Curr Fungal Infect Rep
DOI 10.1007/s12281-012-0108-8

GENOMICS AND PATHOGENESIS (S SHOHAM, SECTION EDITOR)

Metal Acquisition and Homeostasis in Fungi

Elisa Flávia Luiz Cardoso Bailão · Ana Flávia Alves Parente · Juliana Alves Parente · Mirelle Garcia Silva-Bailão · Kelly Pacheco de Castro · Lívia Kmetzsch · Charley Christian Staats · Augusto Schrank · Marilene Henning Vainstein · Clayton Luiz Borges · Alexandre Melo Bailão · Célia Maria de Almeida Soares

© Springer Science+Business Media, LLC 2012

Abstract Transition metals, particularly iron, zinc and copper, have multiple biological roles and are essential elements in biological processes. Among other micronutrients, these metals are frequently available to cells in only limited amounts, thus organisms have evolved highly regulated mechanisms to cope and to compete with their scarcity. The homeostasis of such metals within the animal hosts requires the integration of multiple signals producing depleted environments that restrict the growth of microorganisms, acting as a barrier to infection. As the hosts sequester the necessary transition metals from invading pathogens, some, as is the case of fungi, have evolved elaborate mechanisms to allow their survival and development to establish infection. Metalloregulatory factors allow fungal cells to sense and to adapt to the scarce metal availability in the environment, such as in host tissues. Here we review recent advances in the identification and function of molecules that drive the acquisition and homeostasis of iron, copper and zinc in pathogenic fungi.

E. F. L. C. Bailão · A. F. A. Parente · J. A. Parente · M. G. Silva-Bailão · K. P. de Castro · C. L. Borges · A. M. Bailão · C. M. de Almeida Soares (✉)
Laboratório de Biologia Molecular, Instituto de Ciências Biológicas II, Campus Samambaia, Universidade Federal de Goiás, 74690-900, Goiânia, Goiás, Brazil
e-mail: cmasoares@gmail.com

E. F. L. C. Bailão
Unidade Universitária de Iporá, Universidade Estadual de Goiás, Iporá, Goiás, Brazil

L. Kmetzsch · C. C. Staats · A. Schrank · M. H. Vainstein
Laboratório de Biologia Molecular, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Keywords Iron · Copper · Zinc · Fungal pathogens

Introduction

Metals such as iron, copper and zinc have numerous biological roles and play a central role at the host–pathogen interface. Mammalian and microbial cells have an essential demand for these metals, which act as both structural and catalytic cofactors for proteins, and are therefore required for biological processes. During infection, the competing demands for these nutrients culminate in a struggle for metal acquisition/utilization at the microbe–host interface [1•, 2•]. In the complex interactions between pathogens and their mammalian hosts, metal homeostasis plays an essential role in both virulence and host defense [3, 4].

Iron and copper participate in several oxidation–reduction reactions because of their ability to lose and gain electrons. This same property permits iron and copper to generate reactive oxygen species (ROS) [5, 6]. Zinc is also an essential cofactor of many enzymes, but in excess, may be toxic to cells [7]. For metal balance, cells usually regulate uptake, storage and consumption. Our understanding of the mechanisms involved in metal excretion is incomplete. This review summarizes the current knowledge regarding the most studied metals that contribute to virulence of fungal pathogens: iron, copper and zinc. We focus on the fungal pathogens *Candida albicans*, *Histoplasma capsulatum*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Paracoccidioides*. Specifically we discuss the struggle for control of transition metals during infection, the molecular mechanisms involved in iron, copper and zinc uptake and the regulation of metal homeostasis in those pathogens. Additionally we review the preferential host iron sources

Published online: 22 September 2012

and fungal genes related to iron acquisition/homeostasis directly involved in infection.

Host Metal Homeostasis During Infectious Processes

Among metals involved in fungal infection, the functions of iron are well characterized. Hosts have evolved mechanisms to efficiently acquire iron and at the same time decrease its availability to pathogens [2•]. Physiological conditions that lead to metal overload contribute to increased infections. For example, administration of exogenous iron results in exacerbation of cryptococcosis [8] and increases in free iron also results in higher fungal load in mouse tissues infected with *Paracoccidioides* [9]. At the interface between iron and immunity, macrophages appear as a cellular factory that manage metal homeostasis [3]. Upon infection, the iron efflux from macrophages is suppressed resulting in 70 % reduction in plasma iron, thus restricting the amount of the metal available to extracellular pathogens. Infected macrophages, conversely, restrict the amount of iron available to intracellular microbes by pumping out iron via the ferroportin transporter route. Mutation-impaired ferroportin function compromises the ability of macrophages to clear pathogens [10].

Lactoferrin is produced by neutrophils and epithelial cells to chelate iron in extracellular compartments resulting in impairment of proliferation of fungal invaders [11]. Induction of ferritin production to facilitate withholding of intracellular iron diminishes the amount of the metal available to intracellular pathogens [2•, 12]. Iron also influences immune functions mediated by macrophages, and cytokines affect systemic iron homeostasis and cellular iron efflux [13]. Reduced iron levels have been found in macrophages activated by exposure to interferon gamma (IFN-γ) or granulocyte macrophage colony-stimulating factor (GM-CSF) [14]. Transferrin can be used by pathogens as an iron source in host tissues. To counteract this process IFN-γ decreases the expression of transferrin receptor in macrophages. Moreover, the production of the cellular iron storage molecule ferritin can be regulated by proinflammatory signals [15]. So, in the complex host–pathogen interaction, the control of iron homeostasis is a battlefield where the host must withdraw the micronutrient from microbes and at the same time uses iron to elaborate an efficient oxidative burst, since this metal is required for generation of ROS.

Since copper is essential, it is not unexpected that both humans and pathogens share the requirement for acquiring sufficient levels of copper [6]. In response to fungal infection, macrophages phagocytose the fungal cells and initiate cellular events that culminate in the oxidative burst [6]. Studies suggest that fungal pathogens must obtain copper to develop an efficient survival mechanism in host tissues, since genes related to copper acquisition/homeostasis are upregulated during infection [16, 17]. *C. neoformans* fights the host defenses to acquire copper, which promotes melanin synthesis, a virulence factor for this fungus [18]. The dependence of fungi upon copper for survival under the host conditions can be related to their response to ROS generation by the host since superoxide dismutase is a copper-dependent enzyme.

Zinc levels are modulated during infectious processes. During inflammation, the liver sequesters zinc, likely limiting zinc bioavailability to pathogenic microbes [19]. Neutrophils display an antimicrobial mechanism based on competition for zinc. This zinc-chelating system, found in neutrophil cytoplasm and abscess fluid, exerts fungistatic activity based on the calcium- and zinc-binding protein calprotectin [20]. Abscess fluid inhibits the growth of several fungi and the addition of zinc results in fungal growth in this fluid [7], reinforcing the view that zinc sequestration is a strategy used by the host to combat fungal infections. A metallomic study has demonstrated that GM-CSF-activated macrophages reduce intracellular zinc concentrations upon *H. capsulatum* infection in order to kill the pathogen [14].

Molecular Mechanisms of Iron, Copper and Zinc Uptake

Iron uptake mechanisms are highly regulated in fungi since excess iron is toxic and iron excretion systems have not yet been described in fungi [21]. Fungi have evolved different mechanisms for iron acquisition [21]. A low-affinity iron uptake system characterized only in *Saccharomyces cerevisiae* involves permeases that transport not only iron, but also other metals. In the reductive high-affinity ferrous uptake, ferrireductases reduce ferric iron (Fe^{3+}) to its soluble ferrous form (Fe^{2+}). Fe^{2+} is then reoxidized by plasma membrane ferroxidases and Fe^{3+} is promptly internalized by a high-affinity permease [5]. Another high-affinity mechanism for iron uptake is mediated by siderophores, small molecules with high affinity for Fe^{3+}, that allow specific recognition and uptake of iron at the cell surface [22]. Most fungi produce and secrete hydroxamate-type siderophores under low-iron growth conditions [23]. Some fungi, such as *C. neoformans*, do not produce siderophores, but can transport molecules produced by other organisms (xenosiderophores) [24].

The *C. albicans* genome contains genes that encode 18 putative ferrireductases and five ferroxidase homologues [1•, 25]. The ferroxidase Fet34 localizes to the plasma membrane and possibly associates with the permease Ftr1 early in the secretory pathway, promoting the high-affinity iron uptake [26•]. *C. albicans* produces a siderophore transporter [27] that displays broad substrate specificity, transporting various hydroxamate-type siderophores [28].

Under iron-limiting conditions, *H. capsulatum* produces three different reductants: a secreted glutathione dependent γ-glutamyltransferase (Ggt1) [29], non-enzymatic reductants with low molecular weight, and cell surface ferric reducing agents [30]. The *H. capsulatum* genome contains genes that encode seven putative ferrireductases [31]. Although a high-affinity acquisition mechanism has not been described for *H. capsulatum*, genomic analysis of the strain G186AR revealed genes coding iron permease (ftr1) and ferroxidase (Fet3) homologues [32]. *H. capsulatum* is also able to produce multiple hydroxamate siderophores under conditions of low iron availability [33]. In addition, *H. capsulatum* can utilize xenosiderophores [34].

In *A. fumigatus*, the ferrireductase FreB has been characterized. After reduction, iron is internalized by the ferroxidase-permease complex FetC–FtrA [35]. *A. fumigatus* synthesizes three types of siderophores, two of which are responsible for iron storage [36–38]. The iron-loaded siderophore is internalized by specific transporters [39] and the ester bonds of triacetylfusarinine C are then hydrolyzed by an esterase [40]. The cleavage products (fusarinines) are excreted, and the free iron can either be used in cell metabolism or bind to intracellular siderophore desferri-ferricrocin for storage [37, 38, 41•].

Uptake of iron is probably mediated by two large groups of transporters in *C. neoformans*: high- and low-affinity systems [42]. Cft1 is a high-affinity iron permease associated with the reductive system. On the other hand *cft2* possibly encodes for a low-affinity uptake system, since no clear iron-related phenotypes could be detected in *cft2* null mutants [43]. Cfo1 and Cfo2 ferroxidases have also been described in *C. neoformans* [44]. Cfo1 is required for high-affinity and reductive iron transport, since mutants lacking the coding gene show reduced growth under low iron conditions and cannot use ferric iron for growth. Moreover, under low iron conditions, Cfo1 expression is increased and localized mainly in the cell surface [44]. Studies have shown the inability of *Cryptococcus* species to produce siderophores. This is supported by genomic analysis, which has revealed the absence of genes involved in steps of siderophore biosynthesis [24, 45]. Despite the inability to synthesize siderophores, *Cryptococcus* species are presumably able to transport xenosiderophores [43].

Molecular mechanisms for reductive iron uptake in the genus *Paracoccidioides* are coming to light. In silico analysis has revealed that the genome of this fungus contains genes that encode redundant ferrireductase homologues [45]. Experiments have demonstrated a significant increase in the expression of genes coding the ferrireductases *fre3*, *fre7*, *frp1* and *ggt1* upon iron restriction (Fig. 1a). *Paracoccidioides* has glutathione-dependent ferrireductase activity [46], an aspect that is corroborated by the presence of a *ggt1* homologue in the fungus genome [45]. Since iron permease homologues were not detected in the *Paracoccidioides* genome, it has been proposed that a zinc permease could function additionally as an iron permease to acquire this metal [45]. The importance of iron acquisition by siderophores in the *Paracoccidioides* genus have been noted and studies on siderophore production and uptake are in

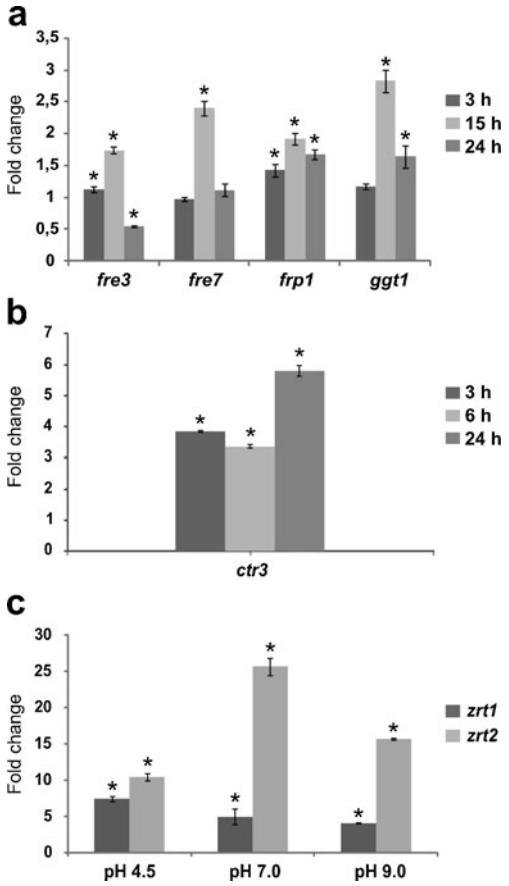

Fig. 1 Expression profile of *Paracoccidioides* (*Pb*01) genes during iron, copper and zinc starvation. *Pb*01 yeast cells were incubated in chemically defined medium containing different concentrations of iron, copper or zinc. Cells were harvested and total RNA was extracted using Trizol and mechanical cell rupture. After in vitro reverse transcription, the cDNAs were submitted to quantitative RT-PCR. The expression values were calculated using the transcripts alpha tubulin or l34 as endogenous controls [9, 82]. Data are presented as fold change relative to experimental controls. **a** Expression of ferrireductases encoding transcripts *fre3*, *fre7*, *frp1*, and *ggt1* evaluated in yeast cells in medium containing 3.5 μM iron (experimental control) or no iron for 3 h, 15 h and 24 h. **b** Expression of copper transporter encoding transcript *ctr3* evaluated in yeast cells in medium containing 50 μM copper (experimental control) or under conditions of copper starvation produced by adding the copper chelator bathocuproine disulphonate (BCS) for 3 h, 6 h and 24 h. **c** Expression level of zinc transporters encoding transcripts *zrt1* and *zrt2* evaluated in yeast cells in medium containing 30 μM zinc (experimental control) or under conditions of zinc depletion produced by adding the zinc chelator *N*,*N*,*N*,*N*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) at different pH values (4.5, 7.0 and 9.0) for 6 h. Data are presented as means ± SD from triplicate determinations. *$p \leq 0.05$, *t* test, in relation to the data obtained from the experimental controls

progress. The major evidence of iron capture by siderophores is supported by the stimulation of fungal growth in the presence of coprogen B and dimerum acid [47]. In silico analysis ha revealed the presence of genes putatively involved in hydroxamate-type siderophore biosynthesis and transport [45]. Corroborating these data, it has been demonstrated by chrome azurol S assays that *Paracoccidioides* is a hydroxamate producer (Silva et al., personal communication).

In fungi, copper and iron homeostasis must be intrinsically linked since iron uptake requires ferroxidases, which are members of the multicopper oxidase family. Copper is first reduced by plasma membrane ferrireductases and then Cu^{1+} is internalized via a high-affinity permease [48]. In *C. albicans*, the ferrireductase c*fl1*/*fre1* is transcriptionally regulated in response to both iron and copper availability [49], indicating that this ferrireductase is also important in copper uptake. Furthermore, the mutant for the *ctr1* copper transporter displays deficient growth in medium low in copper and iron indicating that in *C. albicans* iron and copper homeostasis are linked [50].

As observed for iron, copper plays fundamental roles in several aspects of *C. neoformans* biology. For instance, the production of melanin pigment is dependent on a copper oxidase [51] and the copper-containing ferroxidases are necessary for iron uptake [44]. Copper is probably reduced in *C. neoformans* by the same enzymes that reduce iron at the cell surface [52]. Two copper transporters have been described in *C. neoformans*. The *ctr1* null mutant shows reduced growth in copper-depleted medium. Ctr4, by contrast, is not essential for cryptococcal development in low-copper medium. However, mutant cells lacking both *ctr1* and *ctr4* transporters display severe growth defects in copper-deprived environments [53•].

The *Paracoccidioides* genome contains genes that encode redundant ferrireductases [45] as cited above, suggesting that these enzymes could function as iron and copper reductases. Furthermore a high-affinity copper transporter, *ctr3*, is present at increased levels during copper shortage (Fig. 1b), reinforcing the view that Ctr3 could be involved in copper uptake in *Paracoccidioides* [45].

The zinc uptake system in most fungi comprises just high-affinity and low-affinity permeases belonging to the ZIP family [54, 55], since this metal does not need to be reduced before internalization. Eight genes encoding proteins of the ZIP family of zinc transporters have been described in *A. fumigatus* [56]. Expression of *zrfA*, *zrfB* and *zrfC* is regulated by both zinc and pH [56, 57]. ZrfA and ZrfB function under acidic, zinc-limiting conditions. It seems that ZrfB is a high-affinity zinc permease, since a *zrfB* transcript was downregulated under high zinc conditions [58]. ZrfC participates in zinc uptake in a neutral or alkaline, zinc-poor environment [56]. Aspf2 putatively contributes to zinc uptake as a zinc-binding protein localized in the fungal periplasm [56].

Paracoccidioides possesses two zinc permease homologues (*zrt1* and *zrt2*), indicating a specific zinc uptake system [45]. The transcriptional response of *zrt* homologues to zinc starvation has been demonstrated by quantitative RT-PCR (Fig. 1c). The *zrt2* transcript, but not *zrt1* transcript, is highly expressed at neutral to alkaline pH during zinc depletion (Fig. 1c), as observed to *A. fumigatus* ZrfC.

Host Iron Sources

A high proportion of circulating iron in humans exists as heme in hemoglobin and hemin, iron-containing porphyrins. *C. albicans* shows hemolytic activity, and membrane proteins capable of binding hemin/hemoglobin have been identified [1•]. *C. albicans* Rbt5, a glycosylphosphatidylinositol-anchored protein, is the major hemoglobin receptor [59]. *hmx1* encodes an intracellular heme oxygenase that breaks down iron-protoporphyrin IX to α-biliverdin and is required for heme-iron utilization [60]. In silico analysis has revealed that *Paracoccidioides* genome contains genes that encode *hmx1* and *rbt5* homologues, suggesting effective hemoglobin iron acquisition by this fungus [45].

Intracellular iron in humans is bound to ferritin. *C. albicans* hyphae are able to obtain iron from ferritin using Als3 protein as a receptor. Als3 is a multifunctional protein since it can also function as an adhesin and an invasin [1•]. Transferrin is a glycoprotein that transports iron in serum. *C. albicans* is able to take up iron from transferrin by the reductive pathway using the ferrous permease Ftr1 and ferrireductase Fre10 [61]. In *H. capsulatum*, ferrireductase activity is higher in the presence of hemin and transferrin, suggesting that this fungus uses the ferrireductases to obtain iron during infection [34]. *Paracoccidioides* is likely to be able to take up iron from transferrin since the fungus has five genes encoding ferrireductases in the genome [45].

Regulation of Iron, Copper and Zinc Homeostasis in Pathogenic Fungi

Fungi have evolved sophisticated control mechanisms for maintenance of optimal levels of iron, copper and zinc. These mechanisms include the regulation of genes involved in metal ion uptake, utilization and storage. In fungi, metal ion homeostasis is mainly achieved by transcriptional regulation of gene expression. A group of iron-responsive GATA-type transcription factors mediates repression of iron acquisition genes in response to iron

sufficiency [62]. These regulators have a cysteine-rich central domain located between two zinc fingers, which directly interact with iron [63].

A range of genes and regulators involved in the response of *C. albicans* to iron starvation have been described [64]. During iron sufficiency, the GATA-type regulator Sfu1 downregulates expression of *arn1* and *hap43* genes encoding a siderophore transporter and a transcription factor, respectively [64, 65]. Under iron-limited conditions, the Cap2 protein represses the expression of Sfu1, activating genes of iron uptake pathways [66•]. Sef1 and Rim101 were also identified as positive regulators of iron acquisition in *C. albicans* [67, 68].

A GATA-type factor, Sre1, has been described in *H. capsulatum*. Sre1 acts as a negative regulator of siderophore biosynthesis genes in response to iron excess [69]. Sre1 also regulates cellular processes other than iron acquisition, such as optimal filamentous growth [70]. The same occurs with the Sre1 homologue SreB in *Blastomyces dermatitidis*. SreB regulates siderophore biosynthesis and also governs phase transition and cell growth at 22 °C in *B. dermatitidis* [71].

During iron sufficiency, high-affinity iron uptake systems (reductive pathway and siderophore production) are repressed by SreA in *A. fumigatus* [72]. During iron starvation, the *A. fumigatus* bZIP-type regulator HapX represses iron-dependent pathways, such as respiration, TCA cycle and heme biosynthesis, to save iron, and activates iron uptake by siderophores [73•]. Thus the transcription factors SreA and HapX act in opposite ways within the cell depending on the environmental iron status. During iron excess, SreA is activated and represses HapX expression, while during iron paucity, HapX represses the expression of SreA. In *A. fumigatus* the transcription factor AcuM stimulates iron acquisition via HapX induction and SreA repression [74].

C. neoformans Cir1 possesses a cysteine-rich domain, but unlike other fungal GATA-type iron regulators, it has only a zinc finger motif [75]. Cir1 is a global transcription factor which senses iron levels and regulates positively and negatively the transcriptional response [75, 76]. The expression of *C. neoformans* virulence attributes, such as capsule formation, growth at host temperature and melanin production, are also controlled by Cir1 [75]. A post-translational mechanism for the control of the amount of Cir1 suggests that under conditions of iron starvation Cir1 protein levels decrease. In contrast, iron availability promotes Cir1 stabilization and consequent repression of iron acquisition genes [77]. The transcriptional response to iron in *C. neoformans* is also regulated by HapX. As well as Cir1, HapX has both a positive and negative influence in the regulation of gene expression. However, unlike Cir1, HapX plays a modest role during infection and probably is important during environmental iron acquisition [78].

Proteomic analysis has revealed that during iron starvation the metabolic status of the pathogenic fungus *Paracoccidioides* is altered. Glycolysis is upregulated while iron-consuming pathways, such as tricarboxylic and glyoxylate cycles, are repressed. It has been demonstrated that under iron-limited conditions the transcript level of the HapX increases [9]. However, the regulatory mechanisms that orchestrate the global changes in response to iron availability in this fungus have not been described and are the subject of current investigation.

Regulatory mechanisms that respond to copper availability among pathogenic fungi have been best studied in *C. neoformans*. The copper-dependent transcription factor, Cuf1, has a cysteine-rich sequence, which contains a putative copper binding motif [79]. Under conditions of copper limitation, Cuf1 induces the expression of the copper transporter encoding genes *ctr1* and *ctr4*. During copper excess, the metallothionein (copper binding and detoxifying protein) genes *cmt1* and *cmt2* are induced by Cuf1 [53•]. A copper-dependent transcriptional regulator, Mac1, found in *C. albicans*, is transcriptionally autoregulated and activates the expression of *ctr1* and *fre7* genes during copper paucity [80].

Although regulation of copper homeostasis has not yet been described in *Paracoccidioides*, studies have revealed that the high-affinity copper transporter, Ctr3, is upregulated under infection conditions [16, 81] and is also a potential adhesin [82]. Analysis of genes potentially involved in copper regulation has demonstrated the presence of a copper metalloregulatory transcription factor, Mac1, in *Paracoccidioides* [45], thus prompting further investigation.

As for copper, the regulation of zinc homeostasis in pathogenic fungi is poorly understood. A zinc-responsive transcription factor has been described in *C. albicans* [83]. The Zap1/Csr1 factor induces expression of the plasma membrane zinc transporters, Zrt1 and Zrt2, and is also involved in the control of efficient hyphae and biofilm matrix formation and production of quorum sensing molecules [83–86].

In *A. fumigatus* the expression of *zrfA* and *zrfB* is induced by the ZafA zinc-responsive transcriptional activator under zinc-limited conditions [87]. However, under neutral zinc-limited conditions, the expression of these transporters is repressed by the transcriptional regulator PacC [57]. Additionally, the expression of *zrfC* is upregulated by ZafA under zinc-limited conditions regardless of the environmental pH and downregulated by PacC under acidic growth conditions [56].

Although zinc metabolism regulation is not well understood in *Paracoccidioides* and *Cryptococcus* pathogens, a homologue of Zap1 zinc-regulated transcription factor has been found in their genomes [45]. Studies focusing on this potential transcriptional regulator are in progress.

Virulence

Despite the close correlation between metal availability and virulence, the fungal genes related to iron acquisition/homeostasis directly involved in host infection are poorly described [88, 89]. Table 1 lists metal acquisition/homeostasis genes and provides information on their role in virulence.

In *C. albicans,* mutants lacking the iron permease coding gene *ftr1* lose virulence [90]. The involvement of genes related to siderophore uptake in virulence was not observed [27, 28]. Moreover virulence attenuation was observed in *C. albicans* mutants lacking the iron-responsive transcriptional regulators *hap43*, *aft2*, *sef1* and *cap2* and the heme oxygenase coding gene *hmx1* [65, 66•, 91, 92, 93•], indicating that all these genes are important during *C. albicans* infection.

Table 1 Roles of genes involved in metal homeostasis and virulence of pathogenic fungi

Gene	Protein function	Role in virulence	Reference
Candida albicans			
ftr1	High-affinity iron permease	The *ftr1Δ* mutatation results in complete loss of the capacity to damage epithelial cells in vitro. Moreover mutants lacking *ftr1* are avirulent in mice infected with *C. albicans* during the early stationary phase	[90]
hap43	Transcriptional regulator	Deletion of *hap43* attenuates the virulence of *C. albicans* in a mouse model of disseminated infection	[65]
cap2	Transcriptional regulator	The *cap2Δ* mutant shows delayed virulence in a mouse model of *C. albicans* infection	[66•]
aft2	Transcriptional regulator	The *aft2Δ*/*aft2Δ* strain shows attenuated virulence in mice with disseminated infection	[91]
hmx1	Heme oxygenase	The homozygous mutant *hmx1Δ*/*hmx1Δ* shows reduced virulence in mice with disseminated infection	[93•]
sef1	Transcriptional regulator	The *sef1Δ* mutant shows significantly decreased virulence compared to wild-type strain in BALB/c mice with disseminated infection	[92]
Aspergillus			
sidA	Involved in siderophore biosynthesis	The *sidAΔ* mutant shows completely attenuated virulence in mice	[37, 94]
sidD	Involved in siderophore biosynthesis	The *sidDΔ* mutant shows severely attenuated virulence in neutropenic mice	[95]
sidF	Involved in siderophore biosynthesis	The *sidFΔ* mutant shows attenuated virulence in neutropenic mice infected intranasally	[95]
hapX	Transcriptional regulator	The *hapXΔ* mutant shows attenuated virulence in immunosuppressed mice	[73•]
acuM	Transcriptional regulator	The *acuMΔ* mutant shows attenuated virulence in neutropenic mice with disseminated infection and invasive pulmonary aspergillosis, resulting in significantly delayed mortality	[74]
zafA	Transcriptional regulator	The *zafAΔ* mutant shows reduced virulence in immunosuppressed mice infected intranasally	[87]
pacC	Transcriptional regulator	The *Aspergillus nidulans pacCΔ* mutant shows attenuated virulence in immunosuppressed mice	[99]
Histoplasma capsulatum			
sid1	Involved in siderophore biosynthesis	The *sid1Δ* strain shows a significant defect in pulmonary colonization compared to wild-type cells in mice infected intranasally	[31]
Cryptococcus neoformans			
cft1	High-affinity iron permease	The *cft1Δ* mutant shows attenuated virulence and reduced fungal burden	[43]
cfo1	Ferroxidase	The *cfo1Δ* mutant shows significantly attenuated virulence in mice	[44]
cir1	Transcriptional regulator	The *cir1Δ* mutant is avirulent in mice	[75]
ctr4	Copper transporter	The *ctr4Δ* null mutant shows reduced spread to tissues and is completely avirulent in infected mice	[79]
ctr1	Copper transporter	*ctr1Δ* mutant presented reduced melanization, reduced capsule and enhanced phagocytosis index	[97]
clc	Chloride channel	The *clc-A* mutant shows attenuated virulence in a mouse cryptococcosis model, since *clc-A* plays a role in capsule and laccase expression, important virulence factors	[98]
ccc2	Copper transporter	*ccc2* mutation results in absence of melanization, an important virulence factor	[18]
cuf1	Transcriptional regulator	The deletion of *cuf1* results in attenuated virulence in a mouse model of cryptococcosis	[79]

In *Aspergillus*, mutants lacking genes involved in siderophore biosynthesis (*sid*) have pointed to the relevance of this iron uptake pathway to virulence. *sidA* deletion in *A. fumigatus* abolishes siderophore biosynthesis and completely attenuates virulence [37, 94]. A similar effect was observed in mutants lacking the *sidA* homologue, *sid1*, in *H. capsulatum* [31]. Genes related to fusarinine C and triacetylfusarinine C production, *sidD* and *sidF,* significantly affect *A. fumigatus* virulence [95]. Furthermore, the deletion of the transcriptional regulators *hapX* and *acuM* causes significant attenuation of virulence in a murine model of infection [73•, 74].

The role of iron acquisition in *Cryptococcus* virulence has been extensively studied in recent years. Almost all *C. neoformans* genes involved in iron homeostasis that have been analyzed are related to cryptococcal virulence, as evaluated in murine models of cryptococcosis using null gene mutants [44, 75–78]. When considering the iron permeases Cft1 and Cft2, virulence attenuation and reduced fungal burden are observed in the *cft1* gene null mutant and in the *cft1*/*cft2* double mutant, but not in the *cft2* knockout strain [76]. The ferroxidase Cfo1 also plays a role in virulence, since null mutants are also attenuated in virulence [44]. In addition, *C. neoformans cir1* null mutants are completely avirulent in murine models of cryptococcosis, which is consistent with the hypocapsular phenotype and its reduced ability to proliferate at 37 °C [75].

Regarding copper, a pivotal biological role of this metal has been already described for *C. neoformans*, since two proteins involved in virulence, Cu/Zn-Sod1 and laccase, require copper as a cofactor for activity [51, 96]. The *ctr4* gene is expressed during infection and is directly associated with virulence, since null mutants show reduced spread to tissues and are completely avirulent in infected mice [79]. The *ctr1* gene also is associated with virulence, as mutants show reduced melanization, a reduced capsule, and an enhanced phagocytosis index [97]. Moreover, *C. neoformans* strains with mutation in genes encoding copper distribution transporters, such as the *clc* chloride channel and the *ccc2* secretory transporter, show reduced virulence or reduced expression of virulence factors [18, 98]. Furthermore, in a mouse model of cryptococcosis, transcriptional regulator *cuf1* null mutants display disruption of several virulence-linked characteristics, such as reduced laccase activity, severe growth defects in low-copper medium, and reduced virulence [79]. Cuf1 is required for infection of the brain but not of the lung in mouse models of cryptococcosis, suggesting that copper is limiting in neurological infections [79].

Studies investigating the role of zinc during pathogenesis are sparse. Investigations are restricted to the importance of zinc-responsive transcription factors during pathogenesis, such as *zaf*A. In *A. fumigatus,* ZafA regulates zinc homeostasis, and mutants lacking this gene show reduced virulence in mice [87]. A similar result was found for the pH-responsive transcriptional factor *pac*C, that plays an essential role in pulmonary infection by *A. fumigatus* [56, 99].

Conclusions

Iron, copper and zinc acquisition is a critical determinant in fungal pathogenesis. To circumvent metal sequestration by the host during infection, pathogenic fungi have evolved mechanisms of metal acquisition. Understanding of the roles of iron, copper and zinc in fungal pathogenicity has advanced in recent years. As discussed above, fungi demonstrate remarkable flexibility in gaining access to and utilizing the transition metals iron, copper and zinc. The sophisticated acquisition and regulation of homeostasis of these metals are surely an efficient weapon facilitating fungal survival within the human host, and represent an important component of virulence.

Acknowledgments Work at Universidade Federal de Goiás and Universidade Federal do Rio Grande do Sul was supported by grants from Financiadora de Estudos e Projetos (FINEP- 01.07.0552.00) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-558923/2009-7 and 478591/2010-1). E.F.L.C.B. and M.G.S.B. are supported by doctoral fellowships from Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). A.F.A.P. and L.K. are supported by postdoctoral fellowships from CAPES. We apologize to colleagues whose work we were not able to cite due to space limitations.

Disclosure E.F. Bailão: grants from Capes, CNPq and FINEP; A.F.A. Parente: grants from CNPq, CAPES and FINEP; J.A. Parente: none; M. Garcia Silva-Bailão: grant from CAPES; K. Castro: grants from FINEP and CNPq; L. Kmetzsch: grants from CAPES, CNPq and FINEP; C. Staats: grants from CNPq and FINEP; A. Schrank: grants from CNPq and FINEP; M. Vainstein: grants from CNPq and FINEP; C. Borges: grants from CNPq and FINEP; A. Bailão: grant from CNPq; C.M. Soares: grants from FINEP and CNPq.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance

1. • Almeida RS, Wilson D, Hube B. Candida albicans iron acquisition within the host. FEMS Yeast Res. 2009;9:1000–12. *This article reviews the iron sources used by* C. albicans *during infection in the human host, that are xenosiderophores, hemoglobin, transferrin and ferritin*.
2. • Nairz M, Schroll A, Sonnweber T, Weiss G. The struggle for iron – a metal at the host-pathogen interface. Cell Microbiol. 2010;12:1691–702. *This article reviews the iron functions at the host–pathogen interface since mammalian and microbial cells have an essential demand for the metal. Microbial iron acquisition pathways are attractive targets for the development of new antimicrobial drugs*.

3. Theurl I, Fritsche G, Ludwiczek S, et al. The macrophage: a cellular factory at the interphase between iron and immunity for the control of infections. Biometals. 2005;18:359–67.
4. Ibrahim AS, Gebermariam T, Fu Y, et al. The iron chelator deferasirox protects mice from mucormycosis through iron starvation. J Clin Invest. 2007;117:2649–57.
5. Kaplan CD, Kaplan J. Iron acquisition and transcriptional regulation. Chem Rev. 2009;109:4536–52.
6. Kim BE, Nevitt T, Thiele DJ. Mechanisms for copper acquisition, distribution and regulation. Nat Chem Biol. 2008;4:176–85.
7. Lulloff SJ, Hahn BL, Sohnle PG. Fungal susceptibility to zinc deprivation. J Lab Clin Med. 2004;144:208–14.
8. Barluzzi R, Saleppico S, Nocentini A, et al. Iron overload exacerbates experimental meningoencephalitis by Cryptococcus neoformans. J Neuroimmunol. 2002;132:140–6.
9. Parente AF, Bailão AM, Borges CL, et al. Proteomic analysis reveals that iron availability alters the metabolic status of the pathogenic fungus Paracoccidioides brasiliensis. PLoS One. 2011;6:e22810.
10. Nevitt T. War-Fe-re: iron at the core of fungal virulence and host immunity. Biometals. 2011;24:547–58.
11. Al-Sheikh H. Effect of lactoferrin and iron on the growth of human pathogenic Candida species. Pak J Biol Sci. 2009;12:91–4.
12. Weinberg ED. Iron loading and disease surveillance. Emerg Infect Dis. 1999;5:346–52.
13. Weiss G. Modification of iron regulation by the inflammatory response. Best Pract Res Clin Haematol. 2005;18:183–201.
14. Winters MS, Chan Q, Caruso JA, Deepe Jr GS. Metallomic analysis of macrophages infected with Histoplasma capsulatum reveals a fundamental role for zinc in host defenses. J Infect Dis. 2010;202:1136–45.
15. Byrd TF, Horwitz MA. Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. Coordinate upregulation by iron transferrin and downregulation by interferon gamma. J Clin Invest. 1993;91:969–76.
16. Bailão AM, Schrank A, Borges CL, et al. Differential gene expression by Paracoccidioides brasiliensis in host interaction conditions: representational difference analysis identifies candidate genes associated with fungal pathogenesis. Microbes Infect. 2006;8:2686–97.
17. Samanovic MI, Ding C, Thiele DJ, Darwin KH. Copper in microbial pathogenesis: meddling with the metal. Cell Host Microbe. 2012;11:106–15.
18. Walton FJ, Idnurm A, Heitman J. Novel gene functions required for melanization of the human pathogen Cryptococcus neoformans. Mol Microbiol. 2005;57:1381–96.
19. Beisel WR. Herman Award Lecture, 1995: infection-induced malnutrition – from cholera to cytokines. Am J Clin Nutr. 1995;62:813–9.
20. Sohnle PG, Collins-Lech C, Wiessner JH. The zinc-reversible antimicrobial activity of neutrophil lysates and abscess fluid supernatants. J Infect Dis. 1991;164:137–42.
21. Haas H, Eisendle M, Turgeon BG. Siderophores in fungal physiology and virulence. Annu Rev Phytopathol. 2008;46:149–87.
22. Harrington JM, Crumbliss AL. The redox hypothesis in siderophore-mediated iron uptake. Biometals. 2009;22:679–89.
23. Renshaw JC, Robson GD, Trinci APJ, et al. Fungal siderophores: structures, functions and applications. Mycol Res. 2002;106:1123–42.
24. Jacobson ES, Petro MJ. Extracellular iron chelation in Cryptococcus neoformans. J Med Vet Mycol. 1987;25:415–8.
25. Jeeves RE, Mason RP, Woodacre A, Cashmore AM. Ferric reductase genes involved in high-affinity iron uptake are differentially regulated in yeast and hyphae of Candida albicans. Yeast. 2011;28:629–44.
26. • Ziegler L, Terzulli A, Gaur R, et al. Functional characterization of the ferroxidase, permease high-affinity iron transport complex from Candida albicans. Mol Microbiol. 2011;81:473–85. *This article demonstrates that Fe trafficking in* C. albicans *involves a complex Fet34-Ftr1 using* S. cerevisiae *as host for the functional expression of the* C. albicans *Fe-uptake proteins*.
27. Heymann P, Gerads M, Schaller M, et al. The siderophore iron transporter of Candida albicans (Sit1p/Arn1p) mediates uptake of ferrichrome-type siderophores and is required for epithelial invasion. Infect Immun. 2002;70:5246–55.
28. Hu CJ, Bai C, Zheng XD, et al. Characterization and functional analysis of the siderophore-iron transporter CaArn1p in Candida albicans. J Biol Chem. 2002;277:30598–605.
29. Zarnowski R, Cooper KG, Brunold LS, et al. Histoplasma capsulatum secreted gamma-glutamyltransferase reduces iron by generating an efficient ferric reductant. Mol Microbiol. 2008;70:352–68.
30. Timmerman MM, Woods JP. Ferric reduction is a potential iron acquisition mechanism for Histoplasma capsulatum. Infect Immun. 1999;67:6403–8.
31. Hwang LH, Mayfield JA, Rine J, Sil A. Histoplasma requires SID1, a member of an iron-regulated siderophore gene cluster, for host colonization. PLoS Pathog. 2008;4:e1000044.
32. Hilty J, George Smulian A, Newman SL. Histoplasma capsulatum utilizes siderophores for intracellular iron acquisition in macrophages. Med Mycol. 2011;49:633–42.
33. Howard DH, Rafie R, Tiwari A, Faull KF. Hydroxamate siderophores of Histoplasma capsulatum. Infect Immun. 2000;68:2338–43.
34. Timmerman MM, Woods JP. Potential role for extracellular glutathione-dependent ferric reductase in utilization of environmental and host ferric compounds by Histoplasma capsulatum. Infect Immun. 2001;69:7671–8.
35. Blatzer M, Binder U, Haas H. The metalloreductase FreB is involved in adaptation of Aspergillus fumigatus to iron starvation. Fungal Genet Biol. 2011;48:1027–33.
36. Charlang G, Ng B, Horowitz NH, Horowitz RM. Cellular and extracellular siderophores of Aspergillus nidulans and Penicillium chrysogenum. Mol Cell Biol. 1981;1:94–100.
37. Schrettl M, Bignell E, Kragl C, et al. Siderophore biosynthesis but not reductive iron assimilation is essential for Aspergillus fumigatus virulence. J Exp Med. 2004;200:1213–9.
38. Eisendle M, Schrettl M, Kragl C, et al. The intracellular siderophore ferricrocin is involved in iron storage, oxidative-stress resistance, germination, and sexual development in Aspergillus nidulans. Eukaryot Cell. 2006;5:1596–603.
39. Haas H. Molecular genetics of fungal siderophore biosynthesis and uptake: the role of siderophores in iron uptake and storage. Appl Microbiol Biotechnol. 2003;62:316–30.
40. Kragl C, Schrettl M, Abt B, et al. EstB-mediated hydrolysis of the siderophore triacetylfusarinine C optimizes iron uptake of Aspergillus fumigatus. Eukaryot Cell. 2007;6:1278–85.
41. • Haas H. Iron – a key nexus in the virulence of Aspergillus fumigatus. Front Microbiol. 2012;3:28. *This article reviews iron homeostasis and its participation in virulence in* Aspergillus *genus. The knowledge of the iron handling between host and fungus might improve therapy and diagnosis of fungal infections*.
42. Jacobson ES, Goodner AP, Nyhus KJ. Ferrous iron uptake in Cryptococcus neoformans. Infect Immun. 1998;66:4169–75.
43. Jung WH, Kronstad JW. Iron and fungal pathogenesis: a case study with Cryptococcus neoformans. Cell Microbiol. 2008;10:277–84.
44. Jung WH, Hu G, Kuo W, Kronstad JW. Role of ferroxidases in iron uptake and virulence of Cryptococcus neoformans. Eukaryot Cell. 2009;8:1511–20.
45. Silva MG, Schrank A, Bailão EF, et al. The homeostasis of iron, copper, and zinc in Paracoccidioides brasiliensis, Cryptococcus neoformans var. grubii, and Cryptococcus gattii: a comparative analysis. Front Microbiol. 2011;2:49.
46. Zarnowski R, Woods JP. Glutathione-dependent extracellular ferric reductase activities in dimorphic zoopathogenic fungi. Microbiology. 2005;151:2233–40.

47. Castaneda E, Brummer E, Perlman AM, et al. A culture medium for Paracoccidioides brasiliensis with high plating efficiency, and the effect of siderophores. J Med Vet Mycol. 1988;26:351–8.
48. Knight SA, Labbe S, Kwon LF, et al. A widespread transposable element masks expression of a yeast copper transport gene. Genes Dev. 1996;10:1917–29.
49. Hammacott JE, Williams PH, Cashmore AM. Candida albicans CFL1 encodes a functional ferric reductase activity that can rescue a Saccharomyces cerevisiae fre1 mutant. Microbiology. 2000;146 (Pt 4):869–76.
50. Marvin ME, Williams PH, Cashmore AM. The Candida albicans CTR1 gene encodes a functional copper transporter. Microbiology. 2003;149:1461–74.
51. Williamson PR. Biochemical and molecular characterization of the diphenol oxidase of Cryptococcus neoformans: identification as a laccase. J Bacteriol. 1994;176:656–64.
52. Nyhus KJ, Jacobson ES. Genetic and physiologic characterization of ferric/cupric reductase constitutive mutants of Cryptococcus neoformans. Infect Immun. 1999;67:2357–65.
53. • Ding C, Yin J, Tovar EM, et al. The copper regulon of the human fungal pathogen Cryptococcus neoformans H99. Mol Microbiol. 2011;81:1560–76. *This article describes a new* C. neoformans *Cu transporter, Ctr1, and some targets of the metalloregulatory transcription factor Cuf1*.
54. Zhao H, Eide D. The ZRT2 gene encodes the low affinity zinc transporter in Saccharomyces cerevisiae. J Biol Chem. 1996;271:23203–10.
55. Zhao H, Eide D. The yeast ZRT1 gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. Proc Natl Acad Sci U S A. 1996;93:2454–8.
56. Amich J, Vicentefranqueira R, Leal F, Calera JA. Aspergillus fumigatus survival in alkaline and extreme zinc-limiting environments relies on the induction of a zinc homeostasis system encoded by the zrfC and aspf2 genes. Eukaryot Cell. 2009;9:424–37.
57. Amich J, Leal F, Calera JA. Repression of the acid ZrfA/ZrfB zinc-uptake system of Aspergillus fumigatus mediated by PacC under neutral, zinc-limiting conditions. Int Microbiol. 2009;12:39–47.
58. Vicentefranqueira R, Moreno MA, Leal F, Calera JA. The zrfA and zrfB genes of Aspergillus fumigatus encode the zinc transporter proteins of a zinc uptake system induced in an acid, zinc-depleted environment. Eukaryot Cell. 2005;4:837–48.
59. Weissman Z, Kornitzer D. A family of Candida cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization. Mol Microbiol. 2004;53:1209–20.
60. Santos R, Buisson N, Knight S, et al. Haemin uptake and use as an iron source by Candida albicans: role of CaHMX1-encoded haem oxygenase. Microbiology. 2003;149:579–88.
61. Knight SA, Vilaire G, Lesuisse E, Dancis A. Iron acquisition from transferrin by Candida albicans depends on the reductive pathway. Infect Immun. 2005;73:5482–92.
62. Rutherford JC, Bird AJ. Metal-responsive transcription factors that regulate iron, zinc, and copper homeostasis in eukaryotic cells. Eukaryot Cell. 2004;3:1–13.
63. Scazzocchio C. The fungal GATA factors. Curr Opin Microbiol. 2000;3:126–31.
64. Lan CY, Rodarte G, Murillo LA, et al. Regulatory networks affected by iron availability in Candida albicans. Mol Microbiol. 2004;53:1451–69.
65. Hsu PC, Yang CY, Lan CY. Candida albicans Hap43 is a repressor induced under low-iron conditions and is essential for iron-responsive transcriptional regulation and virulence. Eukaryot Cell. 2011;10:207–25.
66. • Singh RP, Prasad HK, Sinha I, et al. Cap2-HAP complex is a critical transcriptional regulator that has dual but contrasting roles in regulation of iron homeostasis in Candida albicans. J Biol Chem. 2011;286:25154–70. *This article describes the roles performed by Cap2 under iron limiting conditions: activation of genes in iron uptake pathways and repression of iron-utilizing and iron-storage genes*.
67. Homann OR, Dea J, Noble SM, Johnson AD. A phenotypic profile of the Candida albicans regulatory network. PLoS Genet. 2009;5: e1000783.
68. Baek YU, Li M, Davis DA. Candida albicans ferric reductases are differentially regulated in response to distinct forms of iron limitation by the Rim101 and CBF transcription factors. Eukaryot Cell. 2008;7:1168–79.
69. Chao LY, Marletta MA, Rine J. Sre1, an iron-modulated GATA DNA-binding protein of iron-uptake genes in the fungal pathogen Histoplasma capsulatum. Biochemistry. 2008;47:7274–83.
70. Hwang LH, Seth E, Gilmore SA, Sil A. SRE1 regulates iron-dependent and -independent pathways in the fungal pathogen Histoplasma capsulatum. Eukaryot Cell. 2012;11:16–25.
71. Gauthier GM, Sullivan TD, Gallardo SS, et al. SREB, a GATA transcription factor that directs disparate fates in Blastomyces dermatitidis including morphogenesis and siderophore biosynthesis. PLoS Pathog. 2010;6:e1000846.
72. Schrettl M, Kim HS, Eisendle M, et al. SreA-mediated iron regulation in Aspergillus fumigatus. Mol Microbiol. 2008;70:27–43.
73. • Schrettl M, Beckmann N, Varga J, et al. HapX-mediated adaption to iron starvation is crucial for virulence of Aspergillus fumigatus. PLoS Pathog. 2010;6:e1001124. *This article describes the functions of transcriptional regulator HapX of* A. fumigatus*, which is important to fungus adaptation in iron starvation conditions and is crucial for virulence in a murine model of infection*.
74. Liu H, Gravelat FN, Chiang LY, et al. Aspergillus fumigatus AcuM regulates both iron acquisition and gluconeogenesis. Mol Microbiol. 2010;78:1038–54.
75. Jung WH, Sham A, White R, Kronstad JW. Iron regulation of the major virulence factors in the AIDS-associated pathogen Cryptococcus neoformans. PLoS Biol. 2006;4:e410.
76. Jung WH, Sham A, Lian T, et al. Iron source preference and regulation of iron uptake in Cryptococcus neoformans. PLoS Pathog. 2008;4:e45.
77. Jung WH, Kronstad JW. Iron influences the abundance of the iron regulatory protein Cir1 in the fungal pathogen Cryptococcus neoformans. FEBS Lett. 2011;585:3342–7.
78. Jung WH, Saikia S, Hu G, et al. HapX positively and negatively regulates the transcriptional response to iron deprivation in Cryptococcus neoformans. PLoS Pathog. 2010;6:e1001209.
79. Waterman SR, Hacham M, Hu G, et al. Role of a CUF1/CTR4 copper regulatory axis in the virulence of Cryptococcus neoformans. J Clin Invest. 2007;117:794–802.
80. Woodacre A, Mason RP, Jeeves RE, Cashmore AM. Copper-dependent transcriptional regulation by Candida albicans Mac1p. Microbiology. 2008;154:1502–12.
81. Dantas SF, Vieira de Rezende TC, Bailão AM, et al. Identification and characterization of antigenic proteins potentially expressed during the infectious process of Paracoccidioides brasiliensis. Microbes Infect. 2009;11:895–903.
82. Bailão AM, Nogueira SV. Rondon Caixeta Bonfim SM, et al. Comparative transcriptome analysis of Paracoccidioides brasiliensis during in vitro adhesion to type I collagen and fibronectin: identification of potential adhesins. Res Microbiol. 2012;163:182–91.
83. Kim MJ, Kil M, Jung JH, Kim J. Roles of zinc-responsive transcription factor Csr1 in filamentous growth of the pathogenic yeast Candida albicans. J Microbiol Biotechnol. 2008;18:242–7.
84. Nobile CJ, Nett JE, Hernday AD, et al. Biofilm matrix regulation by Candida albicans Zap1. PLoS Biol. 2009;7:e1000133.
85. Finkel JS, Xu W, Huang D, et al. Portrait of Candida albicans adherence regulators. PLoS Pathog. 2012;8:e1002525.

86. Ganguly S, Bishop AC, Xu W, et al. Zap1 control of cell-cell signaling in Candida albicans biofilms. Eukaryot Cell. 2011;10:1448–54.
87. Moreno MA, Ibrahim-Granet O, Vicentefranqueira R, et al. The regulation of zinc homeostasis by the ZafA transcriptional activator is essential for Aspergillus fumigatus virulence. Mol Microbiol. 2007;64:1182–97.
88. Kehl-Fie TE, Skaar EP. Nutritional immunity beyond iron: a role for manganese and zinc. Curr Opin Chem Biol. 2010;14:218–24.
89. Weinberg ED. Iron availability and infection. Biochim Biophys Acta. 2009;1790:600–5.
90. Ramanan N, Wang Y. A high-affinity iron permease essential for Candida albicans virulence. Science. 2000;288:1062–4.
91. Liang Y, Wei D, Wang H, et al. Role of Candida albicans Aft2p transcription factor in ferric reductase activity, morphogenesis and virulence. Microbiology. 2010;156:2912–9.
92. Chen C, Pande K, French SD, et al. An iron homeostasis regulatory circuit with reciprocal roles in Candida albicans commensalism and pathogenesis. Cell Host Microbe. 2011;10:118–35.
93. • Navarathna DH, Roberts DD. Candida albicans heme oxygenase and its product CO contribute to pathogenesis of candidemia and alter systemic chemokine and cytokine expression. Free Radic Biol Med. 2010;49:1561–73. *This article describes the heme oxygenase Hmx1 as a virulence factor in* C. albicans*. The authors observed that mutants lacking hmx1 gene were not affected during initial kidney colonization, but Hmx1 absence clearly affects infection progression*.
94. Hissen AH, Wan AN, Warwas ML, et al. The Aspergillus fumigatus siderophore biosynthetic gene sidA, encoding L-ornithine N5-oxygenase, is required for virulence. Infect Immun. 2005;73:5493–503.
95. Schrettl M, Bignell E, Kragl C, et al. Distinct roles for intra- and extracellular siderophores during Aspergillus fumigatus infection. PLoS Pathog. 2007;3:1195–207.
96. Cox GM, Harrison TS, McDade HC, et al. Superoxide dismutase influences the virulence of Cryptococcus neoformans by affecting growth within macrophages. Infect Immun. 2003;71:173–80.
97. Chun CD, Madhani HD. Ctr2 links copper homeostasis to polysaccharide capsule formation and phagocytosis inhibition in the human fungal pathogen Cryptococcus neoformans. PloS One. 2010;5.
98. Zhu X, Williamson PR. A CLC-type chloride channel gene is required for laccase activity and virulence in Cryptococcus neoformans. Mol Microbiol. 2003;50:1271–81.
99. Bignell E, Negrete-Urtasun S, Calcagno AM, et al. The Aspergillus pH-responsive transcription factor PacC regulates virulence. Mol Microbiol. 2005;55:1072–84.