains require a 10-fold-higher drug concentration to inhibit sterol 14α-demethylase (119), rendering the drug clinically unfeasible. The molecular basis for differential enzyme function has been identified in several common C. neoformans strains (120). One documented fluconazole- and amphotericin-resistant C. neoformans patient isolate showed reduced relative sterol content and a defect in δ-8-isomerase, depleting ergosterol, and accumulated aberrant δ-8- and δ-8,δ-9-doubled-kersterol precursors (121, 122), suggesting the ability to form membrane pores due to aggregation and formation of amphotericin-sterol complexes. Another study evaluating fluconazole- and amphotericin-resistant isolates observed reduced ergosterol content in the isolates, as well as reduced sensitivity of P450 14α-demethylase to inhibition by fluconazole, and a defect in sterol Δ8-Δ7 isomerase (123). Another C. neoformans strain with defective sterol Δ8-Δ7 isomerase was discovered in an amphotericin B-resistant isolate from an AIDS patient (124). These mutations in sterol synthesis enzymes explain resistance evolution and generate targets to fight it with. This information can also help in rational drug design methodologies.

Identification of key virulence-related enzymes is yet another route toward finding an effective drug target. Glycosphingolipids are essential to regulate survival and/or replication of C. neoformans in the phagolysosome, as well as in the extracellular environment of the host (125–127). Glucosylceramide plays a critical role in pathogenicity of C. neoformans, since glucosylceramide synthase (Gcs1) is required for virulence in the murine model of infection (128). gcs1Δ mutants corroborate the crucial role of the glycosphingolipid synthesis in regulation of this considerable aspect of C. neoformans virulence (127). Thus, the glycosphingolipid pathway may also be a reasonable target for antifungal therapies.

Laccase has been considered a drug target in C. neoformans because melanization is critical to virulence. Inhibition of fungal melanization in murine infection using the herbicide glyphosate prolonged average mouse survival. Glyphosate is an inhibitor of both the shikimate acid pathway and L-dopa polymerization (129). Thus, therapies directed at melanization may also be a potential target for antifungal drug design.

Occasionally, a drug proven to work on one microbial pathogen will also be effective against another. This appears to be the case with several viral medications. Drugs such as indinavir and osetlamivir inhibit human immunodefi ciency virus (HIV) protease or influenza virus neuraminidase, respectively, and demonstrate the impact an enzymatic inhibitor can have in the clinic (130, 131). The use of protease inhibitors has shown positive effects on C. neoformans and Candida albicans infections, where drug treatment was associated with inhibition of fungal growth and proliferation in vitro (132, 133). These are likely inhibiting the fungal proteases, both cell associated and as part of the fungal secretome.

CONCLUSION
Recent advances in genomics, proteomics, transcriptomics, and mass spectrometry have facilitated the identification and characterization of new fungal enzymes, including those specific to both fungi and C. neoformans. These enzymes are required for many important biological processes, including growth and infection. The importance of the secretome in cryptococcal pathogenesis is apparent from the fact that strain differences in secreted enzymes correlate with their virulence (134). Nonetheless, important questions remain. Future research on cryptococcal enzymology will not only identify new enzymes and their roles during infection but also pinpoint enzymatic targets for the development of antifungal agents.

ADDENDUM IN PROOF
There are, of course, many enzymes involved in signaling cascades, most of which were not discussed in this review. One such enzyme is vital to stress response in C. neoformans and other pathogenic fungi and thus merits a well-deserved mention: the calcium-dependent phosphatase calcineurin (W. J. Steinbach, J. L. Geddes JM, Griffiths EJ, Choi J, Cadieux B, Caza M, Attarian R. 2012. Adaptation of Cryptococcus neoformans to mammalian hosts: integrated regulation of metabolism and virulence. Eukaryot Cell 11:109–118. http://dx.doi.org/10.1128/EC.05273-11).

REFERENCES
1. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TA. 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23:525–530. http://dx.doi.org/10.1097/QAD.0b013e2828f1f8ec.
2. Heitman J, Kozel TR, Kwon-Chung J, Perfect JR. Casadevall A. 2011. Cryptococcus: from human pathogen to model yeast. ASM Press, Washington, DC.
3. Nielsen K, De Obaldia AL, Heitman J. 2007. Cryptococcus neoformans mates on pigeon guano: implications for the realized ecological niche and globalization. Eukaryot Cell 6:949–959. http://dx.doi.org/10.1128/EC.00097-07.
4. Mitchell TG, Perfect JR. 1995. Cryptococcosis in the era of AIDS—100 years after the discovery of Cryptococcus neoformans. Clin Microbiol Rev 8:515–548.
5. Williamson PR. 1997. Laccase and melanin in the pathogenesis of Cryptococcus neoformans. Front Biosci 2:e99–e107.
6. Casadevall A, Rossa AL, Nosanchuk JD. 2000. Melanin and virulence in Cryptococcus neoformans. Curr Opin Microbiol 3:354–358. http://dx.doi.org/10.1016/S1369-5274(00)00103-X.
7. Casadevall A, Perfect JR. 1998. Cryptococcus neoformans. ASM Press, Washington, DC.
8. Kronstad J, Saikia S, Nielsen ED, Kretschmer M, Jung W, Hu G, Geddes JM, Griffiths EJ, Choi J, Cadieux B, Caza M, Attarian R. 2012. Adaptation of Cryptococcus neoformans to mammalian hosts: integrated regulation of metabolism and virulence. Eukaryot Cell 11:109–118. http://dx.doi.org/10.1128/EC.05273-11.
Minireview

O’Meara TR, Alspaugh JA. 2009. The capsule of the fungal pathogen Cryptococcus neoformans lacks capsular O-acetylation or xylosyl side chains. Infect Immun 77:2868–2875. http://dx.doi.org/10.1128/IAI.71.5.2868-2875.2003.

Heiss C, Klutts JS, Wang Z, Doering TL, Azapi P. 2003. Antigenic and biological characteristics of mutant strains of Cryptococcus neoformans lacking capsular O-acetylation or xylosyl side chains. Infect Immun 71:2868–2875. http://dx.doi.org/10.1128/IAI.71.5.2868-2875.2003.

15. Rodrigues ML, Nimrichter L. 2012. Evidence for branching in cryptococcal capsular polysaccharides and mannoprotein occupy spatially separate and discrete regions in the capsule of Cryptococcus neoformans. Virulence 1:500–508. http://dx.doi.org/10.4161/viru.1.6.13451.

13. McFadden DC, De Jesus M, Casadevall A. 2009. The physical properties of the capsule polysaccharides. J Biol Chem 284:14237–14354. http://dx.doi.org/10.1074/jbc.M808927200.

17. Frases S, Pontes B, Nimrichter L, Viana NB, Rodrigues ML, Casadevall A, Vecchiarelli A, Monari C. 2006. A yeast under a sword and a shield. Clin Microbiol Rev 19:42:5482–5488.

14. Cherniak R, Reiss E, Turner SH. 2002. A novel and a shield. Clin Microbiol Rev 15:401–404. http://dx.doi.org/10.1128/CMR.00012-12.

10. Cheriniak R, Reiss E, Turner SH. 1992. A galactoxylomannan antigen of Cryptococcus neoformans serotype A. Carbohydr Res 103:239–250. http://dx.doi.org/10.1016/0008-6215(80)80866-2.

9. Cheriniak R, Sundstrom JB. 1994. Polysaccharide antigens of the capsule of Cryptococcus neoformans. Infect Immun 62:1507–1512.

18. Rosas AL, Casadevall A. 2010. The second capsule gene of Cryptococcus neoformans, CAP64, is essential for virulence. Infect Immun 84:1977–1983.

24. Cherniak R, Kwon-Chung KJ. 1999. Isolation, characterization, and localization of a capsule-associated gene, CAP10, of Cryptococcus neoformans. J Bacteriol 181:5636–5643.

26. Cherniak R, Penoyer LA, Kwon-Chung KJ. 1996. The second capsule gene of Cryptococcus neoformans, CAP64, is essential for virulence. Infect Immun 64:1977–1983.

20. Park YD, Shin S, Panepinto J, Ramos J, Qiu J, Frases S, Albuquerque P, Doering TL, Zhang N, Himmelreich U, Beenhouwer D, Bennett JE, Casadevall A, Williamson PR. 2014. A role for LHC1 in higher order structure and complement binding of the Cryptococcus neoformans capsule. PLoS Pathog 10:e1004037. http://dx.doi.org/10.1371/journal.ppat.1004037.

28. White CW, Cheriniak R, Jacobson ES. 1990. Side group addition by xylosyltransferase and glucuronyltransferase in biosynthesis of capsular polysaccharide in Cryptococcus neoformans. J Med Vet Mycol 28:289–301. http://dx.doi.org/10.1080/0268121980000381.

29. Castle SA, Owuor EA, Thompson SH, Garney MR, Klutts JS, Gooding TL, Levery SB. 2008. Beta-1,2-Xylosyltransferase Cxt1p is solely responsible for xylose incorporation into Cryptococcus neoformans glycosphingolipids. Eukaryot Cell 7:1611–1615. http://dx.doi.org/10.1083/jcb.2004042193.tb01702.x.

23. Tscheske S, Tyndall RL. 1975. Pigment production by Cryptococcus neoformans from para- and ortho-diphenol ethers by the nitrogen source. J Clin Microbiol 1:509–514.
62. Jacobson ES, Tinnell SB.

61. Dos Reis Almeida FB, de Oliveira LL, de Sousa MV, Barreira MCR, Wann Y, Casadevall A.

58. Edberg SC, Chaskes SJ, Alturere-Berber E, Singer JM. 1980. Esculin-based medium for isolation and identification of Cryptococcus neoformans. J Clin Microbiol 12:332–335.

57. Kwon-Chung KJ, Tom WK, Costa JL. 1983. Utilization of indole compounds by Cryptococcus neoformans to produce a melanin-like pigment. J Clin Microbiol 18:1419–1421.

56. Polacheck I, Hearing VJ, Kwon-Chung KJ. 1982. Biochemical studies of phenoloxidase and utilization of catecholamines in Cryptococcus neoformans. J Bacteriol 150:1212–1220.

55. Polacheck I, Platt Y, Arionovitch J. 1990. Catecholamines and virulence of Cryptococcus neoformans. Infect Immun 58:2919–2922.

54. Strachan AA, Yu RJ, Blank F. 1971. Pigment production of Cryptococcus neoformans grown with extracts of Guizotia abyssinica. Appl Microbiol 22:478–479.

53. Wang HS, Zennis RT, Roberts GD. 1977. Evaluation of a caffeic acid ferrocitr test for rapid identification of Cryptococcus neoformans. J Clin Microbiol 6:445–449.

52. Jacobson ES, Compton GM. 1996. Discordant regulation of phenoxidiase and capsular polysaccharide in Cryptococcus neoformans. J Med Vet Mycol 34:289–291. http://dx.doi.org/10.1016/0268-1219(96)00004-1.

51. Missall TA, Moran JM, Corbett JA, Lodge JK. 2005. Distinct stress responses of two functional laccases in Cryptococcus neoformans are revealed in the absence of the thiol-specific antioxidant Tsa1. Eur Ykaryot Cell 4:202–208. http://dx.doi.org/10.1128/EJC.4.1.202-208.2005.

50. Pukkila-Worley R, Geralda QD, Kraus PR, Boily MJ, Davis MJ, Giles SS, Cox GM, Heitman J, Alspaugh JA. 2005. Transcriptional network of multiple capsule and melanin genes governed by the Cryptococcus neoformans cyclic AMP cascade. Eur Ykaryot Cell 4:190–201. http://dx.doi.org/10.1128/EJC.4.1.190-201.2005.

49. Zhu X, Williamson PR. 2004. Role of laccase in the biology and virulence of Cryptococcus neoformans. FEMS Yeast Res 5:1–10. http://dx.doi.org/10.1016/j.femsyr.2004.04.004.

48. Zhu XD, Gibbons J, Garcia-Rivera J, Casadevall A, Williamson PR. 2001. Laccase of Cryptococcus neoformans is a cell wall-associated virulence factor. Infect Immum 69:5589–5596. http://dx.doi.org/10.1128/IAI.69.9.5589-5596.2001.

47. Salas SD, Bennett JE, Kwon-Chung KJ, Perfect JR, Williamson PR. 1996. Effect of the laccase gene CNLAC1, on virulence of Cryptococcus neoformans. J Exp Med 184:377–386. http://dx.doi.org/10.1084/jem.184.2.377.

46. Wang X, Casadevall A. 1994. Susceptibility of melanized and nonmelanized Cryptococcus neoformans to nitrogen- and oxygen-derived oxidants. Infect Immum 62:3004–3007.

45. Wang X, Aisen P, Casadevall A. 1995. Cryptococcus neoformans melanin and virulence: mechanism of action. Infect Immum 63:3131–3136.

44. Jacobson ES, Tinnell SB. 1993. Antioxidant function of fungal melanin. J Bacteriol 175:710–7104.

43. van Duin D, Casadevall A, Nosanchuk JD. 2002. Melanization of Cryptococcus neoformans and Histoplasma capsulatum reduces their susceptibilities to amphotericin B and capsafungin. Antimicrob Agents Chemother 46:3394–3400. http://dx.doi.org/10.1128/AAC.46.11.3394-3400.2002.

42. Ikeda R, Sugita T, Jacobson ES, Shinoda T. 2003. Effects of melanin upon susceptibility of Cryptococcus to antifungals. Microb Immunol 47:271–277. http://dx.doi.org/10.1011/j1.1348-0421.2003.tb03395.x.

41. Wang XL, Casadevall A. 1994. Growth of Cryptococcus neoformans in presence of l-dopa decreases its susceptibility to amphotericin B. Antimicrob Agents Chemother 38:2684–2690. http://dx.doi.org/10.1128/AAC.38.11.2684.

40. Almeida FB, Conqueira FM, Silva Rdo N, Ulhoa CJ, Lima AL. 2007. Mycoparasitism studies of Trichodernara harzianum strains against Rhi- zoctonia solani: evaluation of coiling and hydrolitic enzyme production. Biotechnol Lett 29:1189–1193. http://dx.doi.org/10.1007/s10529-007-9372-z.

39. Dos Reis Almeida FB, de Oliveira LL, de Sousa MV, Barreira MCR, Hanna ES. 2010. Paracoccidioides brasiliensis mitochondria purification through affinity with chitin and identification of N-acetyl-beta-t-glucosaminidase activity, Yeast 27:67–76.

38. Dos Reis Almeida FB, Carvalho FC, Mariano VS, Alegre ACP, Silva RD, Hanna ES, Roque-Barreca MC. 2011. Influence of N-glycosylation on the morphogenesis and growth of Paracoccidioides brasiliensis and on the biological activities of yeast proteins. PLoS One 6:e29216. http://dx.doi.org/10.1371/journal.pone.0029216.
phages. Infect Immun 71:173–180. http://dx.doi.org/10.1128/IAI.71.1 .173-180.2003.

90. Jacobson ES, Jenkins ND, Todd JM. 1994. Relationship between superoxide-dismutase and melalin in a pathogenic fungus. Infect Immun 62:4085–4086.

91. Chen SC, Muller M, Zhou JZ, Wright LC, Sorrell TC. 1997. Phospholipase activity in Cryptococcus neoformans: a new virulence factor? J Infect Dis 175:414–420. http://dx.doi.org/10.1086/1572.4.14.

92. Chen SC, Wright LC, Santangelo RT, Muller M, Moran VR, Kuchel PW, Sorrell TC. 1997. Identification of extracellular phospholipase B, lysophospholipase, and acyltransferase produced by Cryptococcus neoformans. Infect Immun 65:405–411.

93. Henry J, Guillotte A, Luberto C, Del Poeta M. 2011. Characterization of inositol phospho-sphingolipid-phospholipase C (Isc) in Cryptococcus neoformans reveals unique biochemical features. FEBS Lett 585:635–640. http://dx.doi.org/10.1016/j.febslet.2011.01.015.

94. Barrett-Bee K, Hayes Y, Wilson RG, Ryley JF. 1985. A comparison of phospholipase activity, cellular adherence and pathogenicity of yeasts. J Gen Microbiol 131:1217–1221.

95. Santangelo R, Zoellner H, Sorrell T, Wilson C, Donald C, Djordjevic J, Shounan Y, Wright L. 2004. Role of extracellular phospholipases and mononuclear phagocytes in dissemination of cryptococcosis in a murine model. Infect Immun 72:2229–2239. http://dx.doi.org/10.1128/IAI.72.4.2229-2239.2004.

96. Chen SC, Wright LC, Golding JC, Sorrell TC. 2000. Purification and characterization of secretory phospholipase B, lysophospholipase and lysophospholipase/transacylase from a virulent strain of the pathogenic fungus Cryptococcus neoformans. Biochem J 347:431–439. http://dx.doi.org/10.1042/bj3470431.

97. Siafakas AR, Sorrell TC, Wilson C, Larsen M, Boadle R, Williamson PR, Djordjevic JT. 2007. Cell wall-linked cryptococcal phospholipase B1 is a source of secreted enzyme and a determinant of cell wall integrity. J Biol Chem 282:37508–37514. http://dx.doi.org/10.1074/jbc.M709120200.

98. Wright LC, Santangelo RM, Ganrendre R, Payne J, Djordjevic JT, Sorrell TC. 2007. Cryptococcal lipid metabolism: phospholipase B1 is implicated in transcellular metabolism of macrophage-derived lipids. Eukaryot Cell 6:57–47. http://dx.doi.org/10.1128/EC.00262-06.

99. Nover MC, Cox GM, Perfect JR, Huffnagle GB. 2003. Role of PLB1 in pulmonary inflammation and cryptococcal eicosanoid production. Infect Immun 71:1538–1547. http://dx.doi.org/10.1128/IAI.71.3.1538-1547.2003.

100. Turner KM, Wright LC, Sorrell TC, Djordjevic JT. 2006. N-linked glycosylation sites affect secretion of cryptococcal phospholipase B1, irrespective of glycosylphosphatidylinositol anchoring. Biochim Biophys Acta 1760:1569–1579. http://dx.doi.org/10.1016/j.bbadis.2006.07.002.

101. Chaves ML, Sorrell TC, Siafakas AR, Wilson CF, Pantarat N, Gerik KJ, Boadle R, Djordjevic JT. 2008. Role and mechanism of phospholipidinositol-specific phospholipase C in survival and virulence of Cryptococcus neoformans. Mol Microbiol 69:809–826. http://dx.doi.org/10.1111/j.1365-2958.2008.06310.x.

102. Lev S, Desmarini D, Li C, Chayakulkeeree M, Traven A, Sorrell TC, Djordjevic JT. 2013. Phospholipase C of Cryptococcus neoformans regulates host/macrophage virulence and virulence by providing inositol trisphosphate as a substrate for Arg1 kinase. Infect Immun 81:1245–1255. http://dx.doi.org/10.1128/IAI.01421-12.

103. Bauman AL, Scott JD. 2002. Kinase- and phosphatase-anchoring proteins: harnessing the dynamic duo. Nat Cell Biol 4:E203–E206. http://dx.doi.org/10.1038/nbc8082-e03.

104. McConnell JL, Wadzinski BE. 2009. Targeting protein serine/threonine phosphatases for drug development. Mol Pharmacol 75:1249–1261. http://dx.doi.org/10.1124/mol.108.053140.

105. Collopy-Junior I, Esteves FF, Nimrichter L, Rodrigues ML, Alviano CS, Meyer-Fernandes JR. 2006. An ectophosphatase activity in Cryptococcus neoformans. FEMS Yeast Res 6:1010–1017. http://dx.doi.org/10.1111/j.1567-1364.2006.00105.x.

106. Levine SK, Crosson B, Cha SY, Desmarini D, Li C, Chayakulkeeree M, Wilson CF, Williamson PR, Sorrell TC, Djordjevic JT. 2014. Identification of Apha1, a phosphate-regulated, secreted, and vacuolar acid phosphatase in Cryptococcus neoformans. mBio 5:e01649-14. http://dx.doi.org/10.1128/mBio.01649-14.

107. Bruese KE. 1986. Proteolytic activity of a clinical isolate of Cryptococcus neoformans. J Clin Microbiol 23:631–633.
tion, and virulence. Curr Biol 15:2013–2020. http://dx.doi.org/10.1016/j.cub.2005.09.047.

163. Mogensen EG, Janbon G, Chaloupka J, Steegborn C, Fu MS, Moyrand F, Kneldt T, Pearson DS, Geeves MA, Buck J, Levin IR, Muhledeg FA. 2006. Cryptococcus neoformans senses CO2 through the carbon anhydrase Can2 and the defense of Cryptococcus neoformans. Eukaryot Cell 5:103–111. http://dx.doi.org/10.1089/EC.1.5.103-111.2006.

164. Wang YN, Liu TB, Patel S, Jiang LH, Xue CY. 2011. The casine kinase I protein Cck1 regulates multiple signaling pathways and is essential for cell integrity and fungal virulence in Cryptococcus neoformans. Eukaryot Cell 10:e145–1464. http://dx.doi.org/10.1083/EC.005207-11.

165. Giles SS, Perfect JR, Cox GM. 2005. Cytochrome c peroxidase contributes to the antioxidant defense of Cryptococcus neoformans. Fungal Genet Biol 42:20–29. http://dx.doi.org/10.1016/j.fgb.2004.09.003.

166. Biondo C, Beninati C, Delfino D, Oggioni M, Mancuso G, Midiri A, Bombaci M, Tomasselli G, Teti G. 2002. Identification and cloning of a cryptococcal deacetylase that produces protective immune responses. Infect Immun 70:2383–2391. http://dx.doi.org/10.1128/IAI.70.5.2383-2391.2002.

167. Kelleher DJ, Banerjee S, Cura AJ, Samuelson J, Gilmore R. 1994. Purification and characterization of malate dehydrogenase from Cryptococcus neoformans identification as “nothing dehydrogenase.” Arch Biochem Biophys 313: 304–309.

168. Safrin RE, Lancaster LA, Davis CE, Braude AI. 1986. Differentiation of Cryptococcus neoformans serotypes by isoenzyme electrophoresis. Am J Clin Pathol 96:204–208.

169. Missall TA, Cherry-Harris JR, Lodge JK. 2005. Two glutathione peroxidases in the fungal pathogen Cryptococcus neoformans are expressed in the presence of specific substrates. Microbiology 151:2573–2581. http://dx.doi.org/10.1099/mi.0.20132-0.

170. Kingsbury JM, McCusker JH. 2008. Threonine biosynthetic genes are essential in Cryptococcus neoformans. Microbiology 154:2767–2775. http://dx.doi.org/10.1099/micro.0.2008/09222-0.

171. Hutter Y, Van Heusden J, Mallett TC. 1994. Purification and characterization of glucose-6-phosphate dehydrogenase from Cryptococcus neoformans identification as “nothing dehydrogenase.” Arch Biochem Biophys 313: 304–309.

172. Jung A, Wu CH, Chen HM, Luo F, Kwon-Chung KJ, Chang YC, LaMunyon CW, Plaa A, Huang SH. 2007. Identification and characterization of CPS1 as a hyaluronic acid synthase contributing to the pathogenesis of Cryptococcus neoformans infection. Eukaryot Cell 6:1486–1496. http://dx.doi.org/10.1083/EC.00120-07.

173. Parker AR, Moore TD, Edman JC, Schwab JM, Davison VJ. 1994. Cloning, sequence analysis and expression of the gene encoding imidazole glycerol phosphate dehydrogenatase in Cryptococcus neoformans. Gene 145:35–138. http://dx.doi.org/10.1016/0378-1119(94)90336-0.

174. Morrow CA, Stamp A, Valkov E, Kobe B, Fraser JA. 2010. Crystalization and preliminary X-ray analysis of mycoheninic acid resistant and mycoheninic acid-sensitive forms of IMP dehydrogenase from the human fungal pathogen Cryptococcus. Acta Crystalllog Sect F Struct Biol Cryst Comm 66:1104–1107. http://dx.doi.org/10.1107/S1744309101023669.

175. Heung U, Luebke C, Plowden A, Hannon YA, Del Poeta M. 2004. The sphingolipid pathway regulates Pck1 through the formation of diacylglycerol in Cryptococcus neoformans. J Biol Chem 279:21144–21153. http://dx.doi.org/10.1074/jbc.M312995200.

176. Cheon SA, Jung KW, Chen YL, Heitman J, Bahn YS, Kang HA. 2011. Unique evolution of the UPR pathway with a novel bZIP transcription factor, Hzl1, for controlling pathogenicity of Cryptococcus neoformans. PLoS Pathog 7:e1002177. http://dx.doi.org/10.1371/journal.ppat.1002177.

177. Rude TH, Toffalletti DL, Cox GM, Perfect JR. 2002. Relationship of the glyoxylate pathway to the pathogenesis of Cryptococcus neoformans. Infection 70:5684–5694. http://dx.doi.org/10.1128/IAI.70.10.5684-5694.2002.

178. Mahmoud YA, el Soud SM, Niehaus WG. 1995. Purification and characterization of malate dehydrogenase from Cryptococcus neoformans. Arch Biochem Biophys 322:679–705. http://dx.doi.org/10.1016/0003-2697.1995.1437.

179. Perfect JR, Rude TH, Wong B, Flynn T, Chaturvedi V, Niehaus W. 1996. Identification of a Cryptococcus neoformans gene that directly expresses the cryptocytic Saccharomyces cerevisiae mannnit dehydrogenase gene. J Bacteriol 178:5257–5262.

180. Poeschel EW, Himmelreich U, Mylonakis E, Rude T, Toffalletti D, Cox GM, Miller JL, Perfect JR. 2006. Characterization and regulation of the trehalase synthesis pathway and its importance in the pathogenicity of
198. Beverley SM, Owens KL, Showalter M, Griffith CL, Doering TL, Jones VC, McNeil MR. 2005. Eukaryotic UDP-galactopyranose mutase (GLF gene) in microbial and metazoal pathogens. Eukaryot Cell 4:1147–1154. http://dx.doi.org/10.1128/EC.4.6.1147-1154.2005.

199. Jacobson ES. 1987. Cryptococcal UDP-glucose dehydrogenase: enzymic control of capsular biosynthesis. J Med Vet Mycol 25:131–135. http://dx.doi.org/10.1080/02681218780000201.

200. Reilly MC, Levery SB, Castle SA, Klutts JS, Doering TL. 2009. A novel xylosylphosphotransferase activity discovered in Cryptococcus neoformans. J Biol Chem 284:36118–36127. http://dx.doi.org/10.1074/jbc.M109.056226.

201. Block ER, Jennings AE, Bennett JE. 1973. 5-Fluorocytosine resistance in Cryptococcus neoformans. Antimicrob Agents Chemother 3:649–656. http://dx.doi.org/10.1128/AAC.3.6.649.

202. Schwarz P, Janbon G, Dromer F, Lortholary O, Dannaoui E. 2007. Combination of amphotericin B with flucytosine is active in vitro against flucytosine-resistant isolates of Cryptococcus neoformans. Antimicrob Agents Chemother 51:383–385. http://dx.doi.org/10.1128/AAC.00446-06.