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Metabolism in Fungal Pathogenesis

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Fungal pathogens must assimilate local nutrients to establish an infection in their mammalian host. We focus on carbon, nitrogen, and micronutrient assimilation mechanisms, discussing how these influence host–fungus interactions during infection. We highlight several emerging trends based on the available data. First, the perturbation of carbon, nitrogen, or micronutrient assimilation attenuates fungal pathogenicity. Second, the contrasting evolutionary pressures exerted on facultative versus obligatory pathogens have led to contemporary pathogenic fungal species that display differing degrees of metabolic flexibility. The evolutionarily ancient metabolic pathways are conserved in most fungal pathogen, but interesting gaps exist in some species (e.g., *Candida glabrata*). Third, metabolic flexibility is generally essential for fungal pathogenicity, and in particular, for the adaptation to contrasting host microenvironments such as the gastrointestinal tract, mucosal surfaces, bloodstream, and internal organs. Fourth, this metabolic flexibility relies on complex regulatory networks, some of which are conserved across lineages, whereas others have undergone significant evolutionary rewiring. Fifth, metabolic adaptation affects fungal susceptibility to antifungal drugs and also presents exciting opportunities for the development of novel therapies.

Nutrient assimilation is a central and fundamental prerequisite for the growth and survival of all living organisms. Pathogenic fungi inhabit dynamic and contrasting niches and must display rapid and effective adaptation to changes in nutrient availability in these microenvironments. To achieve this, they regulate specific nutrient uptake mechanisms and modulate their metabolism, displaying an impressive degree of metabolic flexibility. This metabolic flexibility, which enhances the fitness of the fungus, is often as essential for pathogenicity as virulence factors, thereby representing an attractive target for potential therapeutic intervention.

The major fungal pathogens of humans have evolved in a polyphyletic manner (i.e., pathogenicity has emerged independently in different

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phylogenetic branches of the fungal kingdom). Furthermore, major fungal pathogens display different lifestyles, and, consequently, their metabolic flexibility has been shaped by different evolutionary pressures. For example, the ascomycete *Candida albicans* is a facultative pathogen that exists primarily as a commensal of the oral cavity, gastrointestinal tract, and urogenital tract but can also persist within other extracellular microenvironments (blood, tissues) and within intracellular microenvironments (inside damaged epithelial and endothelial cells, and even the phagolysosome of macrophages). During its commensal stage, *C. albicans* must adapt to the nutrients present on mucosal surfaces and compete with cohabitating microflora, whereas during infection, the fungus exploits an alternative array of nutrients available in host tissues. The apparent lack of a significant environmental niche for *C. albicans* (Odds 1988) means that, in recent evolutionary time, the metabolic flexibility of this fungus has been tuned to these host niches. In contrast, fungi such as the basidiomycete *Cryptococcus neoformans* and the ascomycete *Histoplasma capsulatum,* are opportunistic pathogens that are associated with environmental niches such as pigeon guano, soil, and trees but can cause chronic pulmonary infections and devastating systemic infections in immunocompromised individuals (Sil 2006; Heitman 2011). The metabolic flexibility of these fungal pathogens ensures survival during the saprophytic phases of their life cycle as well as promoting their ability to cause pulmonary and disseminated infections (Kronstad et al. 2012). Therefore, different evolutionary pressures have been imposed on the processes that mediate metabolic adaptation in major fungal pathogens.

In addition, there are significant differences between fungal pathogens regarding the degree of evolutionary adaptation to their mammalian hosts. For example, the filamentous ascomycete, *Aspergillus fumigatus* is probably an accidental pathogen. This "grass eater" (Tekaia and Latgé 2005) lives mainly as a saprophyte, degrading plant and other organic material in the environment but can cause severe pulmonary infections in immunocompromised patients. At the other end of the spectrum, *Pneumocystis* species are obligate fungal pathogens that appear to have coevolved with their mammalian hosts to such an extent that they have shed several metabolic pathways and, so far, it has not been possible to culture them in vitro (Cushion 2004; Cushion et al. 2007; Hauser et al. 2010). *C. albicans* and *Candida glabrata* lie between these extremes, apparently being obligate parasites of warm-blooded animals (Odds 1988) and yet retaining a high degree of metabolic flexibility in vitro and in vivo (Brown et al. 2007; Wilson et al. 2009). Clearly the metabolic flexibility of fungal pathogens has been influenced by the evolutionary time scales over which environmental selection pressures have been exerted, as well as by the nature of these pressures.

In this review, we focus on the metabolic flexibility of major fungal pathogens during the infection process: commensalism, colonization, and disease progression. We address major fungal pathogens that are highly significant in clinical settings, such as *C. albicans*, *A. fumigatus*, and *C. neoformans*, mentioning other pathogens where appropriate. We concentrate on nitrogen and carbon metabolism, and micronutrient assimilation, which are both critical for pathogenesis and well characterized in pathogenic fungi.

NITROGEN ASSIMILATION

Nitrogen is required for almost all biosynthetic processes and, like carbon, must be assimilated in large quantities. Therefore, the acquisition of both nitrogen and carbon compounds from the host is essential for pathogenic fungi to survive, grow, and persist within a host. The high degree of metabolic flexibility is reflected in the different types of nitrogen sources exploited by fungal pathogens. In the absence of preferred nitrogen sources, such as ammonia or certain amino acids, these fungi can use compounds like proteins or polyamines. Some pathogenic fungi (e.g., pathogenic *Aspergillus* species) can also reduce nitrate to ammonia (Zhou et al. 2002). However, the pathogenic *Candida* and *Cryptococcus* species are unable to use nitrate as a nitrogen source.

Although fungal nitrogen utilization has been extensively studied in vitro, it is less clear which nitrogen sources are used by human pathogenic fungi in vivo. However, transcriptional profiling studies using microarrays or serial analysis of gene expression (SAGE) in ex vivo or in vivo infection models have provided insights into the nitrogen metabolism of pathogenic fungi such as *C. albicans* (Fradin et al. 2005; Thewes et al. 2007; Zakikhany et al. 2007; Walker et al. 2009; Wilson et al. 2009; Cheng et al. 2013), *C. glabrata* (Kaur et al. 2007), *A. fumigatus* (McDonagh et al. 2008), *C. neoformans* (Fan et al. 2005; Steen et al. 2003), and dermatophytes (Staib et al. 2010) during infection (reviewed by Cairns et al. 2010).

Proteases, Oligopeptide, and Amino Acid Transporters

During infection, *C. albicans* expresses various secreted aspartic proteases (Saps), one of the best-investigated virulence attributes of this fungus (Naglik et al. 2003). Liberated oligopeptides and amino acids are then taken up by dedicated oligopeptide transporters (Opt1–8) (Reuss and Morschhauser 2006) and a family of 22 predicted amino acid permeases (Sychrova and Souciet 1994; Kraidlova et al. 2011). When expressed in host tissues, these factors enable *C. albicans* to use a broad range of nitrogen sources over the course of an infection, driving host tissue damage and invasion (Villar et al. 2007; Naglik et al. 2008; Dalle et al. 2010; Cheng et al. 2013). This proteolytic activity might also promote immune evasion, as factors of the immune system, antimicrobial proteins, and peptides may be degraded (Naglik et al. 2003; Gropp et al. 2009; Meiller et al. 2009). Similarly, the aspartic proteases of *C. glabrata* and *Candida parapsilosis* have been implicated in survival following phagocytosis by macrophages (Kaur et al. 2007; Horvath et al. 2012).

Extracellular proteases have also been reported to be virulence attributes for the most common airborne pathogenic fungus, *A. fumigatus* (Monod et al. 1999), which contains multiple protease genes (Nierman et al. 2005). Although an oligopeptide transporter and a key regulator for extracellular proteolysis are required for in vitro growth of *A. fumigatus* on complex substrates, these proteins are not crucial for survival in vivo (Hartmann et al. 2011). Similarly, *C. albicans* Sap2, a protease essential for the growth on protein as the sole nitrogen source, is not essential for survival in vivo (Hube et al. 1994, 1997). Furthermore, similar observations have been made for dermatophytes such as *Arthroderma benhamiae* and *Trichophyton rubrum*. Proteases have always been considered de facto to be a key virulence attribute of dermatophytes, as these fungi infect keratin-containing tissue and are necessarily keratinolytic. Indeed, their genomes display a clear enrichment in protease genes (Burmester et al. 2011). However, those protease genes most highly expressed during growth on keratin in vitro, and hence for which an important role in virulence would be expected, differ from those genes that are highly up-regulated during infection (Zaugg et al. 2009; Staib et al. 2010). This highlights the fact that in vitro growth conditions often differ significantly from in vivo microenvironments.

Nitrogen Acquisition in Host Niches

Little is known about the nitrogen sources that pathogenic fungi assimilate in host niches. Presumably the types and concentrations of nitrogen source differ significantly between certain niches, such as mucosal surfaces, the gastrointestinal (GI) tract, and the bloodstream. Most information about fungal nitrogen assimilation has been gleaned through fungal transcriptomics using ex vivo infection models combined with limited molecular dissection via targeted gene mutation.

The environment inside the phagosome of phagocytes is thought to be poor in nitrogen. This is reflected in the transcriptional response of *C. albicans* and *C. glabrata* after internalization by macrophages (Lorenz et al. 2004; Seider et al. 2010; Roetzer et al. 2011; Brunke and Hube 2013; Mayer et al. 2013; Miramon et al. 2013). For example, *C. albicans* up-regulates amino acid biosynthetic pathways, suggesting that the fungus at least transiently faces amino acid deprivation. The arginine biosynthetic pathway is

the only amino acid biosynthesis pathway significantly up-regulated in *C. albicans* cells after phagocytosis by macrophages (Lorenz et al. 2004). Engulfment by neutrophils induces the up-regulation of arginine, leucine, lysine, and methionine anabolic pathways as well as *GCN4* (Rubin-Bejerano et al. 2003; Fradin et al. 2005), the gene encoding the master regulator of amino acid synthesis (Tripathi et al. 2002). *C. glabrata* also up-regulates the synthesis of arginine and lysine on internalization by macrophages (Kaur et al. 2007).

The specific contributions of these amino acids to fungal survival within the phagocyte remain to be tested. However, arginine biosynthetic gene induction might play a critical role in the fungal response to macrophage-derived reactive oxygen species (Jimenez-Lopez et al. 2013). Arginase can convert arginine to urea, which in turn is degraded by urea amidolyase to produce ammonia and CO_2. These breakdown products can contribute to neutralization of the acidic pH of the phagolysosome, promoting the intracellular induction of hyphal formation and allowing fungal escape and killing of the host cell (Ghosh et al. 2009). Similarly, the extracellular ureases of *C. neoformans* and *Cryptococcus gattii* (Ure1) degrade urea, which is abundant in the cerebrospinal fluid. The liberated CO_2 acts as a signal to induce capsule formation, a well-studied virulence factor in this fungus (Frazzitta et al. 2013).

Under sugar-limiting conditions, *C. albicans* cells can exploit amino acids as a carbon source (Fig. 1). The amino acids are imported via amino acid permeases, the expression of which is up-regulated by the transcription factor Stp2. The amino acids are metabolized in a glucose-repressible manner, and the excess nitrogen is excreted as ammonia via Ato5, a putative ammonia transporter, thereby raising environmental pH and autoinducing hypha formation (Vylkova et al. 2011). Similarly, Dur31 is a sodium/substrate symporter for the polyamine spermidine, and the amino groups of spermidine can potentially be used to generate ammonia. In fact, a *dur31*Δ mutant is unable to actively increase the local ambient pH. This suggests that Dur31 is an integral component of the

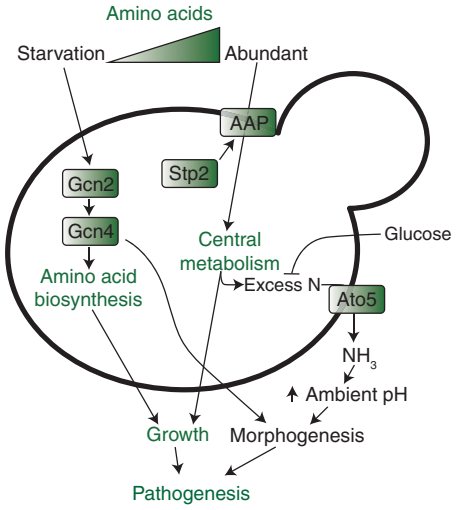

Figure 1. *Candida albicans* responds to amino acid starvation by activating the GCN pathway, which up-regulates amino acid biosynthesis via Gcn2 and Gcn4 to support the resumption of growth (Tripathi et al. 2002; Tournu et al. 2005). The transcription factor Gcn4 also activates filamentation (Tripathi et al. 2002). When amino acids are abundant, and in the absence of glucose, they are imported via amino acid permeases (AAP), the expression of which is activated by the transcription factor Stp2 (Vylkova et al. 2011). These amino acids are used to sustain central metabolism and the excess nitrogen is exported as ammonia via transporters such as Ato5. This leads to alkalinization of the local microenvironment, which stimulates morphogenesis (Vylkova et al. 2011). Metabolism fuels the growth of *C. albicans* cells and host colonization, and morphogenesis promotes the virulence of this pathogen (see text).

mechanisms that promote extracellular alkalinization and hyphal autoinduction. Indeed, *DUR31* is required for morphogenesis (Mayer et al. 2012). Interestingly, Dur31 not only transports spermidine but also histatin 5, a highly cytotoxic antimicrobial peptide (Mayer et al. 2012). Therefore, Dur31 appears to have dual functions as a virulence attribute and as an avirulence factor.

The response of *C. albicans* to neutrophils also includes the up-regulation of the ammonium permease genes *MEP1* and *MEP2* (Fradin et al. 2005). By analogy to the amino acid transporters and the spermidine transporter Dur31, the roles of these ammonium transporters ex-

tend beyond the uptake of nitrogen, because Mep2 can activate hyphal formation in response to ammonium limitation. This signaling role appears to be independent of the role in the extracellular alkalinization hyphal autoinduction pathway (Biswas and Morschhauser 2005).

Interestingly, *C. glabrata* can form pseudohyphae-like structures under certain nitrogen starvation conditions (Csank and Haynes 2000). However, unlike *C. albicans,* this filamentous morphology is not used as an escape mechanism from macrophages. Instead, *C. glabrata* continues to replicate as yeasts inside the phagosome until the host cell bursts by an unknown mechanism (Seider et al. 2011). This intracellular survival and replication depends on a specialized form of autophagy (pexophagy) to survive internalization by macrophages (Roetzer et al. 2010).

In general, the recycling of cellular proteins is a common strategy of fungi to overcome nitrogen starvation. For example, *C. albicans* vacuolar protease genes associated with intracellular protein degradation such as *APR1*, *PRB1*, *PRB2*, or *PRC1* are up-regulated in response to phagocytosis by either macrophages (Lorenz et al. 2004) or neutrophils (Fradin et al. 2005). A second, ubiquitin-dependent pathway contributes to protein recycling under nutrient-limiting conditions in *C. albicans* (Leach et al. 2011). This might explain why autophagy is not essential for this fungus to survive and to form hyphae within macrophages. *C. albicans* cells with defects in autophagy and cytoplasm-to-vacuole trafficking (*atg9*Δ) can survive macrophages and retain the ability to kill host cells (Palmer et al. 2007). Meanwhile, *C. albicans* polyubiquitin (*ubi4*) mutants display attenuated virulence (Leach et al. 2011). Similarly, *A. fumigatus* does not require autophagy for full virulence in a neutropenic mouse model (Richie et al. 2007), probably because its hyphae are not taken up by macrophages (Palmer et al. 2008). In contrast, fungi that can reside inside the phagosome for a long time, such as *C. glabrata* and *C. neoformans*, seem to require autophagy for full virulence, and the inactivation of the autophagic system reduces the virulence of *C. neoformans* in mice (Hu et al. 2008).

Regulation of Nitrogen Metabolism

Flexibility in nitrogen assimilation requires sensing of the available nitrogen sources and appropriate regulation of nitrogen assimilation genes. There are strong similarities between *C. albicans* and the model yeast, *Saccharomyces cerevisiae*, with respect to the regulation of key nitrogen acquisition pathways. In *C. albicans*, this occurs mainly via the SPS sensor mechanism, comprising the amino acid receptor Csy1 (a Ssy1 homolog), the scaffold protein Ptr3, and the signaling endopeptidase Ssy5 (Ljungdahl 2009). Similar to *S. cerevisiae*, the presence of extracellular amino acids is detected by the SPS sensor, leading to the proteolytic processing, activation, and nuclear translocation of the transcription factors Stp1 and Stp2 (Andreasson and Ljungdahl 2002; Martinez and Ljungdahl 2005). In an interesting twist, *C. albicans* Stp1 and Stp2 each activate a specific subset of nitrogen acquisition-related genes. Stp1 activates the transcription of genes involved in protein utilization, such as the genes encoding the secreted protease Sap2 and the oligopeptide transporter Opt1. On the other hand, Stp2 activates transcription of amino acid permease genes, for example, *GAP1*, *GAP2*, and *CAN1* (Martinez and Ljungdahl 2005). As Stp1 levels are also strongly reduced in the presence of amino acids, this system allows *C. albicans* to use free amino acids when they are available, and to obtain nitrogen from extracellular proteins under amino acid-limiting conditions. In the host, this allows optimal utilization of available nitrogen sources, and hence contributes to the pathogenicity potential of *C. albicans* (Ljungdahl 2009).

Microarray data suggest a key role for the Gpa1-cAMP–PKA pathway in the survival of *C. neoformans* within macrophages, which includes the up-regulation of amino acid transporters (Fan et al. 2005). This pathway not only regulates the transcription of genes encoding the major virulence attributes of *C. neoformans*, capsule, and melanin production but also responses to nutrient limitation within phagosomes. Consequently, *gpa1*Δ and *pka1*Δ mutants display reduced growth within macro-

phages (Fan et al. 2005). Microarray experiments also indicate that *Paracoccidioides brasiliensis* regulates amino acid metabolism during interactions with macrophages (Tavares et al. 2007), and that there is a close link between amino acid assimilation and the infectious and pathogenic states of *H. capsulatum* as amino acid transporters are differentially regulated in conidia, yeast, and mycelia (Inglis et al. 2013).

Amino acid starvation in *C. albicans* triggers the induction of genes on essentially all amino acid biosynthetic pathways via general amino acid control (the GCN response) (Fig. 1) (Tripathi et al. 2002; Yin et al. 2004; Tournu et al. 2005). This response is analogous to the GCN response in *S. cerevisiae* (Hinnebusch 1988; Natarajan et al. 2001; Hinnebusch and Natarajan 2002) and cross-pathway control in *Aspergillus* and *Neurospora* species (Paluh et al. 1988; Hoffmann et al. 2001; Krappmann et al. 2004). Briefly, amino acid starvation activates Gcn2, which phosphorylates eIF2α thereby decreasing the activity of this essential translation initiation factor. This reduces the overall rate of mRNA translation while enhancing the translation of *GCN4* via upstream open reading frames (uORFs) in the unusually long 5′-leader sequence of this mRNA. Hence, Gcn4 levels increase, leading to the transcriptional activation of amino acid biosynthetic genes via GCN response elements in their promoters. In *S. cerevisiae*, *GCN4* expression is regulated primarily at the translational level (Hinnebusch 1988), whereas in *C. albicans* Gcn4 synthesis is primarily regulated at the transcriptional level (Tripathi et al. 2002; Tournu et al. 2005). Also, in bakers' yeast purine biosynthesis is induced by the GCN response, unlike in *C. albicans* (Yin et al. 2004). Furthermore, in *C. albicans* Gcn4 interacts with the Ras-cAMP pathway to induce morphogenesis in an Efg1-dependent fashion (Fig. 1) (Tripathi et al. 2002). The GCN response contributes to biofilm formation in *C. albicans* but is not required for the virulence of this pathogen in the mouse model of systemic infection (Brand et al. 2004; Garcia-Sanchez et al. 2004). In contrast, the corresponding CPC response is required for *A. fumigatus* virulence in the murine model of pulmonary aspergillosis (Krappmann et al. 2004). These contrasting observations might reflect differential transcriptional outputs of the *C. albicans* Gcn4 and *A. fumigatus* CpcA transcription factors, and/or differential amino acid availabilities in the mouse lung and kidney.

CARBON ASSIMILATION

Carbon assimilation is essential for the generation of new biomass, and rapid fungal growth in the host relies on the efficient uptake and metabolism of available carbon sources. These can include fermentable sugars (such as glucose, fructose, and galactose) and nonfermentable carbon sources (such as amino acids and organic acids) (Lorenz and Fink 2001; Lorenz et al. 2004; Piekarska et al. 2006; Vieira et al. 2010; Ueno et al. 2011). Fungal pathogens have evolved different carbon assimilation profiles that presumably reflect their different niches. For example, saprobes that are opportunistic pathogens (such as *A. fumigatus*) have retained the ability to use a broad range of carbon sources. *C. albicans* does not display any known auxotrophies, can metabolize a broad range of sugars, and can use all amino acids as sole nitrogen sources (Odds 1988; Kaur et al. 2005). However, *C. glabrata* lacks certain metabolic pathways that exist in other pathogenic yeasts as a result of gene losses that have occurred during its evolution (Dujon et al. 2004). *C. glabrata* cannot catabolize galactose (loss of *GAL1*, *7*, *10*) or allantoin (*DAL1–4*, *7*), and is auxotrophic for pyridoxine (*SNO1*, *2*, *3*), thiamine, and nicotinic acid (*BNA1–6*) (Dujon et al. 2004; Wong and Wolfe 2005). Presumably, these nutritional restrictions are overcome in the specific host niches that are colonized by *C. glabrata*. More dramatic gene loss has occurred during the evolution of *Pneumocystis* species, which appears to have shed significant numbers of metabolic genes, retaining only two of 20 amino acid biosynthetic pathways. Based on gene content, glycolysis, the tricarboxylic acid (TCA) cycle, mitochondrial function, and energy metabolism appear to have remained intact (Cushion 2004; Cushion et al. 2007; Hauser et al. 2010). However, the inability to culture pathogenic

Pneumocystis species in vitro has prevented the direct exploration of their metabolic capacity.

Most pathogenic fungi prefer to assimilate sugars because their catabolism via glycolysis and respiration is energetically favorable. During the assimilation of alternative carbon sources, energetically demanding pathways such as gluconeogenesis and the glyoxylate cycle must be invoked to generate the hexose and pentose sugars required for cell wall and nucleotide synthesis, for example. Fungi can be classified into Crabtree-negative and Crabtree-positive species based on their carbon utilization patterns. Crabtree-negative species up-regulate the pyruvate dehydrogenase complex in the presence of glucose, such that most of this carbon flows into the TCA cycle to generate biomass and CO_2 (Chambergo et al. 2002; Maeda et al. 2004; Xie et al. 2004). Crabtree-positive fungi ferment most of the glucose to generate ethanol (Klein et al. 1998), which is thought to promote the competitive ability of yeasts within polymicrobial microenvironments owing to the antiseptic nature of ethanol (Crabtree 1928).

Regulatory Rewiring of Central Carbon Metabolism

In general, the central metabolic pathways are conserved across the fungal kingdom. However, *S. cerevisiae* and *C. albicans* do display some significant differences in their carbon metabolism gene sets. Indeed, genome-wide comparisons have revealed striking differences, with *C. albicans* harboring additional genes with roles in respiration and oxidative metabolism (Jones et al. 2004). In addition, there are significant differences between *C. albicans* and *S. cerevisiae* with respect to the regulatory networks that control central metabolism. *S. cerevisiae* is a Crabtree-positive yeast, the balance between fermentation and respiratory metabolism being modulated by glucose concentration, oxygen availability, and growth rate (Gancedo 1998). In contrast, *C. albicans* has been classified as a Crabtree-negative yeast because it retains respiratory activity even in the presence of glucose (Niimi et al. 1988). Bioinformatic and transcriptomic analyses have revealed significant regulatory rewiring between these yeasts, and close links between pathogenicity and metabolism in *C. albicans* (Ihmels et al. 2005; Martchenko et al. 2007; Askew et al. 2009; Lavoie et al. 2009). For instance, although galactose utilization genes display a similar syntenic organization in *C. albicans* and *S. cerevisiae*, their upstream regulatory regions are completely distinct (Martchenko et al. 2007). In *S. cerevisiae* the transcription factor Gal4 activates the galactose utilization genes *GAL1*, *GAL7*, and *GAL10*, whereas in *C. albicans,* Gal4 regulates the balance between respiration and fermentation in a carbon-source-dependent fashion (Askew et al. 2009). This presumably reflects the importance of galactose as a carbon source for the pathogenic fungus (Sabina and Brown 2009), particularly in lactating mothers and their infants. The glycolytic transcriptional circuit has also undergone significant transcriptional rewiring. *C. albicans* lacks homologs of the *S. cerevisiae* regulators, Gcr1 and Gcr2, which induce glycolysis regulators in this benign yeast (Askew et al. 2009). Instead Tye7 acts as the key regulator of glycolytic genes in *C. albicans* with *gal4*Δ *tye7*Δ mutants displaying altered glycolytic regulation under hypoxia and attenuated virulence in mouse models of infection (Askew et al. 2009). This highlights the importance of balancing carbon flux between respiration and fermentation in host niches during disease progression.

Recent work has revealed that this regulatory rewiring extends to the posttranscriptional circuitry. Many yeast species, including *S. cerevisiae*, repress pathways involved in the utilization of alternative carbon sources in the presence of glucose with a view to prioritizing sugar utilization over less favorable carbon sources (Flores et al. 2000). This repression operates at multiple levels. In *S. cerevisiae*, the transcription of genes involved in alternative carbon source utilization (for example, gluconeogenic and glyoxylate cycle genes) is down-regulated via AMP kinase and cAMP signaling, and the transcriptional repressor Mig1 (Gancedo 1998; Carlson 1999; Johnston 1999; Turcotte et al. 2010). In addition, *S. cerevisiae* enzymes involved in the assimilation of alternative carbon sources, such as Fbp1, Icl1, and Pck1, are target-

ed for ubiquitin-mediated degradation (Schork et al. 1995; Gancedo and Gancedo 1997; Schule et al. 2000; Horak et al. 2002; Regelmann et al. 2003). However, in *C. albicans* the evolutionary rewiring of ubiquitination targets allows these enzymes to persist longer in the cell following glucose exposure (Sandai et al. 2012) even though their genes are subject to strong transcriptional repression in response to glucose (Lorenz et al. 2004; Rodaki et al. 2009). Therefore, unlike *S. cerevisiae*, *C. albicans* is able to express glycolytic, gluconeogenic, and glyoxylate cycle enzymes at the same time, allowing it to assimilate glucose and alternative carbon sources simultaneously (Sandai et al. 2012). This has presumably evolved to promote the efficient assimilation of complex mixtures of carbon sources, and hence to enhance the fitness of this pathogen in the host (Lorenz 2013).

Regulation of Central Carbon Metabolism in Host Niches

In some host niches, the assimilation of certain alternative carbon sources is essential for fungal proliferation. This is the case for *C. glabrata*, which relies on lactate assimilation to survive in the mouse intestine (Ueno et al. 2011). The importance of central carbon metabolism in fungal pathogenesis has been reinforced, for example, by the targeted mutational disruption of specific metabolic functions in *C. albicans* (Lorenz and Fink 2001; Barelle et al. 2006). Glyoxylate cycle (Icl1), glycolytic (Pyk1), and gluconeogenic enzymes (Pck1) are all required for the full virulence of *C. albicans* in the murine model of systemic candidiasis (Lorenz and Fink 2001; Barelle et al. 2006). These requirements are paralleled in *C. neoformans* to a reasonable extent, as mutants with glycolytic defects (*pyk1*Δ and *hxk1*Δ *hxk2*Δ) are severely attenuated in a murine inhalation model of cryptococcosis and display decreased persistence in the central nervous system (Price et al. 2011).

Additional evidence for the significant impact of central carbon metabolism on fungal pathogenicity has been generated by transcript profiling of fungal cells exposed to macrophages. For example, *C. albicans* glyoxylate cycle and fatty acid β-oxidation genes are induced following phagocytosis by macrophages (Lorenz et al. 2004). Following phagocytosis, *C. albicans* displays a starvation response, reprogramming its metabolism to generate hexose sugars via lipid catabolism, the glyoxylate cycle, and gluconeogenesis (Lorenz et al. 2004). This reprogramming is absent in the benign yeast *S. cerevisiae*, suggesting that *C. albicans* has evolved transcriptional programs to match its pathogenic lifestyle (Lorenz and Fink 2001; Lorenz et al. 2004).

Intriguingly, both glycolytic genes (e.g., *PFK2*, *ENO1*, *PYK1*) and glyoxylate cycle genes (e.g., *ICL1*, *MLS1*, *MDH1*) were shown to be induced in *C. albicans* cells within 20 min of exposure to human blood (Fradin and Hube 2003). At the time, this was surprising because *C. albicans* was thought to follow the *S. cerevisiae* paradigm in which cells do not transcribe glycolytic and gluconeogenic genes at the same time (Yin et al. 2003; Lorenz et al. 2004; Rodaki et al. 2009). Indeed, both species strongly down-regulate gluconeogenic and glyoxylate cycle genes in response to glucose concentrations lower than those found in the bloodstream (about 0.07%) (Yin et al. 2003; Rodaki et al. 2009). Yet the *C. albicans* glyoxylate cycle is required for virulence during systemic infection (Lorenz and Fink 2001). The paradoxical activation of competing carbon metabolism pathways was further complicated by analyses of fungal cells from different infection models. Both mucosal and intraperitoneal infection models were characterized by the simultaneous induction of glycolytic, gluconeogenic, and TCA cycle genes (Thewes et al. 2007; Wilson et al. 2007; Zakikhany et al. 2007). These observations were thought to reflect the heterogeneous nature of fungal populations in complex host microenvironments. For example, phagocytosed cells that are exposed to carbon starvation activate the glyoxylate cycle, whereas nonphagocytosed cells retain access to glucose and activate glycolysis (Brown et al. 2007; Wilson et al. 2007).

To address this paradox regarding the concomitant roles of opposing central metabolic pathways during *C. albicans* pathogenesis, single cell profiling was performed with specific

GFP (green fluorescent protein) fusions to monitor the activation of the relevant metabolic pathways (*PCK1*, a gluconeogenic-specific enzyme; *ICL1*, a glyoxylate cycle enzyme; *PFK2* and *PYK1*, two glycolysis-specific enzymes) (Barelle et al. 2006; Miramon et al. 2012). These studies confirmed that gluconeogenesis and glyoxylate cycle were up-regulated in *C. albicans* following phagocytosis by macrophages or neutrophils, and that these pathways are indeed repressed by physiologically relevant concentrations of glucose in the majority of fungal cells infecting the kidney. These studies also confirmed the heterogeneity of *C. albicans* cell populations in the kidney and, hence, the complexity of these microenvironments. However, many of these *C. albicans* cells expressed both glycolytic and gluconeogenic genes. Subsequently, this apparent paradox was resolved with the discovery that the rewiring of ubiquitin targets in central metabolism permits the relaxation of catabolite repression in *C. albicans* (Sandai et al. 2012).

In *A. fumigatus*, the isocitrate lyase Icl1, which is part of the glyoxylate cycle, is not required for full virulence (Schobel et al. 2007; Olivas et al. 2008), although this enzyme is constitutively expressed in conidia during germination within macrophages and not in resting conidia (Ebel et al. 2006). *C. neoformans* also up-regulates Icl1 after phagocytosis by macrophages, although the *ICL1* gene is not necessary for full virulence or growth within macrophages (Rude et al. 2002). In these species, Icl1 has been regarded as a marker for lipid utilization, and, hence, these observations suggest that lipid utilization is not required for infection. Similarly, the *C. neoformans* malate synthase (Mls1) is dispensable for pathogenicity despite its up-regulation during infection (Idnurm et al. 2007; Kronstad et al. 2012). Nevertheless, peroxisomal and mitochondrial fatty acid β-oxidation affects capsule production and is required for the virulence of *C. neoformans* (Kretschmer et al. 2012). In contrast, fatty acid catabolism does not appear to be required for the virulence of *C. albicans* as a *fox2*Δ mutant, which lacks a key enzyme of fatty acid β-oxidation, displays only a minor defect in virulence in the murine model of systemic candidiasis (Piekarska et al. 2006; Ramirez and Lorenz 2007). Although the glyoxylate cycle and fatty acid catabolism might not be virulence determinants for some species, the gluconeogenic pathway might be crucial during the latter stages of infection and for persistence within infected tissues. Indeed, *C. neoformans* cells recovered from mouse lungs in the later stages of infection displayed increased levels of enzymes involved in gluconeogenesis, glyoxylate cycle, and β-oxidation, indicative of a glucose-limited environment (Hu et al. 2008). Moreover, a *C. neoformans pck1*Δ mutant, which has a block in gluconeogenesis, displays a virulence defect (Panepinto et al. 2005).

Impact of Carbon Metabolism on Fungal Virulence Factors

As well as being essential for proliferation in the host, the assimilation of alternative carbon sources can profoundly influence the fitness, physiology, and pathogenicity of fungal pathogens. For example, glucose represses *C. neoformans* melanization (Zhu and Williamson 2004), and growth on mannitol increases capsule size both in vitro and in vivo, relative to growth on glucose (Guimaraes et al. 2010). Both the capsule and melanization represent important virulence factors in *C. neoformans*. For *C. albicans*, growth on lactate rather than glucose modifies the composition and architecture of the cell wall, and, hence, the interaction of *C. albicans* cells with innate immune cells and host recognition (Ene et al. 2012a,c, 2013). This cell wall remodeling also impacts on adherence, biofilm formation, stress, and drug resistance (Ene et al. 2012a,c). On the other hand, for *C. albicans* cells grown on lactate, transient exposure to glucose leads to the activation of stress-responsive pathways, increasing their resistance to reactive oxygen species and to an azole antifungal agent (Rodaki et al. 2009). Therefore, changes in carbon source affect many aspects of host–pathogen interactions, and can dramatically impact the virulence of *C. albicans* and its susceptibility to therapeutic intervention (Fig. 2) (Ene et al. 2012c).

An early indication of the intimate links between the regulation of carbon metabolism and

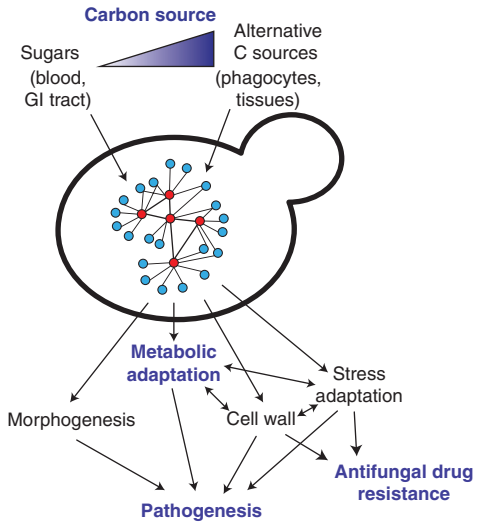

Figure 2. *Candida albicans* adapts to changes in carbon sources (C sources) via a complex regulatory network involving sugar signaling and other regulatory modules to tune its metabolism, cell wall architecture, morphology, and stress resistance to the available nutrients (Nantel et al. 2002; Doedt et al. 2004; Hudson et al. 2004; Maidan et al. 2005; Rodaki et al. 2009; Sabina and Brown 2009; Ene et al. 2012c). These changes affect the antifungal drug susceptibility of *C. albicans* cells and strongly influence their virulence (Ene et al. 2012c) (see text).

virulence was provided by the observation that glycolytic transcript levels are modulated in *C. albicans* cells undergoing the yeast-hyphal transition, a morphogenetic program that is crucial for the virulence of *C. albicans* (Swoboda et al. 1994). Also, this morphogenetic transition is influenced by nutritional cues such as glucose and amino acids (Hudson et al. 2004; Maidan et al. 2005; Vylkova et al. 2011). Furthermore, transcript-profiling studies have revealed that, in addition to blocking morphogenesis, the inactivation of the morphogenetic regulator Efg1 has a significant impact on the expression of metabolic genes (Nantel et al. 2002; Doedt et al. 2004). Efg1 induces glycolytic genes while repressing gluconeogenic, TCA cycle, and respiratory functions (Doedt et al. 2004), thereby stimulating fermentative metabolism and repressing respiratory metabolism. Furthermore, a recent report has highlighted the importance of Efg1 during gastrointestinal colonization (Pierce et al. 2013). *C. albicans* cells that lack Efg1 induce metabolic pathways (carnitine and fatty acid metabolism) that promote hypercolonization of the GI tract in mice (Pierce et al. 2013). Indeed, there is now considerable evidence to suggest that metabolic reprogramming and virulence attributes are integrated by key regulators such as Efg1, Tup1, and Gcn4, which are at the intersections of these regulatory networks (Braun and Johnson 1997; Murad et al. 2001a,b; Tripathi et al. 2002; Doedt et al. 2004).

The white-opaque switch, a developmental program that regulates sexual reproduction in *C. albicans*, is also accompanied by extensive metabolic rewiring. Glycolytic genes are down-regulated in opaque cells, the mating-competent form of *C. albicans*, whereas TCA cycle and fatty acid β-oxidation genes are up-regulated (Lan et al. 2002; Tuch et al. 2010). White cells appear to favor fermentative metabolism, whereas opaque cells favor respiratory metabolism, and these programs are presumably associated with their preference for different anatomical niches in the host.

MICRONUTRIENT ASSIMILATION

Microbial survival, persistence, and growth within the host are not only dependent on macronutrients, such as nitrogen and carbon, but also on micronutrients such as trace metals. Among the most important metals are iron, zinc, manganese, and copper, all of which are required for the functionality of many proteins and enzymes. Fungal pathogens must have sufficient access to these essential metals to achieve host colonization, and yet all sterile extracellular niches in humans are essentially devoid of unbound metals. Fungal infections disturb global metal homeostasis in the mammalian host (Potrykus et al. 2013). However, the host has evolved sophisticated mechanisms that restrict microbial access to these metals via a process called "nutritional immunity" (Ganz 2009; Weinberg 2009; Hood and Skaar 2012). Meanwhile, fungal pathogens have evolved elaborate strategies to circumvent this immunity. For example, all studied fungal pathogens can scav-

enge iron from the host, and this process is essential for their virulence (Almeida et al. 2009; Schrettl and Haas 2011; Kronstad et al. 2012).

The battle over iron during systemic *C. albicans* infections provides an excellent example of this interplay between pathogen and host (Potrykus et al. 2013). Red blood cell recycling in the spleen is inhibited during systemic candidiasis, leading to the accumulation of heme-associated iron in the kidney. Meanwhile, at a local level, the immune infiltrates surrounding the fungal lesions in the renal cortex impose nutritional immunity thereby limiting the availability of iron to the invading fungus. *C. albicans* then responds to this host restriction by up-regulating its heme-iron-acquisition mechanisms (Potrykus et al. 2013).

Iron Assimilation

Free iron in human blood is limited to low concentrations (10^{-24} M), thereby restricting its availability to invading microbes. *C. albicans* has evolved three main iron-acquisition systems to counteract this restriction (Fig. 3) (Almeida et al. 2009). First, *C. albicans* does not synthesize siderophores (low molecular mass ferric iron-specific chelators) but it can exploit siderophores synthesized by other microorganisms (xenosiderophores) via the Sit1 transporter—a microbial strategy also described as "iron parasitism" (Heymann et al. 2002). Sit-mediated iron uptake appears universally conserved in the fungal kingdom, having been described in *S. cerevisiae*, *Candida* species, *Aspergillus* species, and *C. neoformans* (Schrettl and Haas 2011). Second, *C. albicans* can bind two of the major iron transport and storage proteins of humans: transferrin and ferritin. Transferrin is bound via an unknown receptor (Knight et al. 2005), whereas ferritin is bound via the hypha-associated adhesin and invasin Als3 during invasion into host cells (Almeida et al. 2009). Fungal iron acquisition from host transferrin and ferritin requires the reductive pathway, which is also the main pathway for the uptake of free iron, if available, for example, following host-cell lysis. The reductive pathway requires reductases, oxidases, and iron permeases, each encoded by large gene families (Almeida et al. 2009). These include the high-affinity iron permease gene Ftr1, and the oxidase Fet3. Third, *C. albicans* can exploit the iron in hemoglobin and other heme proteins. The fungus expresses hemolysins that disrupt red blood cells (Watanabe et al. 1999) and then binds and uses the resultant hemoglobin and heme proteins via the Rbt5/Hmx1 system (Pendrak et al. 2004; Weissman and Kornitzer 2004).

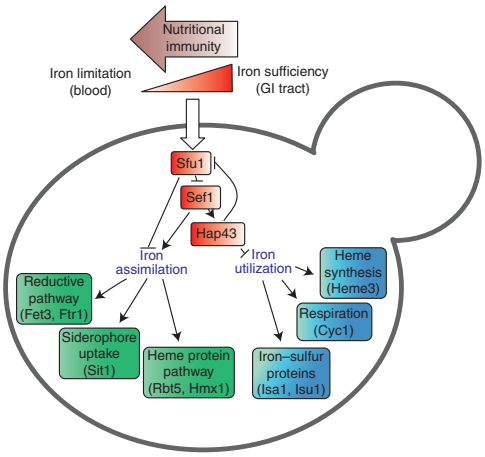

Figure 3. *Candida albicans* regulates iron assimilation and iron utilization pathways in response to iron availability within host niches via a regulatory network involving Sfu1, Sef1, and Hap43 (Chen et al. 2011). Host niches differ markedly with respect to iron availability, the local concentrations of free iron within host tissues being reduced by nutritional immunity (Potrykus et al. 2013). The fungus differentially exploits three main iron assimilation pathways depending on the nature of the available iron (Almeida et al. 2009; Potrykus et al. 2013) (see text).

C. glabrata expresses a reductive iron-uptake pathway but is not known to use host ferritin or transferrin as iron sources. Unlike *C. albicans*, *C. glabrata* cannot use bacterial siderophores as an iron source (Nevitt and Thiele 2011). However, *C. glabrata* can use hydroxamate-type xenosiderophores of fungal origin (ferrichrome, ferrirubin, or coprogen) via a Sit1 homolog, and this significantly increases fungal fitness and survival after phagocytosis by macrophages (Nevitt and Thiele 2011).

The macrophage phagosome is characterized by low iron concentrations, and therefore, phagocytosed microbes must rely on internal

iron reserves or exploit alternative strategies to scavenge iron and survive within macrophages. *H. capsulatum*, for example, gains iron via at least three different strategies. First, the fungus inhibits phagosomal acidification thereby promoting the gradual release of iron from transferrin (Eissenberg et al. 1993). Second, *H. capsulatum* synthesizes and secretes siderophores within macrophages, in contrast to the iron parasitism displayed by *C. albicans* and *C. glabrata* (Hwang et al. 2008). Third, *H. capsulatum* produces surface-bound and secreted enzymes with iron-reducing activity (for example, a secreted γ-glutamyltransferase, Ggt1), which generates an efficient ferric reductant (Zarnowski et al. 2008).

In contrast to the species discussed above, *A. fumigatus* seems unable to scavenge iron from host proteins. Instead, to acquire iron, this pathogen depends on a low-affinity ferrous iron acquisition system (which exists in other fungi and can also transport zinc) as well as two high-affinity iron uptake systems that involve reductive iron assimilation and siderophore-assisted iron uptake (reviewed in Schrettl and Haas 2011). *A. fumigatus* excretes two different siderophores, fusarinine C (FsC) and triacetylfusarinine C (TAFC), to mobilize extracellular iron. This pathogen also synthesizes two intracellular siderophores, hyphal ferricrocin (FC) and conidial hydroxyferricrocin (HFC), for the distribution and storage of iron inside the cell (Schrettl et al. 2007; Wallner et al. 2009). In addition, like other fungi, *A. fumigatus* probably uses vacuolar iron storage, as suggested by the iron-inducible expression of CccA (Schrettl et al. 2008), which is an ortholog of the vacuolar iron importer Ccc1p in *S. cerevisiae* (Kaplan and Kaplan 2009).

C. neoformans also possesses several complementary iron acquisition strategies. *C. neoformans* expresses cell surface reductases that reduce ferric iron to its ferrous state, and also exports reductants such as 3-hydroxyanthranilic acid (Nyhus et al. 1997; Jacobson et al. 1998; Nyhus and Jacobson 1999; Kronstad et al. 2012). Cfo1 and Cft1, which are orthologs of Fet3 and Ftr1, are essential for ferric iron uptake and iron acquisition from transferrin. However, they are not required for iron acquisition from heme or the siderophore ferrioxamine. Like *C. albicans* and *C. glabrata*, *C. neoformans* does not produce its own siderophores but expresses a Sit1 transporter that facilitates ferrioxamine B uptake (Tangen et al. 2007). Inactivation of Sit1 does not attenuate the virulence of *C. neoformans* but inhibits growth under iron-limiting conditions.

Regulation of Fungal Iron Homeostasis

Clearly, evolutionarily diverse fungal pathogens exploit common strategies to scavenge iron from their hosts via parallel pathways that include high-affinity ferroxidase/permease complexes, heme protein utilization, and siderophore uptake systems. However, iron uptake and storage must be tightly regulated because iron is an essential element but excess iron is toxic, generating reactive oxygen species. This is beautifully illustrated by the Sef1-Sfu1 transcriptional circuitry in *C. albicans*, which differentially controls iron sequestration and iron toxicity in host niches with contrasting iron availabilities. The levels of available iron in the gastrointestinal tract are much higher than in the bloodstream, in which free iron is limited by the host (Martin et al. 1987; Miret et al. 2003). Under iron-limiting conditions (e.g., in the bloodstream), the zinc finger transcription factor Sef1 activates iron assimilation functions and down-regulates the transcriptional repressor Sfu1 (Fig. 3) (Chen et al. 2011). When iron is abundant (e.g., in the gastrointestinal tract), Sfu1 strongly down-regulates Sef1 activity. Sfu1 represses *SEF1* expression and inhibits Sef1 functionality by regulating its cellular localization, as well as by promoting Sef1 phosphorylation and destabilization (Chen and Noble 2012). Consequently, Sef1 promotes systemic candidiasis, whereas Sfu1 enhances gastrointestinal commensalism (Chen et al. 2011; Chen and Noble 2012).

An analogous negative-feedback loop regulates iron homeostasis in *A. fumigatus*. Iron starvation triggers a transcriptional response that is regulated by two interconnected transcription factors, SreA and HapX (Schrettl and Haas

2011). Under conditions of iron sufficiency, SreA represses iron acquisition pathways to limit iron toxicity, and represses the expression of HapX to derepress iron-consuming pathways such as heme biosynthesis, the TCA cycle, and respiration. During iron starvation, HapX represses SreA to up-regulate iron acquisition, and down-regulates iron-consuming pathways such that the available iron is used sparingly. A deficiency in HapX (but not SreA) attenuates the virulence of *A. fumigatus*, reinforcing the crucial role of iron adaptation mechanisms in the host (Schrettl et al. 2010).

Hap proteins (HapX, Hap3, and Hap5) combine with the Cir1 transcription factor to regulate the response to changing iron levels in *C. neoformans* (Jung et al. 2006, 2010; Jung and Kronstad 2011). Cir1 is related to *C. albicans* Sfu1, *H. capsulatum* Sre1, and *Blastomyces dermatitidis* SREB (Lan et al. 2004; Chao et al. 2008; Gauthier et al. 2010; Kronstad et al. 2012). Cir1 up-regulates siderophore transport genes via HapX, and down-regulates reductive iron uptake genes. *C. neoformans hapX* mutants display a minor virulence defect, whereas *cir1*Δ cells are avirulent (Jung et al. 2006, 2010). The strong virulence defect of *C. neoformans cir1*Δ mutants could also reflect the roles of Cir1 in the expression of key virulence factors that include growth at 37°C, melanin production, and capsule formation. Cir1 represents a key node in the complex regulatory network, involving HapX, Sre1, Cir1, Tup1, and Gat1, which integrate iron acquisition with critical virulence factors in *C. neoformans* (Jacobson and Hong 1997; Kronstad et al. 2012). This is further complicated by the influence of ambient pH, which modulates iron homeostasis via the transcriptional regulator Rim101 (Kronstad et al. 2012).

Zinc Assimilation

Zinc is the second most abundant metal in most living organisms and a critical cofactor for many proteins. The mammalian host actively limits zinc availability during bacterial infections via nutritional immunity (Corbin et al. 2008), and the same is probably true during systemic fungal infections. To successfully colonize the host, the fungus must counter this zinc restriction.

C. albicans sequesters host zinc by a "zincophore" system (Citiulo et al. 2012). Analogous to siderophore-mediated iron acquisition, the fungus secretes the zinc-binding protein Pra1 (the "zincophore"), which sequesters the metal from host cells. Pra1 then binds to Zrt1 at the fungal cell surface. Zrt1 is a plasma membrane zinc transporter that mediates the uptake of zinc into the fungal cell. The *PRA1* and *ZRT1* genes are coregulated in response to zinc availability through their divergent transcription from a common promoter region (Citiulo et al. 2012). The components of this zincophore system, which are essential for *C. albicans* zinc scavenging during host-cell invasion, are conserved across the fungal kingdom (Wilson et al. 2012). For example, *PRA1* and *ZRT1* orthologs exist in *A. fumigatus* (Aspf2 and ZrfC, respectively), and these are organized in an analogous syntenic arrangement, are regulated by environmental zinc levels, and are required for growth under zinc starvation (Amich et al. 2010). However, some fungal pathogens appear to lack a Pra1 orthlog. These species must rely on alternative zinc acquisition systems or rely solely on transporters for zinc uptake (Wilson et al. 2012). For example, *C. glabrata* apparently lacks both Pra1 and Zrt1. Instead, *C. glabrata* could acquire zinc via two homologs of the low-affinity *S. cerevisiae* zinc transporter Zrt2 (Brunke and Hube 2013). Alternatively, other convergently evolved, secreted zinc-binding protein(s) may be deployed. Some fungi may secrete small molecule zinc chelators to sequester this essential metal (Wilson et al. 2012).

Other essential metals such as manganese (Kehl-Fie and Skaar 2010) and copper (Hodgkinson and Petris 2012) may play important roles in host–pathogen interactions. However, their uptake systems in pathogenic fungi remain largely uncharacterized. A putative manganese transporter (Ccc1) and a copper transporter (Ctr1) have been identified in *C. albicans* (Inglis et al. 2012) but their roles in virulence have not yet been experimentally defined.

CONCLUDING REMARKS

In conclusion, our review has highlighted the fundamental importance of nitrogen, carbon, and micronutrient assimilation for fungal pathogenicity. Clearly fungal pathogens must assimilate the available nutrients within host niches to colonize them. These niches are dynamic, complex, and varied, including the skin, mucosal surfaces, the urogenital and gastrointestinal tracts, the bloodstream, and internal organs. These niches differ with respect to the types and concentrations of available carbon and nitrogen sources, and with regard to micronutrient levels. Fungal pathogens respond by tuning their nutrient acquisition and assimilation mechanisms accordingly (Steen et al. 2003; Lorenz et al. 2004; Fan et al. 2005; Fradin et al. 2005; Kaur et al. 2007; Thewes et al. 2007; Zakikhany et al. 2007; McDonagh et al. 2008; Walker et al. 2009; Wilson et al. 2009; Cairns et al. 2010; Staib et al. 2010; Cheng et al. 2013). In some instances, the host actively reduces the availability of essential nutrients in an attempt to limit fungal colonization. For example, nutritional immunity limits iron availability in the renal cortex during systemic candidiasis, and *C. albicans* responds by up-regulating its heme-assimilation mechanisms (Potrykus et al. 2013).

Fungal pathogens have evolved to address these metabolic challenges but the evolutionary outcomes often differ for several reasons. First, pathogenicity has evolved independently in different phylogenetic branches of the fungal kingdom. Second, fungal pathogens have been subject to differing evolutionary pressures, possibly over different periods of evolutionary time. Some pathogens, such as *A. fumigatus*, *C. neoformans*, and *H. capsulatum*, have retained significant environmental niches, whereas others, such as *C. albicans* and *Pneumocystis jirovecii*, appear to be obligately associated with the host. Third, pathogens differ with respect to their routes of infection. Environmental pathogens such as *A. fumigatus*, *C. neoformans*, and *H. capsulatum* are often inhaled and initially establish pulmonary infections. In contrast, commensal organisms such as *C. albicans* cause systemic infections when mucosal or gastrointestinal barriers and host immune defenses are compromised. Despite these differences, fungal pathogens display common, evolutionarily ancient strategies to address certain metabolic challenges. These include the tight coordination of iron acquisition and iron-consuming pathways via transcriptional circuitry involving multiple negative-feedback loops, and the global activation of amino acid biosynthesis in response to amino acid starvation via a central transcriptional regulator (Gcn4/CpcA). On the other hand, some conserved metabolic pathways have undergone significant evolutionary rewiring at both transcriptional and posttranscriptional levels during the evolution of fungal pathogens (Ihmels et al. 2005; Martchenko et al. 2007; Askew et al. 2009; Lavoie et al. 2009; Sandai et al. 2012).

The essentiality of nutrient assimilation for fungal pathogenicity represents an opportunity for the development of novel antifungal therapies. Large-scale screens have revealed metabolic targets that might be amenable to pharmaceutical intervention (Roemer et al. 2003), and in some cases, significant differences in assimilation pathways or the catalytic mechanisms of essential mammalian and fungal enzymes enhance their attractiveness as potential antifungal targets (Rodaki et al. 2006; Schrettl and Haas 2011). Recent data have revealed that the susceptibility of fungal pathogens to the currently available antifungal drugs is strongly influenced by nutrient availability. In particular, changes in carbon source affected the resistance of *C. albicans* cells to azole, polyene, and echinocandin antifungals (Ene et al. 2012c), suggesting that fungal adaptation to different nutrients in host niches affects their susceptibility to therapeutic intervention. Changes in carbon source also affect the stress resistance of *C. albicans* and exert major effects on the architecture and content of the cell wall, thereby affecting the recognition and killing of *C. albicans* cells by innate immune cells and the virulence of this pathogen (Rodaki et al. 2009; Ene et al. 2012b,c, 2013).

Although the study of metabolic flexibility and reprogramming has set new paradigms for how microbes adapt to their environment, these processes are fundamental for fungi as they

drive both commensalism and infection. These observations highlight the need to investigate the impact of fungal nutrient adaptation on immune recognition and pathogenicity and might help the design of more effective therapeutic strategies.

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