Published in final edited form as:

Med Mycol. 2009 ; 47(Suppl 1): S72–S79. doi:10.1080/13693780802455313.

***Aspergillus fumigatus* metabolism: Clues to mechanisms of *in vivo* fungal growth and virulence**

Sven D. Willger[#], Nora Grahl[#], and Robert A. Cramer Jr.^{*}

Department of Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717

Summary

Aspergillus fumigatus is a saprophytic fungus commonly found in soil and compost piles. In immunocompromised patients it takes on a sinister form as a potentially lethal opportunistic human pathogen. We currently have a limited understanding of the *in vivo* growth mechanisms used by *A. fumigatus* during invasive pulmonary aspergillosis (IPA). The ability of *A. fumigatus* to adapt to various microenvironments encountered during growth in the human host may explain why *A. fumigatus* is the most frequently occurring opportunistic pathogenic mold. The transcriptional and metabolic responses to changing microenvironments found in the mammalian lung require the activation of pathways implicated in resistance to unique stresses. Thus, the production of primary metabolites *in vivo* may give clues to the critical pathways used by *A. fumigatus* to cause disease in human hosts. We recently have identified primary metabolites in the mammalian lung typically associated with fungal growth under hypoxic environments suggesting that *A. fumigatus* may encounter low oxygen tensions during IPA. These and other studies on *A. fumigatus* metabolism are the focus of this review.

Keywords

Aspergillus fumigatus; hypoxia; fungal growth; invasive aspergillosis; metabolism; virulence

Introduction

Aspergillus fumigatus is a saprophytic, asexual reproducing fungus commonly found in soil and compost piles. Its primary ecological function is to recycle carbon and nitrogen through the environment [1-3]. However with the rising number of immunocompromised patients, *A. fumigatus* has become a leading cause of mortality in this patient population [4,5]. While *A. fumigatus* is responsible for a number of clinically relevant diseases, invasive pulmonary aspergillosis (IPA) is the most lethal with mortality rates ranging from 60-90% [4,6]. *A. fumigatus* is the most prevalent causal organism of IPA, but we still do not understand how the fungus survives and thrives in the harsh microenvironments of the mammalian lung.

To cause disease, *A. fumigatus* must face and overcome a number of *in vivo* challenges once it is inhaled in the mammalian host (Fig. 1). It has been proposed that *A. fumigatus* possess unique virulence factors, which allow it to cause disease, but arguably no unique *A. fumigatus* virulence factor has been discovered. It seems most likely then, that *A. fumigatus* possess robust adaptive attributes that allow it to thrive in immunocompromised patients [2, 7]. From an evolutionary perspective, these attributes are not true virulence factors (i.e. factors

^{*}Corresponding author: Robert A. Cramer Jr., Ph.D., PO Box 173610, Bozeman, MT 59717, USA, Tel: +1 (406) 994 7467, FAX: +1 (406) 994 4303, rcramer@montana.edu.

[#]These authors contributed equally to this work

that have arisen from selective pressures to overcome and evade a host defense response); however, they allow *A. fumigatus* to cause disease in mammalian hosts and likely differentiate it from other saprophytic moulds not associated with invasive mycoses.

Mammalian organisms present a broad variety of microenvironments in which *A. fumigatus* must survive to cause disease. These microenvironments, from the initial engulfment of conidia by alveolar macrophages and neutrophils to the hyphal invasion of alveoli in the lung, place unique stresses on the fungal organism. How does the fungus obtain the necessary nutrients, and what are these nutrient sources, to survive *in vivo*? How does the fungus overcome the low oxygen tensions typically found at sites of inflammation and infection to persist and cause disease? The focus of this short review is on recent findings that are beginning to elucidate the *in vivo* requirements for fungal growth in mammalian hosts. We conclude the review with a brief discussion of a recent finding by our laboratory on the production of primary metabolites associated with fungal growth in hypoxic environments in a mouse model of IPA.

Primary Metabolism and *in vivo* fungal growth

During invasive growth, *A. fumigatus* has to deal with unique microenvironments found in the mammalian lung, and further, these environments can rapidly change depending on the current stage of the infection. Thus, the metabolic and developmental response to changing environments *in vivo* requires the transcription of specific genes and the subsequent translation of the mRNA into proteins. For example, amino acids are required for translation and necessary for fungal growth and development. Fungi get amino acids from their environment either by transport processes, from precursors, which are derivatives of carbon and nitrogen primary metabolism, or from degradation of proteins which are no longer required under specific conditions. All three processes have to be coordinated and are carefully regulated in fungi (reviewed in Braus et al. [8]).

In numerous yeasts as well as filamentous fungi, amino acid starvation activates a complex genetic network, the 'cross-pathway control' (CPC) system (also known as General Control [GC] of amino acid biosynthesis) [9-11]. This conserved system consists of a sensor kinase (eIF2 α kinase CpcC/Gcn2p) and a transcriptional activator protein (CpcA/Gcn4p). The sensor kinase detects the accumulation of uncharged tRNA molecules, which is a sign for amino acid depletion. During amino acid starvation, the sensor kinase is activated and leads to the down-regulation of general translation. At the same time, the expression of the transcriptional activator protein (CpcA/Gcn4p) is not repressed [12]. This activator induces the expression of many genes, for example, genes involved in the biosynthesis of amino acids, purines, pyrimidines, and vitamins, as well as genes involved in peroxisomes, amino acid transport, and mitochondrial carriers [13].

In *A. nidulans* the activation of the cross-pathway system results in impaired sexual fruit body formation [14], whereas in the human pathogenic dimorphic yeast *Candida albicans* Gcn4p affects filament formation [15]. The influence of this system on fungal pathogenicity has been studied in *A. fumigatus*. Krappmann et al. (2004) [16] observed that *A. fumigatus* requires the transcription factor CpcA during periods of amino acid starvation and for full disease development. These results suggest that *A. fumigatus* needs the cross-pathway control system to survive in specific microenvironments within the mammalian host. In addition, this result suggests that not all nutrients can be obtained from the host by the fungus during pathogenesis.

During pathogenesis, it appears that the biosynthesis of uracil/uridine and lysine is required by the fungus for *in vivo* growth. In *A. fumigatus*, two auxotrophic markers based on the biosynthesis of uracil/uridine and lysine are well characterized and used in generating genetically modified strains of the fungus. The strain lacking the *pyrG* gene encoding orotidine-5'-phosphate decarboxylase (and therefore auxotrophic for uridine or uracil) [17] and

the strain lacking the *lysF* gene encoding for homoaconitase (and therefore auxotrophic for lysine) [18], however, are not capable of establishing disease in experimental murine models of IPA. The *pyrG* mutant conidia remained ungerminated in alveolar macrophages [17]; whereas conidia from the *lysF* mutant strains were able to persist and germinate likely due to lysine stored in conidia [18]. However, lysine appears to be limited in the lung, because the Δ *lysF* strain was not able establish invasive hyphae and cause disease [18]. In both cases, full virulence could be restored by complementing the mutant gene or by supplementing the drinking water of the animals with the missing amino acid. These findings demonstrate the importance for the fungus to synthesize amino acids on its own with specific metabolic pathways or by uptake from the environment. However, as shown for lysine and uracil, not all amino acids are readily available in the mammalian host during infection.

It is also likely that *A. fumigatus* obtains important nutrients *in vivo* from destruction of host tissue. There are hints that *A. fumigatus* metabolizes proteins *in vivo* to satisfy the need for nutrients during infection [19]. The genome of *A. fumigatus* contains several genes most likely coding for proteases [20] and it has been shown that *A. fumigatus* produces several proteases during lung infections [21]. However, different proteases may play unique or overlapping roles during pathogenesis, and thus, this is a difficult hypothesis to obtain evidence for with traditional gene replacement technologies. Still, the advantage of *in vivo* protein degradation to the fungus is clear and can result in the allocation of all building blocks necessary for energy and biomass production.

Evidence that the degradation of amino acids is important in *A. fumigatus* pathogenesis was recently obtained from generation of a gene replacement mutant in a key enzyme in the methylcitrate cycle, methylcitrate synthase. Methylcitrate synthase is essential for the degradation of propionyl-CoA, which is a degradation product of valine, methionine and isoleucine [22-24] in fungi and some bacteria. Ibrahim-Granet et al. (2008) [19] observed that methylcitrate synthase has a strong impact on the pathogenicity of *A. fumigatus* which supports their hypothesis that proteins are indeed metabolized by the fungus during infection.

However, much less is known about potential carbon sources *in vivo* for the fungus. Based on *in vitro* data, the production of gliotoxin *in vivo* suggests access to simple, fermentable sugars such as glucose that stimulate production of this toxin. An additional potential source of carbon is via the glyoxylate cycle, a metabolic pathway occurring in plants and several microorganisms but not mammals. This pathway allows organisms to use fats for the synthesis of carbohydrates, which most vertebrates, including humans, cannot perform. Studies on the biological function of the glyoxylate cycle correlated its activity with the virulence of the bacterium *Mycobacterium tuberculosis* [25], the plant-pathogenic fungi *Leptosphaeria maculans* [26] and *Magnaporthe grisea* [27], and the human pathogenic yeast *C. albicans* [28,29]. Results from these studies and others concluded that fatty acids and lipids account for the major carbon sources in infected tissue [30]. It was therefore hypothesized that the glyoxylate cycle plays an important role during IPA.

Recent studies on *A. fumigatus* and *A. nidulans* have shown that the glyoxylate cycle is essential for growth when fatty acids or C2 compounds are the sole carbon source [31,32]. In contrast to other pathogens, however, this pathway does not appear to be important for the development of IPA in a murine infection model as isocitrate lyase mutants are still capable of causing disease [33,34]. This is similar to the finding that isocitrate lyase was not required for pathogenesis by the human pathogenic yeast *Cryptococcus neoformans* [35]. These results imply that *A. fumigatus* has access to other carbon sources *in vivo*, and is not dependent upon fatty acids or C2 compounds to cause disease.

Metabolism, however, cannot proceed without energy, and thus the focus of our next discussion is on how *A. fumigatus*, an obligate aerobe, may generate energy *in vivo* during fungal pathogenesis to drive the metabolic pathways required for fungal growth and disease development.

Fungal Respiration *in vivo*

During infection, *A. fumigatus* causes significant damage to host tissue, and when present, recruitment of effector cells to sites of inflammation (Fig. 2). While direct measurements have not been taken during *A. fumigatus* infections, sites of inflammation are known to contain significantly low levels of oxygen. Most eukaryotic cells obligatorily use oxygen to carry out many of their biochemical reactions. Oxygen is a key component of energy production where it functions as a terminal electron acceptor in the formation of ATP from glucose during aerobic respiration. Thus, during an infection, pathogens are often exposed to extremely low levels of oxygen, and oxygen levels can rapidly change depending upon the tissue infected and current status of the immune response.

Pathogens, therefore, must have alternative respiration pathways to generate energy in low oxygen environments found *in vivo* in mammalian hosts. In fact, respiratory flexibility, switching from aerobic respiration to various forms of anaerobic respiration has been implicated as an important virulence attribute in prokaryotic pathogens [36,37]. For example, the prokaryote *M. tuberculosis*, Shi et al. [38] used transcriptional profiling and specific gene deletions to show that respiratory flexibility was an essential component of *M. tuberculosis* adaptation to the host immune response. How pathogenic fungi respond to low oxygen tensions found *in vivo* at sites of infection is largely unknown.

In the model organism *Saccharomyces cerevisiae*, a mechanism of adaptation to hypoxic conditions has been described. In aerobic conditions, heme biosynthesis activates the transcriptional regulator Hap1p [39], which induces genes involved in respiration and oxidative stress-responses. In addition, the transcriptional repressors Rox1p and Mot3p are activated and repress transcription of genes required for hypoxic adaptation [40]. In hypoxic conditions, Rox1p and Mot3p are transcriptionally induced and this leads to transcription of genes involved in the adaptation to hypoxic conditions [41]. To allow expression of hypoxic genes, transcription factors are required that utilize Rox1p-binding sequences, low oxygen-response elements (LORE) or other regulatory elements within promoters [42,43]. Bioinformatic analyses of the *A. fumigatus* genome in our laboratory, however, have so far failed to identify a potential Rox1p homolog in this infectious mould. Given the facultative anaerobic life-style of *S. cerevisiae* and the obligate aerobic life-style of *A. fumigatus*, it is not surprising that mechanisms to deal with hypoxia are likely different in these fungi.

The human pathogenic yeast *C. albicans* also responds differently to hypoxic conditions than *S. cerevisiae*. Setiadi et. al [44] have determined the transcriptional response of to hypoxia and have shown that the *C. albicans* ScRox1 homolog, Rfg1, is not involved in the repression of hypoxic genes but has a function in filamentous growth. In addition, a homolog of ScHap1 could not be found in *C. albicans*. In this transcriptional screen, no global regulator could be identified, although Upc2, which was characterized as the *C. albicans* homolog of Sre1, a sterol regulatory element binding protein [45], was 1.6 fold upregulated [44].

The recent discovery of a putative oxygen sensor, Sre1, in the fission yeast *Schizosaccharomyces pombe*, however, has opened the door to discovering the potential role of hypoxia tolerance in eukaryotic fungal pathogenesis. In mammals, lipid homeostasis is regulated by a family of membrane-bound transcription factors designated sterol regulatory element-binding proteins (SREBPs). SREBPs directly activate the expression of more than 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triglycerides and

phospholipids [46]. Recently, a SREBP homolog in fungi, Sre1, was discovered in the yeast *S. pombe* [47]. As in mammalian cells, Sre1 activates sterol biosynthesis enzymes in fungi, which require oxygen, but apparently also acts as an indirect sensor of oxygen levels by regulating the transcription of genes required for adaptation to hypoxic environments in this yeast.

In the human fungal pathogen *C. neoformans*, a Sre1 ortholog has been identified and characterized [48,49]. A transcriptional profile of *C. neoformans* cells grown under normoxic conditions compared to cells grown under hypoxic conditions revealed that 347 transcripts showed changes in expression. Transcriptionally induced genes were involved in stress regulation, carbohydrate metabolism and respiration, and transcriptionally repressed genes included those involved in translation, vesicle trafficking and cell wall synthesis [49]. The authors also discovered that Sre1 is required for hypoxic induction of genes encoding for oxygen-dependent enzymes involved in ergosterol synthesis. Importantly, mutants in the SREBP pathway displayed defects in their ability to proliferate in host tissues and cause disease in a murine model of cryptococcosis [49]. Thus, the SREBP pathway has great potential to yield insights into how fungi adapt to and generate energy in hypoxic conditions *in vivo*.

The mechanism(s) of hypoxic adaptation in *A. fumigatus* are currently unknown. It seems likely that this saprophytic mould has evolved mechanisms to deal with low oxygen tensions commonly found in the soil and in compost piles. Preliminary transcriptional profiling experiments of *A. fumigatus* grown in hypoxic conditions has begun to reveal how this filamentous fungus adapts to hypoxia (Grahl et al. unpublished data). In these studies, we identified a putative Sre1 homolog in *A. fumigatus*, SrbA (SreA is already in use in *A. nidulans* for an unrelated protein). We hypothesize that this putative SREBP homolog plays an important role in adaptation to hypoxic microenvironments found *in vivo* during IPA. To address our hypothesis, we have generated an SrbA null mutant, which is no longer capable of growth in hypoxic conditions (Willger et al., unpublished data). Studies are underway to examine whether this SREBP signaling pathway is required for *A. fumigatus* pathogenesis, and how this signaling pathway may regulate genes involved in generating energy in hypoxic conditions.

Another potential mechanism for dealing with low oxygen levels is, of course, fermentation. It is known that the obligate aerobe *A. nidulans* can withstand significant periods of anaerobic stress by converting pyruvate to ethanol [50,51]. For *A. parasiticus* it has been shown that the fungus produces ethanol in shake cultures in response to anoxic conditions [52]. In addition, several other obligate aerobic filamentous fungi were also found to have the capability to survive prolonged periods of hypoxia presumably through switching their respiration to an alcohol fermentation pathway. Cultures of *A. fumigatus* grown on rich carbon sources such as glucose have also been reported to produce ethanol, indicating the presence of an alcohol fermentation pathway in this infectious mould [53,54].

Using a murine model of invasive pulmonary aspergillosis we recently have found by-products of alcohol fermentation in the lungs of *A. fumigatus* infected mice (Cramer et al., unpublished data). This suggests that *A. fumigatus* has switched from aerobic respiration (or at least partially utilizes) to alcohol fermentation due to the hypoxic conditions found at the site of infection. Alcohol fermentation should allow the fungus to replenish sources of NAD⁺ and thus generate ATP through continued functioning of glycolysis.

To test our hypothesis that *A. fumigatus* utilizes alcohol fermentation in hypoxic conditions, we grew two wild-type strains under normoxic and hypoxic (1% O₂) conditions on minimal medium with glucose, ethanol, or glycerol as the sole carbon source (Fig. 3). Our results suggest that both wild-type strains of *A. fumigatus*, AF293 and CEA10, grow well under hypoxic

conditions with glucose as the sole carbon source (Fig. 3). Interestingly, CEA10 does not produce conidia under hypoxic conditions and seems to grow faster than strain AF293. Importantly, however, both strains grew less when glycerol, a non-fermentable carbon source, was the sole carbon source under hypoxic conditions. While growth was not completely inhibited, the significant reduction in growth suggests that *A. fumigatus* utilizes fermentation as one mechanism to adapt to hypoxic conditions. We currently are testing mutants in the putative alcohol fermentation pathway to further explore our hypotheses (Grah et al., unpublished data).

Another oxygen-independent energy-producing metabolic pathway that supports anaerobic growth of yeasts and filamentous fungi is ammonia fermentation. This pathway has recently been found to be widely distributed among fungi [55]. Ammonia fermentation consists of the dissimilatory reduction of nitrate to ammonium coupled with the catabolic oxidation of electron donors (ethanol) to acetate and substrate-level phosphorylation [55,56]. One key reaction in the ethanol-oxidizing pathway is catalyzed by the CoA-acylating aldehyde dehydrogenase that is specifically produced under anaerobic conditions to generate acetyl-CoA, which is a key compound that mediates numerous biosynthetic and energy-yielding metabolic pathways as well as regulates several key metabolic reactions [56]. During ammonia fermentation the acetyl-CoA synthetase catalyzes the conversion of acetyl-CoA to acetate. In contrast to other fungi, *A. nidulans* uses this reaction to produce ATP [56]. It is unknown if the same is true for *A. fumigatus*, and whether this pathway is important for *in vivo* growth and virulence.

Conclusion

We are just beginning to understand the intricacies of *A. fumigatus* metabolism *in vivo* during infection, and it is clear that much remains to be learned about the requirements for *in vivo* fungal growth. However, it is very likely that mechanisms of *in vivo* growth will be uncovered that are unique to *A. fumigatus*. An understanding of the host microenvironment is important for developing new therapies for IPA. The host environment, for example, can play an important role in determining the efficacy of antifungal drugs. Recently it was shown that the MIC for Amphotericin B was reduced under hypoxic conditions, indicating the importance of understanding microenvironments found *in vivo* during IPA [57]. Moreover, targeting unique microbial respiration pathways has already been observed to be an effective antimicrobial strategy. For example, the discovery of new anti-tubercular drugs that target respiratory components of *M. tuberculosis* and ATP synthesis suggest that uncovering similar mechanisms in fungal pathogens may also yield new antifungal drugs [58-60]. In addition, other adjunctive therapies such as the use of hyperbaric oxygen may also become more warranted as we uncover the mechanisms of *in vivo* fungal growth and metabolism.

Acknowledgments

RAC is currently supported by funding from the National Institutes of Health, COBRE grant RR020185, and the Montana State University Agricultural Experiment Station. The authors would like to thank Dr. Yohannes G. Asfaw, Duke University Medical Center, for assistance with histopathology, and Dr. John Perfect and Dr. Judy Rhodes for their constructive comments.

References

1. Millner PD, Marsh PB, Snowden RB, Parr JF. Occurrence of *Aspergillus fumigatus* during composting of sewage sludge. *Appl Environ Microbiol* 1977 Dec;34:765–772. [PubMed: 339835]
2. Tekaiia F, Latge JP. *Aspergillus fumigatus*: saprophyte or pathogen? *Curr Opin Microbiol* 2005 Aug; 8:385–392. [PubMed: 16019255]
3. Wilson DM, Mubatanhema W, Jurjevic Z. Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. *Adv Exp Med Biol* 2002;504:3–17. [PubMed: 11922097]

4. Latge JP. *Aspergillus fumigatus* and aspergillosis. Clin Microbiol Rev 1999 Apr;12:310–350. [PubMed: 10194462]
5. Latge JP. The pathobiology of *Aspergillus fumigatus*. Trends Microbiol 2001 Aug;9:382–389. [PubMed: 11514221]
6. Perfect JR, Cox GM, Lee JY, et al. The impact of culture isolation of *Aspergillus* species: a hospital-based survey of aspergillosis. Clin Infect Dis 2001 Dec 1;33:1824–1833. [PubMed: 11692293]
7. Rhodes JC. *Aspergillus fumigatus*: growth and virulence. Med Mycol 2006 Sep;44(Suppl 1):S77–81. [PubMed: 17050423]
8. Braus, G.; Pries, R.; Duevel, K.; Valerius, O. Molecular biology of fungal amino acid biosynthesis regulation. 2nd. Berlin-Heidelberg-New York-Tokyo: Springer Press; 2004.
9. Hinnebusch AG. The general control of amino acid biosynthetic genes in the yeast *Saccharomyces cerevisiae*. CRC Crit Rev Biochem 1986;21:277–317. [PubMed: 3536302]
10. Carsiotis M, Jones RF. Cross-pathway regulation: tryptophan-mediated control of histidine and arginine biosynthetic enzymes in *Neurospora crassa*. J Bacteriol 1974 Sep;119:889–892. [PubMed: 4368541]
11. Carsiotis M, Jones RF, Wesseling AC. Cross-pathway regulation: histidine-mediated control of histidine, tryptophan, and arginine biosynthetic enzymes in *Neurospora crassa*. J Bacteriol 1974 Sep; 119:893–898. [PubMed: 4368540]
12. Braus G, Sasse C, Krappmann S. Amino acid acquisition, cross-pathway control, and virulence in *Aspergillus*. Medical Mycology 2006;44:91–94.
13. Natarajan K, Meyer MR, Jackson BM, et al. Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. Mol Cell Biol 2001 Jul;21:4347–4368. [PubMed: 11390663]
14. Hoffmann B, LaPaglia SK, Kubler E, et al. Developmental and metabolic regulation of the phosphoglucosyltransferase-encoding gene, *pgmB*, of *Aspergillus nidulans*. Mol Gen Genet 2000 Jan; 262:1001–1011. [PubMed: 10660061]
15. Tripathi G, Wiltshire C, Macaskill S, et al. Gcn4 co-ordinates morphogenetic and metabolic responses to amino acid starvation in *Candida albicans*. Embo J 2002 Oct 15;21:5448–5456. [PubMed: 12374745]
16. Krappmann S, Bignell EM, Reichard U, et al. The *Aspergillus fumigatus* transcriptional activator CpcA contributes significantly to the virulence of this fungal pathogen. Mol Microbiol 2004 May; 52:785–799. [PubMed: 15101984]
17. D'Enfert C, Diaquin M, Delit A, et al. Attenuated virulence of uridine-uracil auxotrophs of *Aspergillus fumigatus*. Infect Immun 1996 Oct;64:4401–4405. [PubMed: 8926121]
18. Liebmann B, Muhleisen TW, Muller M, et al. Deletion of the *Aspergillus fumigatus* lysine biosynthesis gene *lysF* encoding homoaconitase leads to attenuated virulence in a low-dose mouse infection model of invasive aspergillosis. Arch Microbiol 2004 May;181:378–383. [PubMed: 15052376]
19. Ibrahim-Granet O, Dubourdeau M, Latge JP, et al. Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. Cell Microbiol 2008 Jan;10:134–148. [PubMed: 17973657]
20. Rementeria A, Lopez-Molina N, Ludwig A, et al. Genes and molecules involved in *Aspergillus fumigatus* virulence. Rev Iberoam Micol 2005 Mar;22:1–23. [PubMed: 15813678]
21. Kogan TV, Jadoun J, Mittelman L, Hirschberg K, Osherov N. Involvement of secreted *Aspergillus fumigatus* proteases in disruption of the actin fiber cytoskeleton and loss of focal adhesion sites in infected A549 lung pneumocytes. J Infect Dis 2004 Jun 1;189:1965–1973. [PubMed: 15143461]
22. Brock M, Buckel W. On the mechanism of action of the antifungal agent propionate. Eur J Biochem 2004 Aug;271:3227–3241. [PubMed: 15265042]
23. Zhang YQ, Keller NP. Blockage of methylcitrate cycle inhibits polyketide production in *Aspergillus nidulans*. Mol Microbiol 2004 Apr;52:541–550. [PubMed: 15066039]
24. Maerker C, Rohde M, Brakhage AA, Brock M. Methylcitrate synthase from *Aspergillus fumigatus*. Propionyl-CoA affects polyketide synthesis, growth and morphology of conidia. Febs J 2005 Jul; 272:3615–3630. [PubMed: 16008561]

25. Munoz-Elias EJ, McKinney JD. *Mycobacterium tuberculosis* isocitrate lyases 1 and 2 are jointly required for in vivo growth and virulence. *Nat Med* 2005 Jun;11:638–644. [PubMed: 15895072]
26. Idnurm A, Howlett BJ. Isocitrate lyase is essential for pathogenicity of the fungus *Leptosphaeria maculans* to canola (*Brassica napus*). *Eukaryot Cell* 2002 Oct;1:719–724. [PubMed: 12455691]
27. Wang ZY, Thornton CR, Kershaw MJ, Debaio L, Talbot NJ. The glyoxylate cycle is required for temporal regulation of virulence by the plant pathogenic fungus *Magnaporthe grisea*. *Mol Microbiol* 2003 Mar;47:1601–1612. [PubMed: 12622815]
28. Lorenz MC, Fink GR. The glyoxylate cycle is required for fungal virulence. *Nature* 2001 Jul 5;412:83–86. [PubMed: 11452311]
29. Lorenz MC, Fink GR. Life and death in a macrophage: role of the glyoxylate cycle in virulence. *Eukaryot Cell* 2002 Oct;1:657–662. [PubMed: 12455685]
30. Bishai W. Lipid lunch for persistent pathogen. *Nature* 2000 Aug 17;406:683–685. [PubMed: 10963578]
31. Armitt S, McCullough W, Roberts CF. Analysis of acetate non-utilizing (*acu*) mutants in *Aspergillus nidulans*. *J Gen Microbiol* 1976 Feb;92:263–282. [PubMed: 3622]
32. Ebel F, Schwienbacher M, Beyer J, et al. 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 Jul;43:476–489. [PubMed: 16603391]
33. Schobel F, Ibrahim-Granet O, Ave P, et al. *Aspergillus fumigatus* does not require fatty acid metabolism via isocitrate lyase for development of invasive aspergillosis. *Infect Immun* 2007 Mar; 75:1237–1244. [PubMed: 17178786]
34. Olivas I, Royuela M, Romero B, et al. 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 Jan;45:45–60. [PubMed: 17616408]
35. Rude TH, Toffaletti DL, Cox GM, Perfect JR. Relationship of the glyoxylate pathway to the pathogenesis of *Cryptococcus neoformans*. *Infect Immun* 2002 Oct;70:5684–5694. [PubMed: 12228298]
36. Smith I. *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. *Clin Microbiol Rev* 2003 Jul;16:463–496. [PubMed: 12857778]
37. Richardson DJ. Bacterial respiration: a flexible process for a changing environment. *Microbiology* 2000 Mar;146(Pt 3):551–571. [PubMed: 10746759]
38. Shi L, Sohaskey CD, Kana BD, et al. Changes in energy metabolism of *Mycobacterium tuberculosis* in mouse lung and under in vitro conditions affecting aerobic respiration. *Proc Natl Acad Sci U S A* 2005 Oct 25;102:15629–15634. [PubMed: 16227431]
39. Zhang L, Hach A. Molecular mechanism of heme signaling in yeast: the transcriptional activator Hap1 serves as the key mediator. *Cell Mol Life Sci* 1999 Oct 30;56:415–426. [PubMed: 11212295]
40. Kastaniotis AJ, Zitomer RS. Rox1 mediated repression. Oxygen dependent repression in yeast. *Adv Exp Med Biol* 2000;475:185–195. [PubMed: 10849660]
41. Kwast KE, Burke PV, Staahl BT, Poyton RO. Oxygen sensing in yeast: evidence for the involvement of the respiratory chain in regulating the transcription of a subset of hypoxic genes. *Proc Natl Acad Sci U S A* 1999 May 11;96:5446–5451. [PubMed: 10318903]
42. Balasubramanian B, Lowry CV, Zitomer RS. The Rox1 repressor of the *Saccharomyces cerevisiae* hypoxic genes is a specific DNA-binding protein with a high-mobility-group motif. *Mol Cell Biol* 1993 Oct;13:6071–6078. [PubMed: 8413209]
43. Vasconcelles MJ, Jiang Y, McDaid K, et al. Identification and characterization of a low oxygen response element involved in the hypoxic induction of a family of *Saccharomyces cerevisiae* genes. Implications for the conservation of oxygen sensing in eukaryotes. *J Biol Chem* 2001 Apr 27;276:14374–14384. [PubMed: 11278521]
44. 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 Aug 18;361:399–411. [PubMed: 16854431]
45. Silver PM, Oliver BG, White TC. Role of *Candida albicans* transcription factor Upc2p in drug resistance and sterol metabolism. *Eukaryot Cell* 2004 Dec;3:1391–1397. [PubMed: 15590814]

46. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002 May;109:1125–1131. [PubMed: 11994399]
47. Hughes AL, Todd BL, Espenshade PJ. SREBP pathway responds to sterols and functions as an oxygen sensor in fission yeast. *Cell* 2005 Mar 25;120:831–842. [PubMed: 15797383]
48. 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 May;64:614–629. [PubMed: 17462012]
49. 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 Feb; 3:e22. [PubMed: 17319742]
50. Kelly JM, Drysdale MR, Sealy-Lewis HM, Jones IG, Lockington RA. Alcohol dehydrogenase III in *Aspergillus nidulans* is anaerobically induced and post-transcriptionally regulated. *Mol Gen Genet* 1990 Jul;222:323–328. [PubMed: 2274033]
51. Lockington RA, Borlace GN, Kelly JM. Pyruvate decarboxylase and anaerobic survival in *Aspergillus nidulans*. *Gene* 1997 May 20;191:61–67. [PubMed: 9210590]
52. Sanchis V, Vinas I, Roberts IN, et al. A pyruvate decarboxylase gene from *Aspergillus parasiticus*. *FEMS Microbiol Lett* 1994 Apr 1;117:207–210. [PubMed: 8181725]
53. Plummer R, Bodkin J, Power D, et al. Effect of caspofungin on metabolite profiles of *Aspergillus* species determined by nuclear magnetic resonance spectroscopy. *Antimicrob Agents Chemother* 2007 Nov;51:4077–4084. [PubMed: 17785514]
54. Scotter JM, Langford VS, Wilson PF, McEwan MJ, Chambers ST. Real-time detection of common microbial volatile organic compounds from medically important fungi by Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS). *J Microbiol Methods* 2005 Nov;63:127–134. [PubMed: 15893831]
55. Zhou Z, Takaya N, Nakamura A, et al. Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. *J Biol Chem* 2002 Jan 18;277:1892–1896. [PubMed: 11713252]
56. Takasaki K, Shoun H, Yamaguchi M, et al. Fungal ammonia fermentation, a novel metabolic mechanism that couples the dissimilatory and assimilatory pathways of both nitrate and ethanol. Role of acetyl CoA synthetase in anaerobic ATP synthesis. *J Biol Chem* 2004 Mar 26;279:12414–12420. [PubMed: 14722082]
57. Warn PA, Sharp A, Guinea J, Denning DW. Effect of hypoxic conditions on in vitro susceptibility testing of amphotericin B, itraconazole and micafungin against *Aspergillus* and *Candida*. *J Antimicrob Chemother* 2004 May;53:743–749. [PubMed: 15044422]
58. Weinstein EA, Yano T, Li LS, et al. Inhibitors of type II NADH:menaquinone oxidoreductase represent a class of antitubercular drugs. *Proc Natl Acad Sci U S A* 2005 Mar 22;102:4548–4553. [PubMed: 15767566]
59. Boshoff HI, Myers TG, Copp BR, et al. The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *J Biol Chem* 2004 Sep 17;279:40174–40184. [PubMed: 15247240]
60. Andries K, Verhasselt P, Guillemont J, et al. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 2005 Jan 14;307:223–227. [PubMed: 15591164]
61. Tomee JF, Kauffman HF. Putative virulence factors of *Aspergillus fumigatus*. *Clin Exp Allergy* 2000 Apr;30:476–484. [PubMed: 10718844]

Fig. 1.

Scheme of different stresses *A. fumigatus* likely encounters within the lung. The cellular defense involving phagocytosis and killing is mediated by alveolar macrophages, polymorphonuclear cells (PMN) and monocytes. The non-cellular defense is mediated by antileukoprotease (ALP), tracheal antimicrobial peptide (TAP) and lysozyme. *A. fumigatus* interferes with the host defense by the release of superoxide dismutase (SOD), *A. fumigatus* diffusible product (AfD) and conidial inhibitory factor (conidial IF) from conidia, and by the release of complement inhibitory factor (complement IF), toxins, and proteases from hyphae [61]. The response of *A. fumigatus* to hypoxia includes the transcription of hypoxia related genes like *srbA* and likely the switch to various pathways of fermentation (e.g. alcohol and ammonia fermentation). The lack of nutrients at the site of infection can be compensated by activating the cross-pathway control system and degradation of proteins to acquire amino acids for protein biosynthesis and possibly the glyoxylate cycle to produce carbohydrates from fats that are available at the site of infection.

Fig 2. Histopathology of *A. fumigatus* infection in an experimental murine model of invasive pulmonary aspergillosis. Hematoxylin and eosin stain of control, uninfected mouse lung showing clear, open alveoli and no inflammation, and a section from a lung infected with *A. fumigatus* 3 days post-infection. Significant tissue necrosis, hyphal invasion, collapse of alveoli, and inflammation are evident (black arrows). Significant hypoxia is likely present at an infection site such as this, and the abundant tissue destruction likely liberates proteins and other nutrients that can be used by the fungus for *in vivo* growth.

Fig 3.

A. fumigatus shows different responses to hypoxic conditions. 1×10^6 conidia were spotted on glucose minimal medium (GMM) (2% glucose), ethanol minimal medium (EMM) (2% ethanol) and glycerol minimal medium (GlyMM) (2% glycerol) and incubated under normoxia (21% oxygen) or hypoxia (1% oxygen) at 37°C for 48 hours.