Published in final edited form as:

Curr Opin Microbiol. 2008 December ; 11(6): 511–516. doi:10.1016/j.mib.2008.09.018.

Fungal Adaptation to the Mammalian Host: It's a New World, After All

Nicole M. Cooney³ and Bruce S. Klein^{1,2,3,4}

¹ Department of Internal Medicine, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison, WI, 53792

² Department of Pediatrics, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison, WI, 53792

³ Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison, WI, 53792

⁴ Comprehensive Cancer Center, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison, WI, 53792

Summary

Adaptation to environmental conditions is key to fungal survival during infection of human hosts. Although the host immune system is often considered the primary obstacle to fungal colonization, invading fungi must also contend with extreme abiotic stresses. Recent work with human pathogenic fungi has uncovered systems for detecting and responding to changes in temperature, carbon source, metal ion availability, pH, and gas tension. These systems play a major role in adaptation to the host niche and are essential factors for persistence in a mammalian host. Future investigations into fungal responses to these and other abiotic components of the host environment have the potential to uncover novel targets for anti-fungal therapy.

Introduction

Fungi survive not in isolation but as inhabitants of environmental niches that are composed of both biotic and abiotic factors. Despite our tendency to think of a niche as a static, consistent environment, many of these factors are highly dynamic and in a constant state of flux. Therefore, for a fungus to survive in its niche, it must be able to adapt to these constantly changing parameters. To this end, fungi possess sensory and regulatory systems whereby a change in a specific environmental component can be detected to induce transcriptional and translational changes that promote survival under the newest environmental conditions.

For fungal pathogens of humans, the requirement for environmental adaptation is extreme, since the transition from a saprophytic lifestyle in the environment to a parasitic lifestyle within a mammalian host entails abrupt changes in a number of environmental parameters. When

Reprints and correspondence to: Bruce Klein, University of Wisconsin-Madison, Rm. 4303 Microbial Sciences Building, 1550 Linden Drive, Madison, WI 53792, 608-263-9217 (telephone), 608-262-8418 (Fax). Email: bsklein@wisc.edu.
Contact information for other authors: Nicole Cooney, University of Wisconsin-Madison, Rm. 4341 Microbial Sciences Building, 1550 Linden Drive, Madison, WI 53706, 608-263-6203, Email: ncooney@wisc.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

considering the interaction between host and pathogen, we commonly think of biotic host components like immune cells as the major antagonists to the survival of fungal pathogens. By considering the host as an environment with its own set of abiotic parameters, however, we can outline a different set of host factors with which pathogens must contend in order to successfully colonize their new niche. As saprophytes, fungi survive in an environment with a moderate ambient temperature and pH, a defined set of sources for essential nutrients like carbon and metal ions, and atmospheric concentrations of key gases like carbon dioxide and oxygen. Upon invasion of a human host, these environmental factors undergo an immediate and drastic change. Ambient temperature is suddenly replaced with the restrictively high temperature of the human body. Ambient pH is replaced with acidic mucosal surfaces or neutral blood and tissues. Familiar sources of carbon and metal ions are missing in an environment where essential nutrients are sequestered from microbes to support host survival. Carbon dioxide and oxygen concentrations are reversed in host tissues, leaving fungi to cope with hypoxia and high levels of carbon dioxide. Here we focus on recently identified systems for adaptation to the abiotic stresses that fungi encounter during colonization and infection of their human hosts and review the role of these systems in fungal survival and virulence.

Temperature Adaptation

Fungal survival at the elevated temperature of a human host is essential for virulence. Despite the universality of thermotolerance among human fungal pathogens, temperature adaptation within the host manifests itself in a variety of ways. The systemic dimorphic fungi *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, and *Penicillium marneffei* undergo a morphological change from hyphal mold to budding yeast. In contrast, *Candida albicans* converts from commensal yeast to invasive hyphae. The fungal pathogens *Cryptococcus neoformans* and *Aspergillus fumigatus* are simply better able to survive at 37°C than their non-pathogenic counterparts.

A *Saccharomyces cerevisiae* pathway controlling filamentous growth during nitrogen starvation has recently been implicated in the survival of pathogenic fungi at 37°C. In this pathway, a G protein-coupled receptor activates a Ras GTPase, triggering the guanine nucleotide exchange factor Cdc24p to activate the GTPase Cdc42p. Downstream signaling, initiated by the Ste20p kinase, triggers transcriptional changes that mediate filamentous growth (1). In *C. neoformans*, Ras1 and Cdc24 mutants show reduced growth at elevated temperatures and are attenuated in a mouse model of cryptococcosis (2). In *P. brasiliensis*, Ras1 expression responds to heat shock, and inhibition of farnesylation, an essential modification of Ras1, causes filamentous growth at 37°C, suggesting that Ras1 is essential for thermal dimorphism (3). An exciting study with *P. marneffei* revealed a temperature-dependent role for the Ste20p homologue PakA in conidial germination. PakA mutants showed minor defects in filamentous germination at ambient temperature, but were severely impaired during germination and subsequent growth as budding yeast at 37°C. This defect was also apparent during growth in host macrophages, suggesting that activation of PakA at 37°C could play a role in virulence (4).

Other fungal proteins have also been implicated in adaptation to elevated temperature. A recent review in the pages of this journal highlighted the identification of Drk1, a hybrid histidine kinase that is essential for growth of *B. dermatitidis* as a yeast at 37°C. This kinase controls expression of the virulence factors Bad1 and α -(1,3)-glucan and is essential for virulence (5, 6). More recently, work in *H. capsulatum* identified Ryp1, a homologue of the *C. albicans* transcriptional regulator Wor1, as essential for thermal dimorphism in response to elevated temperature. Ryp1 binds its own promoter and likely acts as an autoregulatory transcriptional regulator in *H. capsulatum* as it does in *C. albicans*. Ryp1 mutants grow as hyphae and are unable to induce expression of the vast majority of yeast phase-specific genes at 37°C (7).

Because yeast phase-specific genes include most known virulence factors for this fungus, Ryp1 is likely to be essential for initiation of histoplasmosis.

Carbon Metabolism

Fungal survival in any environment requires assimilation of available carbon via metabolic enzymes. It was shown in 2001 that *C. albicans* requires isocitrate lyase (ICL), a key component of the glyoxylate cycle, for full virulence (8). This suggested that in the host, rich carbon sources like glucose, which repress ICL expression, are limited and *C. albicans* activates the glyoxylate cycle for utilization of poor carbon sources like acetate and fatty acids. This hypothesis, however, was contradictory to the fact that glucose levels in the blood are high enough to prevent ICL expression. The use of a *C. albicans* strain expressing GFP behind the ICL promoter demonstrated that ICL expression in the host is confined to specific micro-environments. ICL expression is repressed in the blood, but induced in the nutrient-limited phagosome of immune cells (9). The role of alternative carbon metabolism in *C. albicans* virulence was further underscored by the elucidation of a network of carbon utilization genes, including those that mediate the glyoxylate cycle, fatty acid oxidation, and gluconeogenesis, which are all required for persistence in the host (10).

Other fungal pathogens have also been shown to upregulate glyoxylate cycle genes during host-simulative conditions. Yeast phase cells of *P. brasiliensis* have increased transcript levels of both glyoxylate cycle enzymes after exposure to host macrophages (11). This suggests that in *P. brasiliensis*, as in *C. albicans*, alternative carbon metabolism plays a role in survival within the host. Work in *P. marneffei* demonstrated a unique temperature-dependent regulation of ICL transcription. In known models of ICL regulation, transcription is induced by acetate and fatty acids through the FacB transcriptional activator (12). In *P. marneffei*, both FacB-dependent acetate induction and AbaA-dependent temperature induction of ICL were observed (13). This finding represents a novel mechanism for regulation of this important metabolic gene in a thermally dimorphic intracellular pathogen.

Increased expression of ICL was also observed in *A. fumigatus* after exposure to host macrophages, leading to the hypothesis that ICL would be required for virulence (14). Surprisingly, subsequent work demonstrated that ICL is dispensable for virulence (15). Further investigation revealed that *A. fumigatus* ICL mutants were still able to grow *in vitro* with lipids as the sole carbon source (16). This suggested that *Aspergillus* might possess additional mechanisms for fatty acid utilization that compensate for loss of ICL during infection. A recent microarray study exploring transcriptional changes in *A. fumigatus* spores upon exposure to host neutrophils demonstrated upregulation of dozens of genes involved in carbon metabolism (17). This pool of genes is likely to contain proteins required for fatty acid utilization in the host and for the development of invasive aspergillosis.

Iron Acquisition

Within the host, iron is sequestered from microbes by iron carrier proteins, creating an iron-limited environment in which fungal pathogens must encode mechanisms for iron acquisition in order to survive (18). Many fungi employ siderophores, high affinity iron chelators, to efficiently bind host iron in the extracellular space and safely store it within the fungal cytoplasm. Recent work in *H. capsulatum* identified the siderophore synthesis gene *SIDI* as an essential factor for growth in low iron environments. This growth defect was extended to include defects in both intracellular replication and virulence within mice (19). Additionally, the iron-dependent transcriptional repressor Sre1 was shown to repress transcription of a suite of siderophore synthesis genes in *H. capsulatum* (20). Recent work in *A. fumigatus* demonstrated for the first time that different types of siderophores are employed for distinct

purposes in different developmental stages and niches, with all identified siderophores being essential for virulence (21).

Fungal pathogens must also possess mechanisms for controlling and coordinating the utilization of acquired iron. The recent identification of the transcriptional regulator HapX in *A. nidulans* has provided insight into a mechanism by which fungi can adapt to iron limitation by reducing iron utilization. HapX acts primarily during iron-depleted conditions as a transcriptional repressor for genes that utilize iron, effectively reducing the cell's iron requirement (22). A related protein in *C. albicans* called Hap43, however, was found to act as a transcriptional activator for the ferric reductases, which are crucial for the removal and utilization of iron from chelators including both siderophores and host carrier proteins (23). A novel mechanism by which *C. albicans* scavenges iron from host hemoglobin was also recently described. Receptor-mediated endocytosis of hemoglobin facilitates extraction of iron, likely by a heme oxygenase in the vacuole (24). This hemoglobin utilization system represents an additional iron acquisition system that will likely be linked to persistence of *C. albicans* within the host.

pH Adaptation

Within human hosts fungal pathogens must adapt to changing pH levels, which range from acidic mucosal surfaces to neutral or slightly alkaline blood and tissues. One pathway implicated in fungal responses to changing pH involves activation of the transcription factor PacC/Rim101 (25). PacC is essential for *A. nidulans* virulence in a model of pulmonary aspergillosis (26). Rim101 is essential for *C. albicans* virulence in models of mucosal invasion and systemic candidiasis (27). PacC/Rim101 is activated downstream of a signaling cascade involving a plasma membrane complex, an endosomal membrane complex, and the proteasome.

The proteins of the plasma membrane signaling complex undergo pH-dependent activation. PalI/Rim9 is responsible for localization of the pH-sensor PalH/Rim21 to the plasma membrane (28). This facilitates PalH-dependent phosphorylation and ubiquitylation of PalF/Rim8. PalF is an arrestin-like protein thought to trigger endocytosis of the plasma membrane complex upon PalH-dependent modification, mediating transduction of the pH signal from the plasma membrane to the endosomal membrane (29). PalH is also required for localization of the endosomal complex protein PalC to the endosomal membrane (30). This occurs through interaction of PalC with the endosomal sorting complex protein Vps32, which is essential for both the pH response and the virulence of *C. albicans* (31). Vps32 also interacts with endosomal membrane complex proteins PalA/Rim20 and PalB/Rim13. PalA recruits the inactive transcription factor PacC to the endosomal membrane complex at neutral or alkaline pH, facilitating cleavage of PacC to a smaller precursor by the protease PalB (25). In the final processing step, PacC is cleaved from inactive precursor to active transcription factor by the proteasome (32). Active PacC translocates to the nucleus and mediates expression changes of pH-responsive genes.

Several effector proteins downstream of PacC/Rim101 are essential for adaptation to the host. In *A. nidulans*, activation of PacC induces upregulation of iron acquisition system genes including siderophore biosynthesis genes (33). Similarly, expression of ferric reductase genes occurs downstream of Rim101 activation in *C. albicans* (23). This links the pH response to known virulence factors and is likely one reason why PacC/Rim101 mutants show reduced virulence. Mucosal invasion by *C. albicans* requires degradation of epithelial cell junctions by the protease Sap5p, which is upregulated downstream of Rim101. Expression of Sap5p in a Rim101 mutant strain restored the ability of *C. albicans* to invade an epithelial barrier in an *in vitro* model (34). Changes in cell wall composition also occur downstream of Rim101

activation in *C. albicans*, with over-expression of several cell wall-modulating proteins partially restoring virulence to a Rim101 mutant strain (35). These results indicate the importance of pH sensing in fungal adaptation to mammalian hosts and highlight components of the pH signaling cascade as potential drug targets.

Gas Tension

Carbon Dioxide

During human infection, fungi are exposed to carbon dioxide (CO₂) concentrations from low atmospheric CO₂ on epithelial surfaces to higher physiological CO₂ within host tissues. Work in *C. albicans* and *C. neoformans* identified carbonic anhydrase (CA) and adenylyl cyclase (AC) as CO₂ sensors. Conversion of atmospheric CO₂ to bicarbonate by CA is essential for fatty acid biosynthesis and growth. Physiological CO₂ levels, however, spontaneously generate sufficient bicarbonate for both growth and stimulation of AC. This activates the cAMP pathway and initiates filamentation and expression of virulence traits (36). Accordingly, CA is required for virulence in models with ambient CO₂ levels, such as *C. albicans* epithelial invasion (37). In contrast, CA is dispensable and AC is essential in models with elevated CO₂, such as systemic infection by both *C. albicans* and *C. neoformans* (37,38,39). Recent work implicates differential regulation of the cAMP-repressor NRG1 in response to host-specific factors in the differential virulence of *Candida* species. Host-specific cues like elevated CO₂ induce *C. albicans* downregulation of NRG1 and activation of the cAMP-dependent filamentation pathway. Non-pathogenic *C. dubliniensis* fails to downregulate NRG1 and maintains cAMP repression. Deletion of *C. dubliniensis* NRG1 increases virulence of this species in several infection models, indicating that activation of cAMP signaling in response to abiotic components of the host environment is a major determinant of virulence in *Candida* species (40).

Oxygen

In order to maintain metabolic and biosynthetic functions in the host, fungal pathogens must also be able to adapt to hypoxia within host tissues. Oxygen levels in mammalian tissues are below atmospheric levels. Moreover, inflammation, thrombosis, and necrosis associated with infection are thought to increase degrees of hypoxia. In *C. albicans*, the response to hypoxia depends on the coordination of specific transcriptional regulators. In hypoxic conditions, the transcription factor Ace2 represses oxidative metabolic processes and promotes filamentation (41). The transcriptional regulator Egl1p, however, antagonizes Ace2 by repressing filamentation during hypoxia (42). The hypoxic environment of the vaginal mucosa has been shown to induce iron uptake protein expression, likely linking the hypoxic response to both iron acquisition and virulence in *C. albicans* (43). In *C. neoformans*, two additional players in hypoxic adaptation have recently been described. The hybrid histidine kinase Tco1 is required for hypoxic growth and appears to play a post-transcriptional role in the hypoxia response (44). Sre1, a conserved regulator of sterol biosynthesis, is required for transcriptional changes that promote growth under hypoxic conditions. Sre1 is activated by proteolytic cleavage that likely occurs in response to reduced activity of oxygen-requiring sterol synthesis enzymes during hypoxia. Two independent laboratories have demonstrated an indispensable role for Sre1 in both hypoxic adaptation and virulence in a mouse model for cryptococcosis (44,45).

Conclusions

All fungal pathogens must possess systems for sensing and responding to changes in abiotic factors during colonization of human hosts. Where tested, these systems have proved essential for normal colonization and full virulence. It is thus clear that components of the fungal response to abiotic factors within the host are promising targets for anti-fungal drugs.

Identification of factors divergent from or absent in mammalian cells will maximize the potential for successful human therapies. The advantages of thinking of the host as a set of environmental conditions become even more apparent if we consider other abiotic stimuli that have not been discussed in this review. In addition to the specific sensory and regulatory systems reviewed above, fungal pathogens must also adapt to changes in nitrogen, calcium, magnesium, and copper sources, pressure, and fluid flow rates. Viewing the host as a new environment for an invading pathogen will foster a more complete understanding of fungal pathogenesis and the advent of novel targets for anti-fungal drugs (Box 1).

BOX

Therapeutic Applications

One goal of studying fungal adaptation to mammalian hosts is the development of potential anti-fungal therapies. This goal came into the spotlight in 1994 when the anti-malarial drug chloroquine was shown to inhibit the growth of *H. capsulatum* within host macrophages. Upon supplementation with iron, intracellular growth was restored, indicating that chloroquine's iron-chelating ability was responsible for the growth restriction. Acquisition of iron within mice proved essential for virulence, as chloroquine treatment led to a resolution of infection (46). Subsequently, chloroquine proved effective against *C. neoformans* and *P. marneffei*, but this time through its impact on a different aspect of the host environment: hydrogen ion concentration. In each of these cases, chloroquine's alkalizing activity was essential for restriction of fungal growth (47,48). Most recently, the story has come full circle, as chloroquine restricts intracellular growth of *P. brasiliensis* in an iron-dependent manner and is therapeutic in a mouse model for paracoccidoidomycosis (49,50). Chloroquine has thus emerged as a drug with broad anti-fungal capacity that acts by altering abiotic parameters of the host environment. These discoveries highlight the clinical importance of understanding the impact of abiotic stresses on fungi during persistence within mammalian hosts.

Acknowledgements

The authors are supported by research project grants and T32 training grant funds from the National Institutes of Health and by a grant from the Hartwell Foundation.

References

1. Pan X, Harashima T, Heitman J. Signal transduction cascades regulating pseudohyphal differentiation of *Saccharomyces cerevisiae*. *Curr Opin Microbiol* 2000;3:567–572. [PubMed: 11121775]
2. Nichols CB, Perfect ZH, Alspaugh JA. A Ras1-Cdc24 signal transduction pathway mediates thermotolerance in the fungal pathogen *Cryptococcus neoformans*. *Mol Microbiol* 2007;63:1118–1130. [PubMed: 17233829]
3. Fernandes L, Paes HC, Tavares AH, Silva SS, Dantas A, Soares CM, Torres FA, Felipe MS. Transcriptional profile of *ras1* and *ras2* and the potential role of farnesylation in the dimorphism of the human pathogen *Paracoccidioides brasiliensis*. *FEMS Yeast Res* 2008;8:300–310. [PubMed: 17927766]
4. Boyce KJ, Andrianopoulos A. A p21-activated kinase is required for conidial germination in *Penicillium marneffei*. *PLoS Pathog* 2007;3:e162. [PubMed: 17983267] This work identifies a downstream effector of the Ras1 signaling pathway as a novel regulator of temperature-dependent transcriptional and developmental changes in a thermally dimorphic fungus
5. Nemecek JC, Wuthrich M, Klein BS. Global control of dimorphism and virulence in fungi. *Science* 2006;312:583–588. [PubMed: 16645097]
6. Klein BS, Tebbets B. Dimorphism and virulence in fungi. *Curr Opin Microbiol* 2007;10:314–319. [PubMed: 17719267]

- 7••. Nguyen VQ, Sil A. Temperature-induced switch to the pathogenic yeast form of *Histoplasma capsulatum* requires Ryp1, a conserved transcriptional regulator. *Proc Natl Acad Sci U S A* 2008;105:4880–4885. [PubMed: 18339808] This work identifies a global transcriptional regulator that is required for morphogenesis and expression of yeast-phase specific genes in a dimorphic fungal pathogen
8. Lorenz MC, Fink GR. The glyoxylate cycle is required for fungal virulence. *Nature* 2001;412:83–86. [PubMed: 11452311]
- 9•. Barelle CJ, Priest CL, Maccallum DM, Gow NA, Odds FC, Brown AJ. Niche-specific regulation of central metabolic pathways in a fungal pathogen. *Cell Microbiol* 2006;8:961–971. [PubMed: 16681837] This work reveals distinct patterns of expression for glyoxylate cycle genes in different micro-environments within the host of a fungal pathogen
10. Ramirez MA, Lorenz MC. Mutations in alternative carbon utilization pathways in *Candida albicans* attenuate virulence and confer pleiotropic phenotypes. *Eukaryot Cell* 2007;6:280–290. [PubMed: 17158734]
11. Derengowski LS, Tavares AH, Silva S, Procopio LS, Felipe MS, Silva-Pereira I. Upregulation of glyoxylate cycle genes upon *Paracoccidioides brasiliensis* internalization by murine macrophages and in vitro nutritional stress condition. *Med Mycol* 2008;46:125–134. [PubMed: 18324491]
12. Todd RB, Kelly JM, Davis MA, Hynes MJ. Molecular characterization of mutants of the acetate regulatory gene *facB* of *Aspergillus nidulans*. *Fungal Genet Biol* 1997;22:92–102. [PubMed: 9367656]
13. Canovas D, Andrianopoulos A. Developmental regulation of the glyoxylate cycle in the human pathogen *Penicillium marneffei*. *Mol Microbiol* 2006;62:1725–1738. [PubMed: 17427290]
14. Ebel F, Schwienbacher M, Beyer J, Heesemann J, Brakhage AA, Brock M. Analysis of the regulation, expression, and localisation of the isocitrate lyase from *Aspergillus fumigatus*, a potential target for antifungal drug development. *Fungal Genet Biol* 2006;43:476–489. [PubMed: 16603391]
15. Schobel F, Ibrahim-Granet O, Ave P, Latge JP, Brakhage AA, Brock M. *Aspergillus fumigatus* does not require fatty acid metabolism via isocitrate lyase for development of invasive aspergillosis. *Infect Immun* 2007;75:1237–1244. [PubMed: 17178786]
16. Olivas I, Royuela M, Romero B, Monteiro MC, Minguez JM, Laborda F, De Lucas JR. Ability to grow on lipids accounts for the fully virulent phenotype in neutropenic mice of *Aspergillus fumigatus* null mutants in the key glyoxylate cycle enzymes. *Fungal Genet Biol* 2008;45:45–60. [PubMed: 17616408]
- 17•. Sugui JA, Kim HS, Zarembek KA, Chang YC, Gallin JI, Nierman WC, Kwon-Chung KJ. Genes differentially expressed in conidia and hyphae of *Aspergillus fumigatus* upon exposure to human neutrophils. *PLoS ONE* 2008;3:e2655. [PubMed: 18648542] This work uses a microarray to identify carbon utilization genes as a major component of the transcriptional response of a fungal pathogen to host cells.
18. Haas H, Eisendle M, Turgeon BG. Siderophores in Fungal Physiology and Virulence. *Annu Rev Phytopathol* 2008;46:149–187. [PubMed: 18680426]
19. Hwang LH, Mayfield JA, Rine J, Sil A. *Histoplasma* requires SID1, a member of an iron-regulated siderophore gene cluster, for host colonization. *PLoS Pathog* 2008;4:e1000044. [PubMed: 18404210]
20. Chao LY, Marletta MA, Rine J. Sre1, an iron-modulated GATA DNA-binding protein of iron-uptake genes in the fungal pathogen *Histoplasma capsulatum*. *Biochemistry* 2008;47:7274–7283. [PubMed: 18549241]
21. Schrettl M, Bignell E, Kragl C, Sabiha Y, Loss O, Eisendle M, Wallner A, Arst HN Jr, Haynes K, Haas H. Distinct roles for intra- and extracellular siderophores during *Aspergillus fumigatus* infection. *PLoS Pathog* 2007;3:1195–1207. [PubMed: 17845073]
22. Hortschansky P, Eisendle M, Al-Abdallah Q, Schmidt AD, Bergmann S, Thon M, Kniemeyer O, Abt B, Seeber B, Werner ER, et al. Interaction of HapX with the CCAAT-binding complex--a novel mechanism of gene regulation by iron. *EMBO J* 2007;26:3157–3168. [PubMed: 17568774]
23. Baek YU, Li M, Davis DA. *Candida albicans* ferric reductases are differentially regulated in response to distinct forms of iron limitation by the Rim101 and CBF transcription factors. *Eukaryot Cell* 2008;7:1168–1179. [PubMed: 18503007]

- 24•. Weissman Z, Shemer R, Conibear E, Kornitzer D. An endocytic mechanism for haemoglobin-iron acquisition in *Candida albicans*. *Mol Microbiol* 2008;69:201–217. [PubMed: 18466294] This paper identifies a novel pathway by which a fungal pathogen acquires iron from host hemoglobin.
25. Penalva MA, Tilburn J, Bignell E, Arst HN Jr. Ambient pH gene regulation in fungi: making connections. *Trends Microbiol* 2008;16:291–300. [PubMed: 18457952]
26. Bignell E, Negrete-Urtasun S, Calcagno AM, Haynes K, Arst HN Jr, Rogers T. The *Aspergillus* pH-responsive transcription factor PacC regulates virulence. *Mol Microbiol* 2005;55:1072–1084. [PubMed: 15686555]
27. Davis D, Edwards JE Jr, Mitchell AP, Ibrahim AS. *Candida albicans* RIM101 pH response pathway is required for host-pathogen interactions. *Infect Immun* 2000;68:5953–5959. [PubMed: 10992507]
28. Calcagno-Pizarelli AM, Negrete-Urtasun S, Denison SH, Rudnicka JD, Bussink HJ, Munera-Huertas T, Stanton L, Hervas-Aguilar A, Espeso EA, Tilburn J, et al. Establishment of the ambient pH signaling complex in *Aspergillus nidulans*: PalI assists plasma membrane localization of PalH. *Eukaryot Cell* 2007;6:2365–2375. [PubMed: 17951518]
29. Herranz S, Rodriguez JM, Bussink HJ, Sanchez-Ferrero JC, Arst HN Jr, Penalva MA, Vincent O. Arrestin-related proteins mediate pH signaling in fungi. *Proc Natl Acad Sci U S A* 2005;102:12141–12146. [PubMed: 16099830]
- 30•. Galindo A, Hervas-Aguilar A, Rodriguez-Galan O, Vincent O, Arst HN Jr, Tilburn J, Penalva MA. PalC, one of two Bro1 domain proteins in the fungal pH signalling pathway, localizes to cortical structures and binds Vps32. *Traffic* 2007;8:1346–1364. [PubMed: 17696968] This work is the first to investigate the role of PalC in the fungal pH signaling cascade and identifies this protein as a potential bridge between the plasma membrane and endosomal signaling complexes.
31. Cornet M, Bidard F, Schwarz P, Da Costa G, Blanchin-Roland S, Dromer F, Gaillardin C. Deletions of endocytic components VPS28 and VPS32 affect growth at alkaline pH and virulence through both RIM101-dependent and RIM101-independent pathways in *Candida albicans*. *Infect Immun* 2005;73:7977–7987. [PubMed: 16299290]
32. Hervas-Aguilar A, Rodriguez JM, Tilburn J, Arst HN Jr, Penalva MA. Evidence for the direct involvement of the proteasome in the proteolytic processing of the *Aspergillus nidulans* zinc finger transcription factor PacC. *J Biol Chem* 2007;282:34735–34747. [PubMed: 17911112]
33. Eisendle M, Oberegger H, Buttinger R, Illmer P, Haas H. Biosynthesis and uptake of siderophores is controlled by the PacC-mediated ambient-pH Regulatory system in *Aspergillus nidulans*. *Eukaryot Cell* 2004;3:561–563. [PubMed: 15075286]
34. Villar CC, Kashleva H, Nobile CJ, Mitchell AP, Dongari-Bagtzoglou A. Mucosal tissue invasion by *Candida albicans* is associated with E-cadherin degradation, mediated by transcription factor Rim101p and protease Sap5p. *Infect Immun* 2007;75:2126–2135. [PubMed: 17339363]
35. Nobile CJ, Solis N, Myers CL, Fay AJ, Deneault JS, Nantel A, Mitchell AP, Filler SG. *Candida albicans* transcription factor Rim101 mediates pathogenic interactions through cell wall functions. *Cell Microbiol*. 2008
36. Bahn YS, Muhlschlegel FA. CO₂ sensing in fungi and beyond. *Curr Opin Microbiol* 2006;9:572–578. [PubMed: 17045514]
37. Klengel T, Liang WJ, Chaloupka J, Ruoff C, Schroppel K, Naglik JR, Eckert SE, Mogensen EG, Haynes K, Tuite MF, et al. Fungal adenylyl cyclase integrates CO₂ sensing with cAMP signaling and virulence. *Curr Biol* 2005;15:2021–2026. [PubMed: 16303561]
38. Bahn YS, Cox GM, Perfect JR, Heitman J. Carbonic anhydrase and CO₂ sensing during *Cryptococcus neoformans* growth, differentiation, and virulence. *Curr Biol* 2005;15:2013–2020. [PubMed: 16303560]
39. Mogensen EG, Janbon G, Chaloupka J, Steegborn C, Fu MS, Moyrand F, Klengel T, Pearson DS, Geeves MA, Buck J, et al. *Cryptococcus neoformans* senses CO₂ through the carbonic anhydrase Can2 and the adenylyl cyclase Cac1. *Eukaryot Cell* 2006;5:103–111. [PubMed: 16400172]
- 40•. Moran GP, MacCallum DM, Spiering MJ, Coleman DC, Sullivan DJ. Differential regulation of the transcriptional repressor NRG1 accounts for altered host-cell interactions in *Candida albicans* and *Candida dubliniensis*. *Mol Microbiol* 2007;66:915–929. [PubMed: 17927699] This work demonstrates that cAMP-dependent filamentation in response to host specific cues plays a major role in the virulence of different *Candida* species.

41. Mulhern SM, Logue ME, Butler G. *Candida albicans* transcription factor Ace2 regulates metabolism and is required for filamentation in hypoxic conditions. *Eukaryot Cell* 2006;5:2001–2013. [PubMed: 16998073]
42. Setiadi ER, Doedt T, Cottier F, Noffz C, Ernst JF. Transcriptional response of *Candida albicans* to hypoxia: linkage of oxygen sensing and Efg1p-regulatory networks. *J Mol Biol* 2006;361:399–411. [PubMed: 16854431]
43. Sosinska GJ, de Groot PW, Teixeira de Mattos MJ, Dekker HL, de Koster CG, Hellingwerf KJ, Klis FM. Hypoxic conditions and iron restriction affect the cell-wall proteome of *Candida albicans* grown under vagina-simulative conditions. *Microbiology* 2008;154:510–520. [PubMed: 18227255]
44. Chun CD, Liu OW, Madhani HD. A link between virulence and homeostatic responses to hypoxia during infection by the human fungal pathogen *Cryptococcus neoformans*. *PLoS Pathog* 2007;3:e22. [PubMed: 17319742] This paper formally identifies the hypoxia response and two of its regulators as essential for virulence of a fungal pathogen.
45. Chang YC, Bien CM, Lee H, Espenshade PJ, Kwon-Chung KJ. Sre1p, a regulator of oxygen sensing and sterol homeostasis, is required for virulence in *Cryptococcus neoformans*. *Mol Microbiol* 2007;64:614–629. [PubMed: 17462012]
46. Newman SL, Gootee L, Brunner G, Deepe GS Jr. Chloroquine induces human macrophage killing of *Histoplasma capsulatum* by limiting the availability of intracellular iron and is therapeutic in a murine model of histoplasmosis. *J Clin Invest* 1994;93:1422–1429. [PubMed: 8163646]
47. Levitz SM, Harrison TS, Tabuni A, Liu X. Chloroquine induces human mononuclear phagocytes to inhibit and kill *Cryptococcus neoformans* by a mechanism independent of iron deprivation. *J Clin Invest* 1997;100:1640–1646. [PubMed: 9294133]
48. Taramelli D, Tognazioli C, Ravagnani F, Leopardi O, Giannulis G, Boelaert JR. Inhibition of intramacrophage growth of *Penicillium marneffei* by 4-aminoquinolines. *Antimicrob Agents Chemother* 2001;45:1450–1455. [PubMed: 11302809]
49. Dias-Melicio LA, Moreira AP, Calvi SA, Soares AM. Chloroquine inhibits *Paracoccidioides brasiliensis* survival within human monocytes by limiting the availability of intracellular iron. *Microbiol Immunol* 2006;50:307–314. [PubMed: 16625052]
50. Dias-Melicio LA, Calvi SA, Bordon AP, Golim MA, Peracoli MT, Soares AM. Chloroquine is therapeutic in murine experimental model of paracoccidioidomycosis. *FEMS Immunol Med Microbiol* 2007;50:133–143. [PubMed: 17456179]