International Journal of Medical Microbiology 301 (2011) 400–407

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.de/ijmm

Mini review

Nutrient acquisition by pathogenic fungi: Nutrient availability, pathway regulation, and differences in substrate utilization

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ARTICLE INFO

Keywords:
Host niche
Glyoxylate cycle
Glycolysis
Gluconeogenesis
Protease

ABSTRACT

All pathogenic microorganisms have in common that they need to feed on nutrients available from their host. Therefore, the specific interruption of metabolic pathways is a promising approach which could lead to the discovery of new antimicrobial drugs. However, nutrient availability strongly varies in respect to the infected host niche and pathogens may possess different strategies to acquire nutrients. This review focuses on the differences in regulation and use of key metabolic pathways during infection by pathogenic fungi, especially in the filamentous fungus *Aspergillus fumigatus* and the dimorphic yeast *Candida albicans*. Besides universal metabolic pathways, emphasis is given on pathways, which are absent in humans and might, therefore, suit as antifungal drug targets. Niche-specific nutrient availability and different physiological strategies complicate the identification of metabolic pathways, which are essential for all pathogens at each step of the infection process.

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Introduction

Nutrition is an essential prerequisite for the onset and manifestation of an infection by pathogenic microorganisms. Several fungi with pathogenic potential, e.g. *Aspergillus fumigatus*, the main causes of invasive aspergillosis (Denning et al., 2002), are free-living microorganisms and commonly found as saprophytes in soil. Therefore, these fungi are trained to cope with competitors for nutrients, and they are well adapted to rapidly changing environmental conditions. Additionally, predators, such as amoeba and nematodes, frequently attack fungi in their natural environment, and strategies to escape these predators have been assumed to represent 'virulence schools' for human infections (Alvarez and Casadevall, 2006; Bliska and Casadevall, 2009). In contrast, the dimorphic yeast *Candida albicans* has never been isolated as free-living organism and may have adapted specifically to mammalian hosts. *C. albicans* is frequently found as a harmless commensal, which persists at different mucosal surfaces (Soll, 2002; Hube, 2004). Therefore, *C. albicans* must possess efficient strategies for cell adhesion (Park et al., 2009; Dalle et al., 2010), which may be more pronounced in this fungus than in others. Additionally, *C. albicans* may have specifically adapted to the nutritional conditions provided by the host (Brock, 2009).

Healthy humans are generally not affected by severe invasive fungal infections, because the interplay of different immune effectors, especially from the innate immune system, successfully combats fungal invaders. However, this situation may change dramatically in immunocompromised patients, either suffering from immunosuppressing diseases or chemotherapy (Shoham and Marwaha, 2010). In these patients, the orchestrating of immune effector cells becomes imbalanced, which may allow fungi colonizing and invading human tissues. However, the residual immune system in these patients still attacks fungal pathogens, which may, e.g. cause hyperinflammatory responses (Lewis and Kontoyiannis, 2009). An example for such a response is given by the massive neutrophil recruitment to lung tissues under corticosteroid treatment after pulmonary infection with *A. fumigatus* conidia. These neutrophils encircle outgrowing hyphae preventing the uncontrolled and rapid spreading of mycelium through the tissue by the cost of severe tissue damages (Ibrahim-Granet et al., 2010). Macrophages and neutrophils may also phagocytose conidia or yeast cells causing environments of acid pH, nutrient limitation, and oxidative stress (Fernández-Arenas et al., 2007; Frohner et al., 2009). Therefore, fungi require strategies to survive and propagate under these hostile conditions. One strategy for escaping phagocytes is the rapid elongation of hyphae, which can destroy the cell membrane of macrophages by mechanical forces (Lorenz et al., 2004; Ghosh et al., 2009) and subsequently allows invasive growth through tissues. However, elongation of hyphae and growth are energy consuming reactions and without nutrition an infection could not establish.

The numbers of antifungals available for combating life-threatening fungal infections are rather limited, and they mainly act on the integrity of the fungal cell membrane and cell wall (Hoehamer et al., 2010). Species-specific resistance has been observed leading to ineffective therapy (Chandrasekar, 2009; Niimi et al., 2010). Therefore, the knowledge about the essential

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doi:10.1016/j.ijmm.2011.04.007

metabolic pathways used by pathogenic fungi during host infection may lead to the discovery of new drugs effective against a broad spectrum of fungi. A basic problem of this idea is the incomplete knowledge on the importance of metabolic pathways during pathogenesis (Brock, 2009). Different routes of infection such as conidia inhalation, epithelial invasion or wound infection provide different host environments. Additionally, the presence or absence of specific immune effector cells may influence the nutritional conditions. Last but not least, regulation and use of metabolic pathways may vary significantly between different fungal species. Therefore, the investigation of a broad range of pathogenic fungi is needed to obtain deeper insights into the essential metabolic pathways to combat fungal infections on the nutritional level.

The host niches

Fungi can infect nearly every body site. However, the nutrient composition varies significantly. Fungi other than dermatophytes rarely infect the skin surface. The dry environment and the extremely limited nutrient supply in combination with immune effector cells in subcutaneous regions prohibit the establishment of an infection unless the skin is harmed by trauma, irritation, or maceration. Exceptions are the superficial infections caused by dermatophytes. These fungi are able to efficiently use the insoluble keratin present at the skin as nutrient even at low water availability, providing them with carbon and nitrogen required for growth (Zaugg et al., 2009). Other fungi are either unable to grow or have very low growth rates on keratin at solid surfaces, which excludes them as pathogens at the skin surface (Scott and Untereiner, 2004).

In contrast, mucosal surfaces, as found in the oral region or the gut, are rich in nutrients from food uptake, but reproduction is limited by competing microorganisms (Basson, 2000), and tissue invasion is restricted by the immune system. In this context, it has been shown in an in vitro model that, e.g. epithelial cells were protected against *C. albicans* invasion and cell injury by a TLR4 response triggered by polymorphonuclear cells (Weindl et al., 2007). The mucosal surface of the lung, in contrast, may provide a more nutrient-limited condition, since it is not in direct contact with nutrients from food intake. Additionally, the surface is covered with a mucus layer, which contains large amounts of antimicrobial factors (Travis et al., 1999). This allows the inactivation of inhaled microorganisms and their embedment for removal by ciliated cells and subsequent coughing. Additionally, alveolar macrophages rapidly phagocytose inhaled microorganisms, and their function is supported by neutrophils and dendritic cells (Behnsen et al., 2007; Mircescu et al., 2009; Bourgeois et al., 2010; Ibrahim-Granet et al., 2010). Therefore, nutrients are not easily available, and acquisition may require the disruption of host cells releasing cellular storage compounds. However, once penetrating the epithelial layers, pathogens may reach the bloodstream. Since blood is the major carrier of nutrients, glucose, proteins, amino acids, and vitamins are present in larger quantities. Therefore, it is essential for the host prohibiting the entry of pathogens to the blood, which is realized by large quantities of immune effector cells keeping the bloodstream virtually pathogen-free. In case of a systemic infection, pathogens can reach different internal organs such as the liver, which is the main storage compartment of glucose in the form of glycogen. Likewise, the brain only contains low amounts of proteins but is rich in glucose and vitamins, which are required for normal brain function. Contrarily, and as mentioned above, phagocytes are present at nearly all body sites and may form extremely nutrient-limited conditions causing severe starvation. This indicates that different metabolic adaptations are required at different host sites, and it remains questionable, whether targeting of a single metabolic pathway can prohibit fungal growth at all host niches.

Glucose and glycolysis: fermentation or oxidation?

Most aerobic microorganisms prefer the use of glucose as carbon source rather than using gluconeogenic nutrients. The explanation for this phenomenon is simple: Glucose as carbon source does not require gluconeogenesis for its synthesis and is easily degraded via glycolysis or the pentose phosphate pathway. The latter not only provides building blocks for nucleic acids and cofactors, but also generates NADPH for biosynthetic processes and for combating oxidative stresses (Thön et al., 2007). Additionally, the use of glucose allows fermentation even under aerobic conditions by gaining energy from glycolysis. Thereby, the use of glucose as carbon source splits fungi into so-called Crabtree-negative and -positive species. Filamentous fungi tend to group with the Crabtree-negative species, showing a strong upregulation of the pyruvate dehydrogenase complex in the presence of glucose (Chambergo et al., 2002; Maeda et al., 2004; Xie et al., 2004). This enables a high flux of acetyl-CoA through the TCA cycle and the complete conversion into CO_2. Analysis of the carbon balance of *A. nidulans* grown on glucose as sole carbon and energy source showed that the total C content was found within the biomass and CO_2 (Brock and Buckel, 2004). In contrast, Crabtree-positive fungi, especially *Saccharomyces cerevisiae*, downregulate activity of the PDH complex in the presence of high glucose concentrations. Pyruvate is not converted to acetyl-CoA for oxidation via the TCA cycle, which leads to the secretion of organic compounds, mainly ethanol, even in the presence of the terminal electron acceptor oxygen (Klein et al., 1998). One possible advantage of this strategy is the rapid consumption of glucose from the medium, which might be beneficial for competition in the environment. As a drawback, energy and biomass yields from glucose are reduced, because some energy and carbon remain fixed in the fermentation products (Merico et al., 2007).

Regulation of glycolysis

In order to favor glycolysis over gluconeogenesis, most fungi seem to contain a carbon catabolite repressor system, which is mainly represented by Mig1 in *S. cerevisiae* and *C. albicans* and by CreA in filamentous fungi (Ruijter and Visser, 1997; Schüller, 2003). In the absence of glucose, Mig1 becomes phosphorylated, leading to its translocation from the nucleus to the cytosol (De Vit et al., 1997). In *S. cerevisiae*, Mig1 also interacts with the glucose-sensing hexokinase Hxk2p, which only exhibits regulatory functions in the nucleus during the simultaneous presence of Mig1p (Ahuatzi et al., 2004). Thereby, Hxk2p can inhibit the phosphorylation of Mig1p by the Snf1 kinase (Ahuatzi et al., 2007). Active Mig1p targets the global repressor complex Tup1p/Ssn6p to the promoters of glucose-repressed genes, which leads to the inactivation of genes involved in metabolism of alternative carbon sources (Elbing et al., 2004). Although Mig1p is also present in *C. albicans* (CaMig1p), some differences in their regulatory properties seem to exist. A disruption mutant of *mig1* in *S. cerevisiae* abolishes the glucose repression of the genes responsible for galactose metabolism (Nehlin et al., 1991), whereas a deletion of the *mig1* homologue in *C. albicans* has no effect on expression of the glucose-repressed gene CaGal1. Moreover, CaMig1 expression does not significantly change between glucose or ethanol as carbon source, and CaMig1p possesses no putative phosphorylation site for CaSnf1p. Therefore, additional proteins may be involved in carbon catabolite repression in *C. albicans*. Nevertheless, CaMig1 was able to complement the phenotypes of a *S. cerevisiae MIG1* deletion strain (Zaragoza et al., 2000).

In Aspergilli, carbon catabolite repression has not been fully understood, but CreA seems the most important key player in this mechanism (Ruijter and Visser, 1997). However, CreA appears

to possess a broader function than simply repressing metabolism of alternative carbon sources, since mutations in the zinc finger domain result in severe morphological changes (Shroff et al., 1997). Furthermore, additional proteins (CreB, CreC, and CreD) are present, whereby mutations in *creB* and *creC* revealed that they also participate in regulation of carbon source utilization (Hynes and Kelly, 1977; Lockington and Kelly, 2002). Additionally, CreD seems to influence the protein interaction between CreB and CreC (Boase and Kelly, 2004). In contrast to *S. cerevisiae*, the localization of CreA is not influenced by the respective carbon source, and no autoregulation in *creA* expression seems to exist (Roy et al., 2008). Therefore, it remains unclear, which specific signals trigger the activation or inactivation of the carbon catabolite repression system in Aspergilli. However, it has been shown that CreA can directly inactivate genes by promoter binding and genes become fully induced in CreA mutants even in the presence of glucose. Other genes, although negatively regulated by CreA, need an additional activator in dependence of the available nutrient source. These genes are not upregulated in CreA mutants in the presence of glucose, but when shifted to the respective inducing carbon source (Mogensen et al., 2006).

Whether there are any additional specific regulatory proteins, which induce the expression of glycolytic genes in Aspergilli, has not been studied in detail. However, comparative studies between *S. cerevisiae* and *C. albicans* have shown that fundamental differences seem to exist, which also contributes to their classification in Crabtree-positive and -negative fungi (Askew et al., 2009). In *S. cerevisiae*, Gcr1p and Gcrp2 represent two important activators for glycolytic genes (Uemura and Fraenkel, 1990; Chambers et al., 1995). Gcr1p binds directly to the promoter region of glycolytic genes, whereas Gcr2p supports Gcr1p action by complex formation (Uemura and Jigami, 1992; Uemura et al., 1997). Deletion mutants in these genes displayed a strong growth defect on glucose, whereas no altered phenotype was observed on non-fermentable carbon sources. Transcription factors, such as Tye7p (=Sgc1p), Rap1p, Abf1p, and Reb1p additionally influence activation of glycolytic genes, but their contribution appears less important than that of Gcr1p and Gcr2p (Scott and Baker, 1993; Nishi et al., 1995). The pyruvate dehydrogenase complex, directing pyruvate to the TCA cycle, seems constitutively expressed in *S. cerevisiae*, but is inhibited on fermentable carbon sources by phosphorylation (James et al., 1995). Therefore, glycolysis strongly increases in the presence of glucose, but complete degradation via the TCA cycle is inhibited.

Interestingly, most Crabtree-negative fungi, including *C. albicans*, lack homologues of Gcr1p and Gcr2p, and alternative regulation mechanisms need to exist. For *C. albicans*, it has recently been shown that Tye7p and Gal4p act as main regulators of glycolysis (Askew et al., 2009). Both proteins are required for full induction of glycolytic genes, especially in the presence of a low oxygen partial pressure. Under these conditions, Tye7p also regulates genes involved in trehalose, glycogen, and glycerol metabolism, whereas Gal4p is responsible for the regulation of the pyruvate dehydrogenase complex. Tye7p seems to act as the central key regulator of carbohydrate metabolism, and Gal4p is responsible for the fine-tuning of gene expression (Askew et al., 2009). Therefore, the upregulation of the PDH complex, as also described for several filamentous fungi, directs pyruvate into the TCA cycle and prevents the Crabtree effect.

Impact of glycolysis on virulence

The impact of glycolytic enzymes on virulence by the direct analysis of glycolytic mutants has hardly been investigated. However, expression analyses from infected tissues, especially performed on *C. albicans*, imply that at least at certain time points glycolytic genes are strongly expressed. As mentioned above, the interaction of *C. albicans* with immune effector cells, such as macrophages and neutrophils, seems to suppress high glycolytic activity, but, in turn, favors induction of gluconeogenesis and (in case of *C. albicans*) the glyoxylate cycle (Fradin et al., 2005). In contrast, once penetrating a tissue and establishing an infection, significant proportions of cells seem to use, at least temporarily, the glycolytic path. Transcriptome analyses on *C. albicans* reisolated after infection of a haemoperfused liver, which served as an ex vivo model, showed an upregulation of the phosphofructokinase (*PFK2*), genes from the pyruvate dehydrogenase complex (*PDA1*; *PDX1*) and some genes of the TCA cycle (*KGD1*; *KGD2*). However, a subpopulation of cells also showed a strong upregulation of the phosphoenolpyruvate carboxykinase (*PCK1*), which generates phosphoenolpyruvate from oxaloacetate and is a key enzyme of gluconeogenesis (Thewes et al., 2007). Additionally, a so-called single cell profiling of *C. albicans* cells infecting the kidneys of mice showed a strong fluorescence of cells expressing the GFP protein under the control of the *PYK1* and *PFK2* promoters, indicating active growth and glycolysis. However, also in this independent approach, at least a subpopulation of cells showed isocitrate lyase (*ICL1*) and *PCK1* expression, indicating that some cells are either starving or utilizing non-fermentable carbon sources (Barelle et al., 2006). Nevertheless, *C. albicans TYE7/GAL4* mutants with disturbed regulation of glycolysis under low oxygen supply revealed an attenuation of virulence in a murine model system. This indicates that glycolysis and shunting of metabolites into the TCA cycle possesses an important contribution to virulence of this fungus (Askew et al., 2009). Therefore, glycolysis may be of temporal importance during growth within host tissues, but may not be essential for the general ability of fungi to cause an infection at all host niches.

Regulation of glyoxylate cycle and gluconeogenesis in yeasts and filamentous fungi

Gluconeogenesis is required on all non-fermentable carbon sources to provide sugars for the synthesis of cell wall components, storage compounds and for feeding the pentose phosphate pathway for nucleotide and cofactor synthesis from ribose. In addition, the glyoxylate cycle is required for the anaplerosis of malate (oxaloacetate) during growth on non-fermentable carbon sources, which exclusively produce acetyl-CoA units such as acetate, ethanol, and fatty acids. Therefore, a direct link between gluconeogenesis and glyoxylate cycle exists. Regulation of the fungal glyoxylate cycle has been studied best in the yeast *S. cerevisiae* and the filamentous fungus *A. nidulans*. Although the basic principles of glyoxylate cycle regulation seem conserved among fungal species, they differ in some important aspects. The main regulatory mechanisms presented here are summarized in a schematic presentation in Fig. 1.

In *A. nidulans*, and most likely also in several other filamentous ascomycetes, induction of the glyoxylate cycle is strongly dependent on the transcriptional activator FacB. This transcription factor of the GAL4-type contains a $Zn(II)_2Cys_6$ binuclear cluster (Todd et al., 1997b) and regulates the acetate-dependent induction of acetyl-CoA synthase, isocitrate lyase, malate synthase, acetamidase, and NADP-isocitrate dehydrogenase (Todd et al., 1997a), but not the pyruvate carboxykinase or other genes of the gluconeogenic path (Hynes et al., 2002). Upregulation of the latter genes seems mainly controlled by AcuM and AcuK (Hynes et al., 2007). Deletion of the *facB* gene in *A. nidulans* prohibits growth on acetate, ethanol, and fatty acids, but does not interfere with growth on other gluconeogenic carbon sources such as glycerol, propionate, or several amino acids. Transcription of *facB* is induced during growth on acetate, but transcript levels are controlled by carbon catabolite

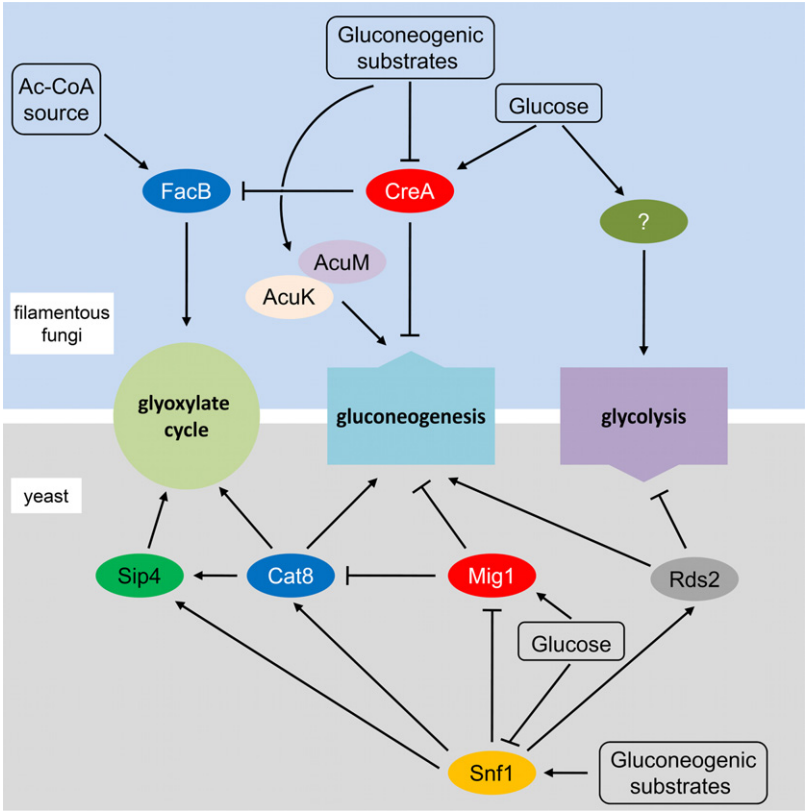

Fig. 1. Simplified scheme for the global regulation of glyoxylate cycle, gluconeogenesis, and glycolysis in filamentous fungi (upper part) and yeasts (lower part). Some major transcription factors are shown. Dead-end lines denote negative effects, whereas arrows represent stimulating effects. Substrates mediating induction or repression are boxed. Ac-CoA source = acetyl-CoA generating carbon sources. In filamentous fungi, CreA is the major carbon catabolite repressor, which inhibits induction of genes required for alternative carbon source utilization in the presence of glycolytic sugars. For glyoxylate cycle activation, the absence of glucose and the presence of an inducing carbon source are required, whereby the transcriptional activator FacB does not induce genes of gluconeogenesis. Glycolysis is stimulated by a yet unknown transcription factor. In yeasts, Mig1 is the major carbon catabolite repressor. Inactivation of Mig1 via phosphorylation by the Snf1 kinase (in *S. cerevisiae*) allows activation of Cat8, which stimulates both, the glyoxylate cycle and gluconeogenesis. Note that for simplification of the scheme the activators of glycolysis Gcr1 and Gcr2 in *S. cerevisiae* and Tye7 and Gal4 in *C. albicans* are not shown. For a more detailed explanation refer to the main text.

repression as shown by the addition of sucrose to acetate medium and investigations on a *creA204* mutant strain (Katz and Hynes, 1989). Therefore, induction of the glyoxylate cycle in *A. nidulans* not only requires the presence of an inducing carbon source, but also the absence of repressing sugars, which activate CreA (Mogensen et al., 2006).

In *S. cerevisiae*, the situation appears somewhat more complicated, but, as a simplified scheme, the key players seem to consist of the Snf1 kinase, Mig1, Cat8, Sip4, and Rds2 (Turcotte et al., 2010). Snf1 kinase is activated under gluconeogenic conditions and inactivates the carbon catabolite repressor Mig1 by phosphorylation. The Snf1 kinase also phosphorylates Cat8, Sip4, and Rds2, leading to their activation. Mig1 possesses its closest homology with the carbon catabolite repressor protein CreA from filamentous ascomycetes and negatively regulates Cat8. Due to the inactivation of Mig1 by the Snf1 kinase and the additional activation of Cat8 by phosphorylation, Cat8 becomes highly active. The DNA binding domain of Cat8 is highly identical to the binding domain of FacB from *A. nidulans*, and the glyoxylate cycle genes in *S. cerevisiae* are under its control. However, Cat8 has a much broader and more general function than FacB in *A. nidulans*. Cat8 also induces its coregulator Sip4, but a deletion of Sip4 only confers weak phenotypes on gluconeogenic carbon sources. Most importantly, a deletion of Cat8 leads to an inability to grow on all non-fermentable carbon sources (Hiesinger et al., 2001). This is mainly due to the fact that Cat8 not only regulates genes of the glyoxylate cycle (as described for FacB), but also several gluconeogenic enzymes (Schüller, 2003). The function of Cat8 and Sip4 is furthermore enhanced by Rds2, which binds to promoters of genes involved in gluconeogenesis and the glyoxylate shunt, enhancing their expression. Additionally, Rds2 is a repressor of negative regulators of gluconeogenesis (Turcotte et al., 2010). Since there seems no regulator present in *S. cerevisiae*, which only targets the genes of the glyoxylate bypass, upregulation of the key enzymes isocitrate lyase and malate synthase may occur under conditions of low glucose without the urgent need for the presence of inducing carbon sources requiring this anaplerotic path. Since most of the transcriptional regulators involved in gluconeogenesis present in *S. cerevisiae* are also found in *C. albicans* (especially Mig1, Cat8, and Rds2, which is called CWT1), similar regulatory mechanisms may exist in this pathogenic fungus. In agreement, a fusion of the isocitrate lyase promoter of *C. albicans* with the gene coding for eGFP reveals high fluorescence not only after growth on acetate, but also on glycerol and peptone, and starvation and activity determinations confirmed the presence of active isocitrate lyase under these conditions (Brock, 2009). In contrast, determination of *A. fumigatus* isocitrate lyase by Western blot analysis showed that isocitrate lyase is not detectable on the gluconeogenic carbon source glycerol. This confirms the separation of regulation of the glyoxylate cycle and gluconeogenesis in Aspergilli (Ebel et al., 2006). However, isocitrate lyase is present in resting conidia of *A. fumigatus* (Ebel et al., 2006), but conidia of *A. fumigatus* mutants with a deletion of the isocitrate lyase showed no germination defect on glucose or glycerol (Schöbel et al., 2007). Therefore, isocitrate lyase may belong to the metabolic toolbox of conidia, which is required for being adapted to a broad

variety of nutrients, which may be found in the natural environment

The glyoxylate cycle: a specific marker for carbon sources or a general responder to glucose starvation?

Several independent studies have shown that genes coding for glyoxylate cycle enzymes are upregulated in *C. albicans* cells after phagocytosis by macrophages (Lorenz et al., 2004) and confrontation with neutrophils (Fradin et al., 2005). Additionally, a deletion of the gene coding for the key enzyme isocitrate lyase results in attenuated virulence (Lorenz and Fink, 2001; Barelle et al., 2006; Piekarska et al., 2006). Therefore, it was at first assumed that lipids and fatty acids, either deriving from the host or from internal degradation of storage compounds, might provide an important nutrient for *C. albicans* during pathogenesis and especially after phagocytosis by macrophages (Lorenz and Fink, 2002). However, as mentioned above, further analyses of *C. albicans* isocitrate lyase expression and mutant phenotype analyses showed that nutrient limitation is not essential to induce isocitrate lyase. Isocitrate lyase was shown to play also an important role on other non-fermentable carbon sources such as lactate, citrate, glycerol, or amino acids (Barelle et al., 2006; Piekarska et al., 2008; Brock, 2009) and, therefore, seems to provide the key pathway in this fungus for the anaplerosis of oxaloacetate required for gluconeogenesis. This assumption coincides with the simultaneous regulation of glyoxylate cycle genes and genes coding for enzymes of the gluconeogenic path, which are under the control of Cat8 in the yeast *S. cerevisiae*. Assuming that the regulatory mechanisms are similar in *C. albicans*, it can be concluded that induction of the glyoxylate cycle is not a marker for lipid metabolism in *C. albicans*, but for non-fermentable carbon sources and gluconeogenesis in general.

This assumption is in sharp contrast to observations on *A. fumigatus*. Although high levels of isocitrate lyase were detected in germinating hyphae after confronting conidia with macrophages, a deletion of the respective gene did not significantly alter virulence in leukopenic or non-leukopenic animal models for bronchopulmonary aspergillosis (Schöbel et al., 2007; Olivas et al., 2008). The high levels of isocitrate lyase in germinating conidia may mainly result from the fact that isocitrate lyase is already present in resting conidia and is not degraded during germination within macrophages due to the low amount of glucose present. Once disrupting the macrophage membrane and reaching the cell culture medium, immunofluorescence specific for isocitrate lyase rapidly disappeared in *A. fumigatus* hyphae (Ebel et al., 2006), which coincides with degradation of glyoxysomes in the presence of glucose as described for *A. nidulans* (Amor et al., 2000). In agreement with high levels of isocitrate lyase inside macrophages, the confrontation of *A. fumigatus* conidia with neutrophils also increased the expression of glyoxylate cycle genes and genes responsible for β-oxidation of fatty acids. This implies that under immune effector cell-mediated stress carbon catabolite repression is negligible, and the presence of phospholipids and lipids may induce glyoxylate cycle activity (Sugui et al., 2008). However, due to the unchanged virulence in non-leukopenic infection models, alternative nutrient sources such as proteins and amino acids could make the anaplerosis of oxaloacetate by the glyoxylate cycle during infection unnecessary. As mentioned above, starvation, glycerol, or peptone did not significantly induce isocitrate lyase in *A. fumigatus*, and the growth phenotype of isocitrate lyase mutants under these conditions is not altered. In contrast, growth of the mutant on acetate, ethanol, or olive oil is completely abolished (Schöbel et al., 2007). Therefore, it can be concluded that in *A. fumigatus*, unlike *C. albicans*, the induction of isocitrate lyase in vegetative mycelium indeed acts as a marker for nutrient sources providing acetyl-CoA units, but cannot serve as a marker for non-fermentable carbon sources per se. These differences in regulation and use of the glyoxylate cycle in the two pathogenic fungi might explain why one of the species is strongly dependent on a functional glyoxylate cycle during pathogenesis, whereas the other is not. However, despite a lack of evidence for the impact of gluconeogenesis in virulence of *A. fumigatus*, it can be assumed that after phagocytosis or within necrotic tissues glucose concentration may become limited. Therefore, although the glyoxylate cycle itself is not of major importance for *A. fumigatus* virulence, disruption of the gluconeogenic path might affect the ability of fungi to persist within infected tissues.

Nitrogen acquisition from proteins and amino acids

Besides the importance of an adequate carbon source, uptake of nitrogen is an essential prerequisite for biomass formation. An elegant way combining the simultaneous uptake of suitable carbon and nitrogen sources is the intake of amino acids. However, the largest proportion of amino acids is fixed in host proteins, requiring the production of proteolytic enzymes.

C. albicans produces several secreted proteases all belonging to the secreted aspartyl proteinases (SAP). Interestingly, production of different proteases seems dependent on fungal morphology and the nutritional status of the cells. The most studied aspartic proteases concerning their contribution to virulence are Sap1–6. Thereby, Sap1, 2, and 3 are mainly associated with the yeast form, whereas Sap4, 5, and 6 are produced in hyphae. Several investigations have shown that the protease Sap2p is secreted in the presence of proteins as sole nitrogen source (Hube et al., 1994; Dabas and Morschhäuser, 2008). Both, the yeast-associated as well as the hyphae-associated proteases contribute to virulence (Hube et al., 1997; Sanglard et al., 1997). However, invasion of epithelial cells and manifestation of vaginal candidiasis seem mainly attributable to Sap1–3 (De Bernardis et al., 1999; Schaller et al., 2000), whereas Sap4–6 appeared more important for murine disseminated and peritoneal infections (Kretschmar et al., 1999). This difference may be due to the different pH optima of the proteases (Hube et al., 1994; White and Agabian, 1995) and the fact that hyphal formation is required for tissue invasion (Felk et al., 2002), whereby Sap5 seems to be the major protease responsible for tissue destruction (Naglik et al., 2008). However, it remains unclear, whether the production of proteases is due to the need of amino acid uptake for nitrogen acquisition, required for the defense against immune effectors, e.g. the degradation of components of the complement system (Gropp et al., 2009), or for facilitating the invasion of cells by weakening the connecting barriers of the host tissues. Probably, all three tasks are of importance, and Sap proteins may specifically contribute to the adaptiveness of *C. albicans* to the human host. Therefore, inhibitors of *C. albicans* proteases might provide suitable antifungals against this pathogen (Stewart and Abad-Zapatero, 2001; Mastrolorenzo et al., 2007; Yordanov et al., 2008).

The role of proteases in the virulence of *A. fumigatus* appears somewhat more difficult to assess, but at least under some conditions, amino acids seem to serve as nutrients during infection. This assumption is supported by different investigations such as: (i) a methylcitrate synthase mutant, which is unable to remove toxic propionyl-CoA deriving from the degradation of isoleucine, valine, and methionine, is attenuated in virulence (Ibrahim-Granet et al., 2008) and (ii) a lysine auxotrophic homocitrate synthase mutant displays normal virulence in a model for disseminated aspergillosis (Schöbel et al., 2010), which indicates that, at least, a sufficient intake of free lysine from the blood is possible. However, this latter mutant is strongly attenuated in a model for murine bronchopulmonary aspergillosis, implying that the free lysine content within

lung tissues is limited. Further investigations showed that resting conidia are not able to release lysine from proteins due to insufficient protease activity. However, once germinated, the mutant was able to grow on proteins, which seems mainly due to the secretion of proteases from hyphae. The main secreted protease produced by *A. fumigatus* is the alkaline serine protease Alp1, which was found to be highly abundant in infected lung tissues (Reichard et al., 1990; Monod et al., 1991; Frosco et al., 1992; Larcher et al., 1992; Kolattukudy et al., 1993) and able to degrade the complement components C3b, C4b, and C5 (Behnsen et al., 2010; Rambach et al., 2010). However, Alp1-deficient strains still show elastinolytic activity, which led to the identification of the metalloprotease Mep1, which is also produced under infectious conditions (Monod et al., 1993). As a third protease, probably important for virulence, a secreted aspartic protease was identified (Reichard et al., 1994, 1995). Additionally, *A. fumigatus* produces several dipeptidyl-peptidases and sedolisins, which may also contribute to the degradation of proteins under different environmental conditions (Beauvais et al., 1997; Reichard et al., 2006). Further proteases have been detected, but will not be listed here (Monod et al., 2009). However, the contribution of all these proteases in virulence is unclear. Several studies showed that the knockout of one or the combination of two proteases is not sufficient to attenuate virulence (Jaton-Ogay et al., 1994; Reichard et al., 1997). However, a more detailed analysis of these studies showed that in most cases animals were used, which were either immunosuppressed by corticosteroid treatment or remained immunocompetent prior to infection. In contrast, investigations on a mutant *A. fumigatus* strain with strongly reduced elastinolytic activity as well as two environmental isolates with a low elastinolytic index showed a strongly reduced virulence in a neutropenic infection model (Kolattukudy et al., 1993; García et al., 2006), but successive inoculations of the environmental strains in neutropenic mice strongly increased their elastinolytic activity (García et al., 2006). Contrarily, mutants carrying a deletion of the major regulator for protease production and secretion, PrtT, did not attenuate virulence when tested in murine infection models regardless of the use of leukopenic or non-leukopenic infection models (Bergmann et al., 2009; Sharon et al., 2009). Although both studies showed that production of all major proteases was significantly reduced under several in vitro conditions in *prtT* deletion strains, it remained unclear, whether proteolytic activity might be induced in a PrtT-independent manner under in vivo infectious conditions.

However, at least in non-leukopenic mice, a reduced impact of *A. fumigatus*-secreted proteases is well conceivable. The immunosuppression regimens with corticosteroids increase the number of circulating neutrophils (Schleimer, 2004), and these are recruited to the site of infection. Although attenuated in their antimicrobial activity, these neutrophils cause severe tissue damage by oxidative bursts and degranulation (Brock et al., 2008; Ibrahim-Granet et al., 2010). Therefore, degranulating neutrophils may substitute for the protease deficiency of the *A. fumigatus* strains. Contrarily, in neutropenic models or patients, the proteolytic activity for destroying connecting tissues, complement evasion, and for feeding off nitrogen from proteins needs to derive from the pathogen, although it remains to be confirmed that these actions are indeed required for establishment of invasive aspergillosis. However, it can be assumed that the immunosuppression regimen might affect the outcome of virulence studies and should be taken into account, when metabolic mutants are investigated.

Conclusions

Taken together, fungi possess different regulatory mechanisms for the induction of key metabolic pathways, resulting in subtle differences in metabolic strategies. This may reason, why disruption of key enzymes of metabolic pathways, such as the isocitrate lyase from the glyoxylate cycle, displays different impacts on virulence in dependence on the investigated species. Additionally, the site of infection requires versatile metabolic adaptations due to vital differences in the nutritional constitution. Furthermore, the immunosuppression regimen influences the composition of immune effector cells, and their attack on fungal pathogens strongly influences the metabolic requirements. Lots of efforts have been made to understand the metabolic needs of *C. albicans* during infection, but additional studies are required to draw a complete picture on metabolism during host infection. For other pathogenic fungi including *A. fumigatus*, the number of investigations is much more limited and the 'metabolic picture' appears rather incomplete. In order to identify common essential metabolic pathways, suitable for the development of new broad-spectrum antifungals, a systematic investigation of metabolic mutants in different infection models seems required.

Acknowledgements

The authors' work is supported by grants from the German Sciences Foundation (DFG, priority program 1160 and BR2216/4), the Jena School for Microbial Communication (JSMC), and the Hans Knöll Institute (HKI).

References

Ahuatzi, D., Herrero, P., de la Cera, T., Moreno, F., 2004. The glucose-regulated nuclear localization of hexokinase 2 in *Saccharomyces cerevisiae* is Mig1-dependent. J. Biol. Chem. 279, 14440–14446.

Ahuatzi, D., Riera, A., Pelaez, R., Herrero, P., Moreno, F., 2007. Hxk2 regulates the phosphorylation state of Mig1 and therefore its nucleocytoplasmic distribution. J. Biol. Chem. 282, 4485–4493.

Alvarez, M., Casadevall, A., 2006. Phagosome extrusion and host-cell survival after *Cryptococcus neoformans* phagocytosis by macrophages. Curr. Biol. 16, 2161–2165.

Amor, C., Domínguez, A.I., De Lucas, J.R., Laborda, F., 2000. The catabolite inactivation of *Aspergillus nidulans* isocitrate lyase occurs by specific autophagy of peroxisomes. Arch. Microbiol. 174, 59–66.

Askew, C., Sellam, A., Epp, E., Hogues, H., Mullick, A., Nantel, A., Whiteway, M., 2009. Transcriptional regulation of carbohydrate metabolism in the human pathogen *Candida albicans*. PLoS Pathog. 5, e1000612.

Barelle, C.J., Priest, C.L., Maccallum, D.M., Gow, N.A., Odds, F.C., Brown, A.J., 2006. Niche-specific regulation of central metabolic pathways in a fungal pathogen. Cell. Microbiol. 8, 961–971.

Basson, N.J., 2000. Competition for glucose between *Candida albicans* and oral bacteria grown in mixed culture in a chemostat. J. Med. Microbiol. 49, 969–975.

Beauvais, A., Monod, M., Wyniger, J., Debeaupuis, J.P., Grouzmann, E., Brakch, N., Svab, J., Hovanessian, A.G., Latgé, J.P., 1997. Dipeptidyl-peptidase IV secreted by *Aspergillus fumigatus*, a fungus pathogenic to humans. Infect. Immun. 65, 3042–3047.

Behnsen, J., Narang, P., Hasenberg, M., Gunzer, F., Bilitewski, U., Klippel, N., Rohde, M., Brock, M., Brakhage, A.A., Gunzer, M., 2007. Environmental dimensionality controls the interaction of phagocytes with the pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*. PLoS Pathog. 3, e13.

Behnsen, J., Lessing, F., Schindler, S., Wartenberg, D., Jacobsen, I.D., Thoen, M., Zipfel, P.F., Brakhage, A.A., 2010. Secreted *Aspergillus fumigatus* protease Alp1 degrades human complement proteins C3, C4, and C5. Infect. Immun. 78, 3585–3594.

Bergmann, A., Hartmann, T., Cairns, T., Bignell, E.M., Krappmann, S., 2009. A regulator of *Aspergillus fumigatus* extracellular proteolytic activity is dispensable for virulence. Infect. Immun. 77, 4041–4050.

Bliska, J.B., Casadevall, A., 2009. Intracellular pathogenic bacteria and fungi–a case of convergent evolution? Nat. Rev. Microbiol. 7, 165–171.

Boase, N.A., Kelly, J.M., 2004. A role for *creD*, a carbon catabolite repression gene from *Aspergillus nidulans*, in ubiquitination. Mol. Microbiol. 53, 929–940.

Bourgeois, C., Majer, O., Frohner, I.E., Tierney, L., Kuchler, K., 2010. Fungal attacks on mammalian hosts: pathogen elimination requires sensing and tasting. Curr. Opin. Microbiol. 13, 401–408.

Brock, M., Buckel, W., 2004. On the mechanism of action of the antifungal agent propionate. Eur. J. Biochem. 271, 3227–3241.

Brock, M., Jouvion, G., Droin-Bergere, S., Dussurget, O., Nicola, M.A., Ibrahim-Granet, O., 2008. Bioluminescent *Aspergillus fumigatus*, a new tool for drug efficiency testing and in vivo monitoring of invasive aspergillosis. Appl. Environ. Microbiol. 74, 7023–7035.

Brock, M., 2009. Fungal metabolism in host niches. Curr. Opin. Microbiol. 12, 371–376.

Chambergo, F.S., Bonaccorsi, E.D., Ferreira, A.J., Ramos, A.S., Ferreira Junior, J.R., Abrahao-Neto, J., Farah, J.P., El-Dorry, H., 2002. Elucidation of the metabolic fate of glucose in the filamentous fungus *Trichoderma reesei* using expressed sequence tag (EST) analysis and cDNA microarrays. J. Biol. Chem. 277, 13983–13988.

Chambers, A., Packham, E.A., Graham, I.R., 1995. Control of glycolytic gene expression in the budding yeast (*Saccharomyces cerevisiae*). Curr. Genet. 29, 1–9.

Chandrasekar, P., 2009. Prophylaxis against *Aspergillus* is not perfect: problems and perils in stem cell transplantation. Med. Mycol. 47 (Suppl. 1), S349–354.

Dabas, N., Morschhäuser, J., 2008. A transcription factor regulatory cascade controls secreted aspartic protease expression in *Candida albicans*. Mol. Microbiol. 69, 586–602.

Dalle, F., Wächtler, B., L'Ollivier, C., Holland, G., Bannert, N., Wilson, D., Labruere, C., Bonnin, A., Hube, B., 2010. Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. Cell. Microbiol. 12, 2471–2482.

De Bernardis, F., Arancia, S., Morelli, L., Hube, B., Sanglard, D., Schäfer, W., Cassone, A., 1999. Evidence that members of the secretory aspartyl proteinase gene family, in particular SAP2, are virulence factors for *Candida* vaginitis. J. Infect. Dis. 179, 201–208.

De Vit, M.J., Waddle, J.A., Johnston, M., 1997. Regulated nuclear translocation of the Mig1 glucose repressor. Mol. Biol. Cell 8, 1603–1618.

Denning, D.W., Anderson, M.J., Turner, G., Latgé, J.P., Bennett, J.W., 2002. Sequencing the *Aspergillus fumigatus* genome. Lancet Infect. Dis. 2, 251–253.

Ebel, F., Schwienbacher, M., Beyer, J., Heesemann, J., Brakhage, A.A., Brock, M., 2006. Analysis of the regulation, expression, and localisation of the isocitrate lyase from *Aspergillus fumigatus*, a potential target for antifungal drug development. Fungal Genet. Biol. 43, 476–489.

Elbing, K., Ståhlberg, A., Hohmann, S., Gustafsson, L., 2004. Transcriptional responses to glucose at different glycolytic rates in *Saccharomyces cerevisiae*. Eur. J. Biochem. 271, 4855–4864.

Felk, A., Kretschmar, M., Albrecht, A., Schaller, M., Beinhauer, S., Nichterlein, T., Sanglard, D., Korting, H.C., Schäfer, W., Hube, B., 2002. *Candida albicans* hyphal formation and the expression of the Efg1-regulated proteinases Sap4 to Sap6 are required for the invasion of parenchymal organs. Infect. Immun. 70, 3689–3700.

Fernández-Arenas, E., Cabezón, V., Bermejo, C., Arroyo, J., Nombela, C., Diez-Orejas, R., Gil, C., 2007. Integrated proteomics and genomics strategies bring new insight into *Candida albicans* response upon macrophage interaction. Mol. Cell. Proteomics 6, 460–478.

Fradin, C., De Groot, P., MacCallum, D., Schaller, M., Klis, F., Odds, F.C., Hube, B., 2005. Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. Mol. Microbiol. 56, 397–415.

Frohner, I.E., Bourgeois, C., Yatsyk, K., Majer, O., Kuchler, K., 2009. *Candida albicans* cell surface superoxide dismutases degrade host-derived reactive oxygen species to escape innate immune surveillance. Mol. Microbiol. 71, 240–252.

Frosco, M., Chase Jr., T., Macmillan, J.D., 1992. Purification and properties of the elastase from *Aspergillus fumigatus*. Infect. Immun. 60, 728–734.

García, M.E., Caballero, J., Blanco, I., Cruzado, M., Costas, E., Blanco, J.L., 2006. Changes in the elastase activity and colonization ability of *Aspergillus fumigatus* after successive inoculations in mice. Rev. Iberoam. Micol. 23, 221–223.

Ghosh, S., Navarathna, D.H., Roberts, D.D., Cooper, J.T., Atkin, A.L., Petro, T.M., Nickerson, K.W., 2009. Arginine-induced germ tube formation in *Candida albicans* is essential for escape from murine macrophage line RAW 264.7. Infect. Immun. 77, 1596–1605.

Gropp, K., Schild, L., Schindler, S., Hube, B., Zipfel, P.F., Skerka, C., 2009. The yeast *Candida albicans* evades human complement attack by secretion of aspartic proteases. Mol. Immunol. 47, 465–475.

Hiesinger, M., Roth, S., Meissner, E., Schüller, H.J., 2001. Contribution of Cat8 and Sip4 to the transcriptional activation of yeast gluconeogenic genes by carbon source-responsive elements. Curr. Genet. 39, 68–76.

Hoehamer, C.F., Cummings, E.D., Hilliard, G.M., Rogers, P.D., 2010. Changes in the proteome of *Candida albicans* in response to azole, polyene, and echinocandin antifungal agents. Antimicrob. Agents Chemother. 54, 1655–1664.

Hube, B., Monod, M., Schofield, D.A., Brown, A.J., Gow, N.A., 1994. Expression of seven members of the gene family encoding secretory aspartyl proteinases in *Candida albicans*. Mol. Microbiol. 14, 87–99.

Hube, B., Sanglard, D., Odds, F.C., Hess, D., Monod, M., Schäfer, W., Brown, A.J., Gow, N.A., 1997. Disruption of each of the secreted aspartyl proteinase genes SAP1, SAP2, and SAP3 of Candida albicans attenuates virulence. Infect. Immun. 65, 3529–3538.

Hube, B., 2004. From commensal to pathogen: stage- and tissue-specific gene expression of *Candida albicans*. Curr. Opin. Microbiol. 7, 336–341.

Hynes, M.J., Kelly, J.M., 1977. Pleiotropic mutants of *Aspergillus nidulans* altered in carbon metabolism. Mol. Gen. Genet. 150, 193–204.

Hynes, M.J., Draht, O.W., Davis, M.A., 2002. Regulation of the *acuF* gene, encoding phosphoenolpyruvate carboxykinase in the filamentous fungus *Aspergillus nidulans*. J. Bacteriol. 184, 183–190.

Hynes, M.J., Szewczyk, E., Murray, S.L., Suzuki, Y., Davis, M.A., Sealy-Lewis, H.M., 2007. Transcriptional control of gluconeogenesis in *Aspergillus nidulans*. Genetics 176, 139–150.

Ibrahim-Granet, O., Dubourdeau, M., Latgé, J.P., Ave, P., Huerre, M., Brakhage, A.A., Brock, M., 2008. Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. Cell. Microbiol. 10, 134–148.

Ibrahim-Granet, O., Jouvion, G., Hohl, T.M., Droin-Bergère, S., Philippart, F., Kim, O.Y., Adib-Conquy, M., Schwendener, R., Cavaillon, J.M., Brock, M., 2010. In vivo bioluminescence imaging and histopathopathologic analysis reveal distinct roles for resident and recruited immune effector cells in defense against invasive aspergillosis. BMC Microbiol. 10, 105.

James, A.G., Cook, R.M., West, S.M., Lindsay, J.G., 1995. The pyruvate dehydrogenase complex of *Saccharomyces cerevisiae* is regulated by phosphorylation. FEBS Lett. 373, 111–114.

Jaton-Ogay, K., Paris, S., Huerre, M., Quadroni, M., Falchetto, R., Togni, G., Latgé, J.P., Monod, M., 1994. Cloning and disruption of the gene encoding an extracellular metalloprotease of *Aspergillus fumigatus*. Mol. Microbiol. 14, 917–928.

Katz, M.E., Hynes, M.J., 1989. Isolation and analysis of the acetate regulatory gene, *facB*, from *Aspergillus nidulans*. Mol. Cell. Biol. 9, 5696–5701.

Klein, C.J., Olsson, L., Nielsen, J., 1998. Glucose control in *Saccharomyces cerevisiae*: the role of Mig1 in metabolic functions. Microbiology 144 (Pt 1), 13–24.

Kolattukudy, P.E., Lee, J.D., Rogers, L.M., Zimmerman, P., Ceselski, S., Fox, B., Stein, B., Copelan, E.A., 1993. Evidence for possible involvement of an elastolytic serine protease in aspergillosis. Infect. Immun. 61, 2357–2368.

Kretschmar, M., Hube, B., Bertsch, T., Sanglard, D., Merker, R., Schröder, M., Hof, H., Nichterlein, T., 1999. Germ tubes and proteinase activity contribute to virulence of *Candida albicans* in murine peritonitis. Infect. Immun. 67, 6637–6642.

Larcher, G., Bouchara, J.P., Annaix, V., Symoens, F., Chabasse, D., Tronchin, G., 1992. Purification and characterization of a fibrinogenolytic serine proteinase from *Aspergillus fumigatus* culture filtrate. FEBS Lett. 308, 65–69.

Lewis, R.E., Kontoyiannis, D.P., 2009. Invasive aspergillosis in glucocorticoid-treated patients. Med. Mycol. 47 (Suppl. 1), S271–281.

Lockington, R.A., Kelly, J.M., 2002. The WD40-repeat protein CreC interacts with and stabilizes the deubiquitinating enzyme CreB in vivo in *Aspergillus nidulans*. Mol. Microbiol. 43, 1173–1182.

Lorenz, M.C., Fink, G.R., 2001. The glyoxylate cycle is required for fungal virulence. Nature 412, 83–86.

Lorenz, M.C., Fink, G.R., 2002. Life and death in a macrophage: role of the glyoxylate cycle in virulence. Eukaryot. Cell 1, 657–662.

Lorenz, M.C., Bender, J.A., Fink, G.R., 2004. Transcriptional response of *Candida albicans* upon internalization by macrophages. Eukaryot. Cell 3, 1076–1087.

Maeda, H., Sano, M., Maruyama, Y., Tanno, T., Akao, T., Totsuka, Y., Endo, M., Sakurada, R., Yamagata, Y., Machida, M., Akita, O., Hasegawa, F., Abe, K., Gomi, K., Nakajima, T., Iguchi, Y., 2004. Transcriptional analysis of genes for energy catabolism and hydrolytic enzymes in the filamentous fungus *Aspergillus oryzae* using cDNA microarrays and expressed sequence tags. Appl. Microbiol. Biotechnol. 65, 74–83.

Mastrolorenzo, A., Rusconi, S., Scozzafava, A., Barbaro, G., Supuran, C.T., 2007. Inhibitors of HIV-1 protease: current state of the art 10 years after their introduction. From antiretroviral drugs to antifungal, antibacterial and antitumor agents based on aspartic protease inhibitors. Curr. Med. Chem. 14, 2734–2748.

Merico, A., Sulo, P., Piskur, J., Compagno, C., 2007. Fermentative lifestyle in yeasts belonging to the *Saccharomyces* complex. FEBS J. 274, 976–989.

Mircescu, M.M., Lipuma, L., van Rooijen, N., Pamer, E.G., Hohl, T.M., 2009. Essential role for neutrophils but not alveolar macrophages at early time points following *Aspergillus fumigatus* infection. J. Infect. Dis. 200, 647–656.

Mogensen, J., Nielsen, H.B., Hofmann, G., Nielsen, J., 2006. Transcription analysis using high-density micro-arrays of *Aspergillus nidulans* wild-type and *creA* mutant during growth on glucose or ethanol. Fungal Genet. Biol. 43, 593–603.

Monod, M., Togni, G., Rahalison, L., Frenk, E., 1991. Isolation and characterisation of an extracellular alkaline protease of *Aspergillus fumigatus*. J. Med. Microbiol. 35, 23–28.

Monod, M., Paris, S., Sarfati, J., Jaton-Ogay, K., Ave, P., Latgé, J.P., 1993. Virulence of alkaline protease-deficient mutants of *Aspergillus fumigatus*. FEMS Microbiol. Lett. 106, 39–46.

Monod, M., Jousson, O., Reichard, U., 2009. *Aspergillus fumigatus* secreted proteases. In: Latgé, J.P., Steinbach, W. (Eds.), *Aspergillus fumigatus* and Aspergillosis. ASM Press, Washington, DC, pp. 87–106.

Naglik, J.R., Moyes, D., Makwana, J., Kanzaria, P., Tsichlaki, E., Weindl, G., Tappuni, A.R., Rodgers, C.A., Woodman, A.J., Challacombe, S.J., Schaller, M., Hube, B., 2008. Quantitative expression of the *Candida albicans* secreted aspartyl proteinase gene family in human oral and vaginal candidiasis. Microbiology 154, 3266–3280.

Nehlin, J.O., Carlberg, M., Ronne, H., 1991. Control of yeast GAL genes by MIG1 repressor: a transcriptional cascade in the glucose response. EMBO J. 10, 3373–3377.

Niimi, M., Firth, N.A., Cannon, R.D., 2010. Antifungal drug resistance of oral fungi. Odontology 98, 15–25.

Nishi, K., Park, C.S., Pepper, A.E., Eichinger, G., Innis, M.A., Holland, M.J., 1995. The GCR1 requirement for yeast glycolytic gene expression is suppressed by dominant mutations in the SGC1 gene, which encodes a novel basic-helix-loop-helix protein. Mol. Cell. Biol. 15, 2646–2653.

Olivas, I., Royuela, M., Romero, B., Monteiro, M.C., Minguez, J.M., Laborda, F., De Lucas, J.R., 2008. 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. 45, 45–60.

Park, H., Liu, Y., Solis, N., Spotkov, J., Hamaker, J., Blankenship, J.R., Yeaman, M.R., Mitchell, A.P., Liu, H., Filler, S.G., 2009. Transcriptional responses of *Candida albicans* to epithelial and endothelial cells. Eukaryot. Cell 8, 1498–1510.

Piekarska, K., Mol, E., van den Berg, M., Hardy, G., van den Burg, J., van Roermund, C., MacCallum, D., Odds, F., Distel, B., 2006. Peroxisomal fatty acid beta-oxidation is not essential for virulence of *Candida albicans*. Eukaryot. Cell 5, 1847–1856.

Piekarska, K., Hardy, G., Mol, E., van den Burg, J., Strijbis, K., van Roermund, C., van den Berg, M., Distel, B., 2008. The activity of the glyoxylate cycle in peroxisomes of *Candida albicans* depends on a functional beta-oxidation pathway: evidence for

reduced metabolite transport across the peroxisomal membrane. Microbiology 154, 3061–3072.
Rambach, G., Dum, D., Mohsenipour, I., Hagleitner, M., Würzner, R., Lass-Flörl, C., Speth, C., 2010. Secretion of a fungal protease represents a complement evasion mechanism in cerebral aspergillosis. Mol. Immunol. 47, 1438–1449.
Reichard, U., Büttner, S., Eiffert, H., Staib, F., Rüchel, R., 1990. Purification and characterisation of an extracellular serine proteinase from *Aspergillus fumigatus* and its detection in tissue. J. Med. Microbiol. 33, 243–251.
Reichard, U., Eiffert, H., Rüchel, R., 1994. Purification and characterization of an extracellular aspartic proteinase from *Aspergillus fumigatus*. J. Med. Vet. Mycol. 32, 427–436.
Reichard, U., Monod, M., Rüchel, R., 1995. Molecular cloning and sequencing of the gene encoding an extracellular aspartic proteinase from *Aspergillus fumigatus*. FEMS Microbiol. Lett. 130, 69–74.
Reichard, U., Monod, M., Odds, F., Rüchel, R., 1997. Virulence of an aspergillopepsin-deficient mutant of *Aspergillus fumigatus* and evidence for another aspartic proteinase linked to the fungal cell wall. J. Med. Vet. Mycol. 35, 189–196.
Reichard, U., Lechenne, B., Asif, A.R., Streit, F., Grouzmann, E., Jousson, O., Monod, M., 2006. Sedolisins, a new class of secreted proteases from *Aspergillus fumigatus* with endoprotease or tripeptidyl-peptidase activity at acidic pHs. Appl. Environ. Microbiol. 72, 1739–1748.
Roy, P., Lockington, R.A., Kelly, J.M., 2008. CreA-mediated repression in *Aspergillus nidulans* does not require transcriptional auto-regulation, regulated intracellular localisation or degradation of CreA. Fungal Genet. Biol. 45, 657–670.
Ruijter, G.J., Visser, J., 1997. Carbon repression in Aspergilli. FEMS Microbiol. Lett. 151, 103–114.
Sanglard, D., Hube, B., Monod, M., Odds, F.C., Gow, N.A., 1997. A triple deletion of the secreted aspartyl proteinase genes SAP4, SAP5, and SAP6 of Candida albicans causes attenuated virulence. Infect. Immun. 65, 3539–3546.
Schaller, M., Schackert, C., Korting, H.C., Januschke, E., Hube, B., 2000. Invasion of *Candida albicans* correlates with expression of secreted aspartic proteinases during experimental infection of human epidermis. J. Invest. Dermatol. 114, 712–717.
Schleimer, R.P., 2004. Glucocorticoids suppress inflammation but spare innate immune responses in airway epithelium. Proc. Am. Thorac. Soc. 1, 222–230.
Schöbel, F., Ibrahim-Granet, O., Ave, P., Latgé, J.P., Brakhage, A.A., Brock, M., 2007. *Aspergillus fumigatus* does not require fatty acid metabolism via isocitrate lyase for development of invasive aspergillosis. Infect. Immun. 75, 1237–1244.
Schöbel, F., Jacobsen, I.D., Brock, M., 2010. Evaluation of lysine biosynthesis as antifungal drug target: biochemical characterization of *Aspergillus fumigatus* homocitrate synthase and virulence studies. Eukaryot. Cell 9, 878–893.
Schüller, H.J., 2003. Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*. Curr. Genet. 43, 139–160.
Scott, E.W., Baker, H.V., 1993. Concerted action of the transcriptional activators REB1, RAP1, and GCR1 in the high-level expression of the glycolytic gene TPI. Mol. Cell. Biol. 13, 543–550.
Scott, J.A., Untereiner, W.A., 2004. Determination of keratin degradation by fungi using keratin azure. Med. Mycol. 42, 239–246.
Sharon, H., Hagag, S., Osherov, N., 2009. Transcription factor PrtT controls expression of multiple secreted proteases in the human pathogenic mold *Aspergillus fumigatus*. Infect. Immun. 77, 4051–4060.
Shoham, S., Marwaha, S., 2010. Invasive fungal infections in the ICU. J. Intensive Care Med. 25, 78–92.
Shroff, R.A., O'Connor, S.M., Hynes, M.J., Lockington, R.A., Kelly, J.M., 1997. Null alleles of *creA*, the regulator of carbon catabolite repression in *Aspergillus nidulans*. Fungal Genet. Biol. 22, 28–38.
Soll, D.R., 2002. *Candida* commensalism and virulence: the evolution of phenotypic plasticity. Acta Trop. 81, 101–110.
Stewart, K., Abad-Zapatero, C., 2001. *Candida* proteases and their inhibition: prospects for antifungal therapy. Curr. Med. Chem. 8, 941–948.
Sugui, J.A., Kim, H.S., Zarember, K.A., Chang, Y.C., Gallin, J.I., Nierman, W.C., Kwon-Chung, K.J., 2008. Genes differentially expressed in conidia and hyphae of *Aspergillus fumigatus* upon exposure to human neutrophils. PLoS One 3, e2655.
Thewes, S., Kretschmar, M., Park, H., Schaller, M., Filler, S.G., Hube, B., 2007. In vivo and ex vivo comparative transcriptional profiling of invasive and non-invasive *Candida albicans* isolates identifies genes associated with tissue invasion. Mol. Microbiol. 63, 1606–1628.
Thön, M., Al-Abdallah, Q., Hortschansky, P., Brakhage, A.A., 2007. The thioredoxin system of the filamentous fungus *Aspergillus nidulans*: impact on development and oxidative stress response. J. Biol. Chem. 282, 27259–27269.
Todd, R.B., Kelly, J.M., Davis, M.A., Hynes, M.J., 1997a. Molecular characterization of mutants of the acetate regulatory gene *facB* of *Aspergillus nidulans*. Fungal Genet. Biol. 22, 92–102.
Todd, R.B., Murphy, R.L., Martin, H.M., Sharp, J.A., Davis, M.A., Katz, M.E., Hynes, M.J., 1997b. The acetate regulatory gene *facB* of *Aspergillus nidulans* encodes a $Zn(II)_2Cys_6$ transcriptional activator. Mol. Gen. Genet. 254, 495–504.
Travis, S.M., Conway, B.A., Zabner, J., Smith, J.J., Anderson, N.N., Singh, P.K., Greenberg, E.P., Welsh, M.J., 1999. Activity of abundant antimicrobials of the human airway. Am. J. Respir. Cell Mol. Biol. 20, 872–879.
Turcotte, B., Liang, X.B., Robert, F., Soontorngun, N., 2010. Transcriptional regulation of nonfermentable carbon utilization in budding yeast. FEMS Yeast Res. 10, 2–13.
Uemura, H., Fraenkel, D.G., 1990. *gcr2*, a new mutation affecting glycolytic gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 10, 6389–6396.
Uemura, H., Jigami, Y., 1992. Role of *GCR2* in transcriptional activation of yeast glycolytic genes. Mol. Cell. Biol. 12, 3834–3842.
Uemura, H., Koshio, M., Inoue, Y., Lopez, M.C., Baker, H.V., 1997. The role of Gcr1p in the transcriptional activation of glycolytic genes in yeast *Saccharomyces cerevisiae*. Genetics 147, 521–532.
Weindl, G., Naglik, J.R., Kaesler, S., Biedermann, T., Hube, B., Korting, H.C., Schaller, M., 2007. Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. J. Clin. Invest. 117, 3664–3672.
White, T.C., Agabian, N., 1995. *Candida albicans* secreted aspartyl proteinases: isoenzyme pattern is determined by cell type, and levels are determined by environmental factors. J. Bacteriol. 177, 5215–5221.
Xie, X., Wilkinson, H.H., Correa, A., Lewis, Z.A., Bell-Pedersen, D., Ebbole, D.J., 2004. Transcriptional response to glucose starvation and functional analysis of a glucose transporter of *Neurospora crassa*. Fungal Genet. Biol. 41, 1104–1119.
Yordanov, M., Dimitrova, P., Patkar, S., Saso, L., Ivanovska, N., 2008. Inhibition of *Candida albicans* extracellular enzyme activity by selected natural substances and their application in *Candida* infection. Can. J. Microbiol. 54, 435–440.
Zaragoza, O., Rodriguez, C., Gancedo, C., 2000. Isolation of the MIG1 gene from *Candida albicans* and effects of its disruption on catabolite repression. J. Bacteriol. 182, 320–326.
Zaugg, C., Monod, M., Weber, J., Harshman, K., Pradervand, S., Thomas, J., Bueno, M., Giddey, K., Staib, P., 2009. Gene expression profiling in the human pathogenic dermatophyte *Trichophyton rubrum* during growth on proteins. Eukaryot. Cell 8, 241–250.