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# War-Fe-re: iron at the core of fungal virulence and host immunity

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**Abstract** Iron acquisition is a bona fide virulence determinant. The successful colonization of the mammalian host requires that microorganisms overcome the Fe aridity of this milieu in which the levels of circulating Fe are maintained exceedingly low both through the compartmentalization of this nutrient within cells as well as the tight binding of Fe to host circulating proteins and ligands. Microbes notoriously employ multiple strategies for high affinity Fe acquisition from the host that rely either on the expression of receptors for host Fe-binding proteins and ligands, its reduction by cell surface reductases or the utilization of siderophores, small organic molecules with very high affinity for $Fe^{3+}$. This review will discuss the multiple mechanisms deployed by fungal pathogens in Fe acquisition focusing on the role of siderophore utilization in virulence as well as host immune strategies of iron withholding and emerging clinical evidence that human disorders of Fe homeostasis can act as modifiers of infectious disease.

**Keywords** Fungal pathogenesis · Iron · Siderophores · Hereditary hemochromatosis

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## Introduction

Iron (Fe) is an essential micronutrient for virtually all organisms. The ability of Fe to readily lose and gain electrons is critical to the activity and is a structural determinant of a plethora of enzymes and proteins that perform fundamental functions in cellular biology, from respiration and DNA replication to oxygen transport and redox-mediated microbial killing. However, the same properties that make Fe such a versatile co-factor in cellular biochemistry can also drive cellular damage. This is because Fe can mediate Fenton/Haber–Weiss type reactions leading to the generation of highly reactive oxygen species (ROS) that cause lipid peroxidation, protein carbonylation and DNA damage (Halliwell and Gutteridge 1985). It is therefore not surprising that organisms have evolved highly regulated mechanisms of Fe acquisition, utilization and storage (Philpott and Protchenko 2008; Hentze et al. 2010).

Fe holds a remarkable stance in the context of infectious disease, particularly in mammals. Whereas, on the one hand, microbial Fe acquisition is a major determinant for infection and persistence within the host (Schaible and Kaufmann 2004), on the other hand, the host actively manipulates local and systemic Fe levels as a *microbiostatic* mechanism to contain microbial proliferation. Furthermore, the successful compartmentalization of Fe away from microorganisms, either within cells or tightly bound to host proteins and ligands, allows the host to not only satisfy its

own requirements for Fe but to also articulate a *microbicidal* response that exploits the ROS-generating activities of Fe and copper (Cu) for microbial killing (White 2009). Macrophage activation correlates with increased Cu uptake and mobilization into the phagosomal compartment where it is required for efficient bacterial killing. The existence of the human X-linked immunodeficiency condition, Chronic granulomatous disease that arises due to mutations in the NADPH oxidase responsible for generating ROS during the oxidative burst in macrophages and neutrophils underscores the importance of this microbicidal mechanism (Holland 2010). As such, the ability of mammals to compartmentalize and manipulate Fe forms a critical component of innate immunity that contributes significantly towards the attenuation of microbial growth and virulence (Ratledge and Dover 2000). The following sections will briefly review the mechanism of mammalian Fe-withholding, the strategies employed by fungi to overcome this host-imposed Fe aridity and emerging clinical evidence for increased fungal burden in human conditions of Fe overload.

## Iron sequestration as a microbiostatic strategy of innate immunity

Given the absence of a dedicated mechanism of Fe excretion, mammalian Fe acquisition and storage are tightly regulated (Hentze et al. 2010). An overview of mammalian Fe homeostasis is presented in Fig. 1. During the acute phase response that follows pathogen recognition and IL-6 cytokine release, storage of Fe within ferritin nanocages is enhanced and circulating transferrin-bound Fe levels decrease in part through elevated transferrin receptor (TfR1) expression on the surface of cells. A member of the transferrin protein family, lactoferrin (Lf) binds Fe with much higher affinity than Tf especially at lower pH (Mazurier and Spik 1980). Lf is present in the plasma and neutrophil granules that are released during inflammation and the increased expression of its cognate receptor (LfR) further decreases serum Fe levels. Iron withholding in response to pathogen recognition therefore requires that signals originating from the innate immune system become integrated into the mechanism of systemic Fe homeostasis. During an inflammatory response, there is a decrease in dietary Fe absorption that correlates with an increased expression of the levels of mRNA encoding for hepcidin, a peptide hormone and central regulator of systemic Fe homeostasis. Hepcidin has long been known to possess antimicrobial properties similar to the small cysteine-rich cationic defensins produced in response to infection (Ganz 2006). This observation was firmly established in studies of turpentine-induced inflammation observed in hepcidin-deficient mice that were unable to generate the serum hypoferremia observed in the wild type mice (Nicolas et al. 2002). A link between inflammation and Fe homeostasis became evident when IL-6 was shown to be required for the inflammatory stimulation of hepcidin expression in vitro and in vivo. Moreover, infusion of IL-6 in human subjects resulted in elevated urinary hepcidin that correlated with low serum Fe and transferrin saturation levels (Wrighting and Andrews 2006). IL-6 is an inflammatory cytokine secreted by activated macrophages and neutrophils that amplifies the local inflammatory response to a systemic response via STAT3-mediated hepcidin expression in the liver. The target of hepcidin is the mammalian Fe exporter ferroportin (Fpn), a transmembrane protein expressed primarily in enterocytes, hepatocytes, macrophages and placental cells (Ganz and Nemeth 2006). Binding of hepcidin to Fpn triggers the internalization of the transporter and its lysosomal degradation, thus preventing the release of dietary Fe from intestinal epithelial cells, and blocking the release of Fe stores from hepatocytes and macrophages into circulation, in concert leading to a systemic reduction of serum Fe concentrations (Nemeth et al. 2004; De Domenico et al. 2007). As an extreme condition, this anemia of inflammation is a chronic hypoferremic condition that arises in a number of pathological situations such as prolonged infection and cancer (Wessling-Resnick 2010).

## Regulation of Fe homeostasis in fungi

Due to the essential but toxic properties of Fe, intracellular Fe levels and Fe handling must be rheostatically regulated. Although Fe homeostasis has perhaps been most extensively studied in the bakers yeast *Saccharomyces cerevisiae*, the mechanism of Fe-responsive transcriptional regulation in this microorganism, as well as its close relative and human pathogen, *Candida glabrata*, differs antagonistically

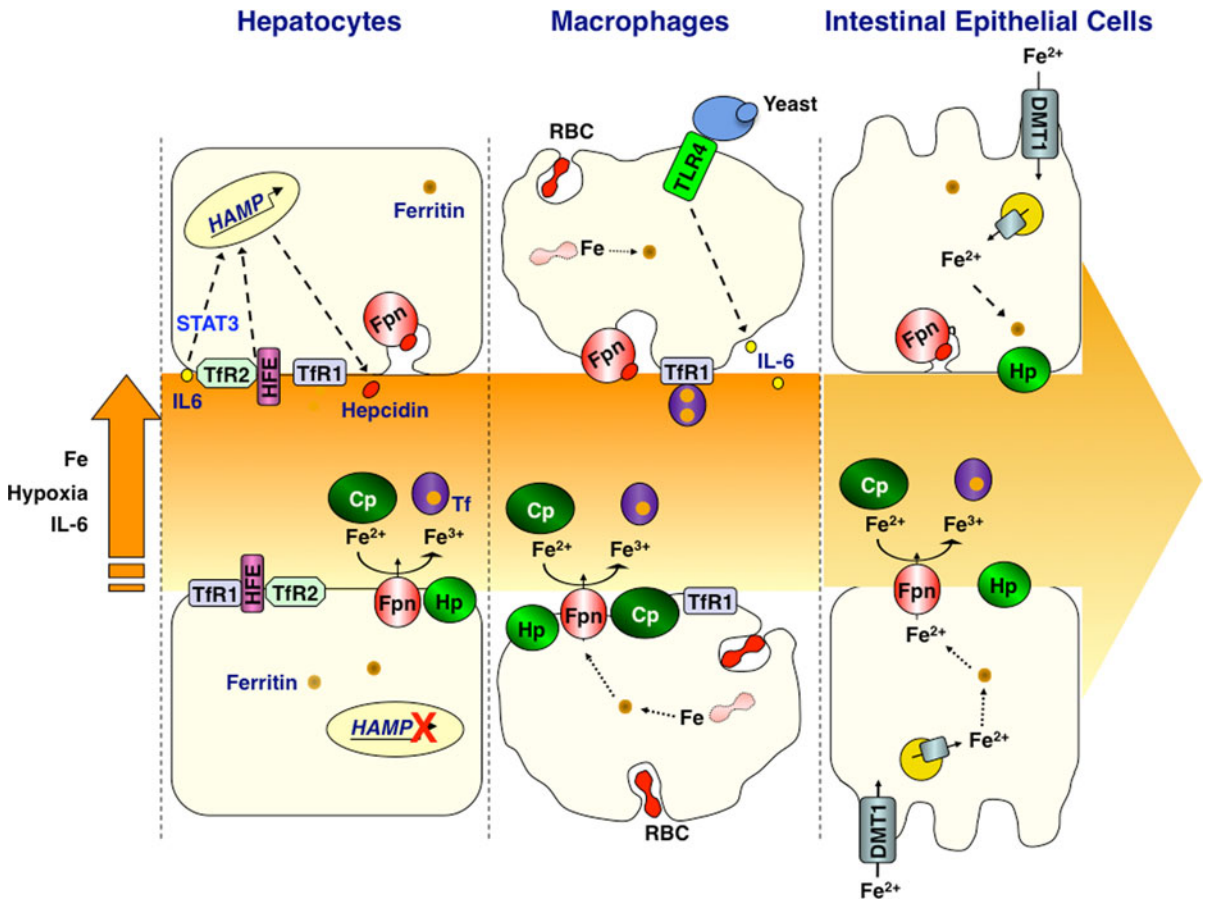
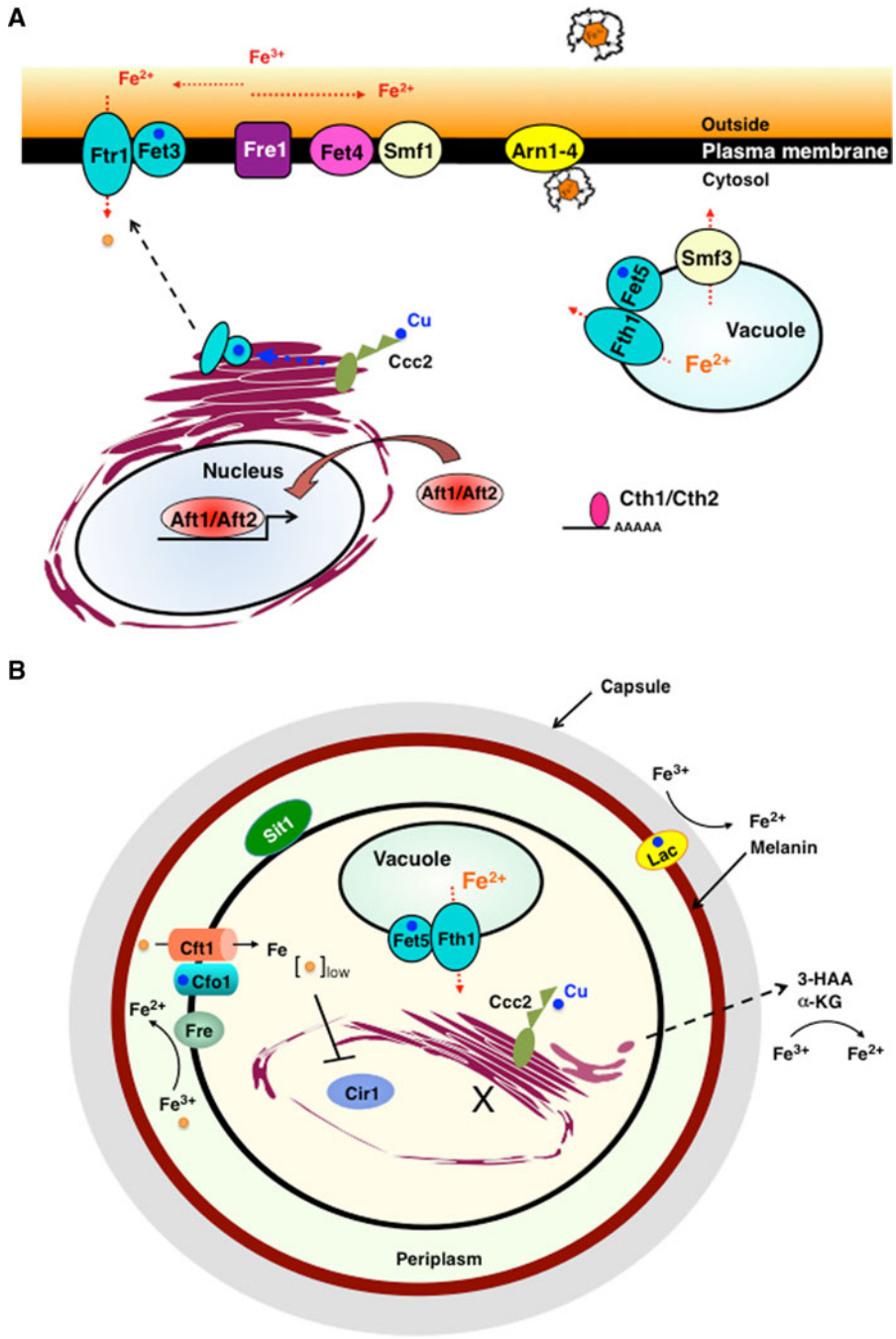



**Fig. 1** Since there is no mechanism of regulated Fe excretion, maintenance of stable serum Fe concentrations implies the recycling of red blood cell (RBC) hemoglobin-bound Fe, primarily by splenic macrophages, its mobilization from hepatic stores as well as by its regulated dietary uptake from intestinal epithelial cells (IEC). The activities of the $Fe^{2+}$-transporter, Ferroportin (Fpn), as well as its negative regulator, hepcidin, play a central role in systemic Fe homeostasis (Hentze et al. 2010). Iron enters IECs through the divalent metal transporter, DMT1 where it is routed for storage in ferritin or exported into systemic circulation through Fpn. Regulated Fpn activity homeostatically releases dietary Fe from IECs and Fe stored in macrophages and hepatocytes into the serum. Ferroxidase-assisted loading of Fe onto transferrin (Tf) by ceruloplasmin (Cp) and hephaestin (Hp) delivers it systemically where Fe is primarily taken up by RBC precursors, which receive 70–90% of Tf-bound Fe. Under conditions of Fe excess and hypoxia, Tf receptor 2 (TfR2) associates with the Major Histocompatibility Factor Class I-like protein, HFE, that together with bone morphogenetic signaling (not shown) in hepatocytes leads to the synthesis and secretion of hepcidin. Hepcidin binds to Fpn, triggering its internalization and degradation and effectively preventing the movement of Fe into the serum. The increased expression of transferrin receptor 1 (TfR1) further depletes circulating Fe levels by receptor-mediated endocytosis of holo-Tf and storage of the released Fe in ferritin. During a pro-inflammatory response, initiated for instance by recognition of yeast cells by Toll-like Receptor 4 (TLR4) on the surface of macrophages and neutrophils, leads to the secretion of the cytokine interleukin-6 (IL-6), which induces hepcidin expression in the liver through STAT3-mediated signalling, thus implementing systemic Fe withholding

from that present in the vast majority of fungi. Whilst under conditions of Fe deficiency, genes involved in the acquisition, mobilization and sparing of intracellular Fe are regulated in *S. cerevisiae* by the Aft1 transcriptional activator, as well as by its paralogue, Aft2, in most fungal species that include *Aspergillus fumigatus* (Schrettl et al. 2008), *Candida albicans* (Lan et al. 2004) and *Cryptococcus neoformans* (Jung et al. 2006) these genes are, in contrast, relieved from repression by a highly conserved GATA-type transcriptional repressor (Fig. 2). Notably, in *Blastomyces dermatitidis*, this repressor, denoted SREB




(Siderophore biosynthesis REpressor in Blastomyces) has been shown to mediate the morphological change from yeast to mold, critical for the production of infectious spores (Gauthier et al. 2010). In *C. neoformans*, deletion of the GATA-type repressor Cir1 leads to a complete loss of virulence in a mouse model of cryptococcosis, highlighting the strict requirement for Fe acquisition for viability under the Fe-limiting conditions of the mammalian host (Jung et al. 2006). Despite the differences in the regulation of the Fe regulon itself the regulated genes, encoding components of high and low affinity Fe and Cu acquisition, as

**Fig. 2** Iron uptake in fungi. **a** In *S. cerevisiae*, under conditions of Fe adequacy the activity of cell surface ferrireductases (Fre proteins) and low-affinity Fe transporters Fet4 and Smf1 is sufficient to meet cellular demand. A shift to Fe deficiency leads to the accumulation of the Aft1 and Aft2 transcriptional activators in the nucleus where they up-regulate target gene expression. Collectively termed the Fe regulon, the vast majority of these genes encode components of high-affinity acquisition of extracellular Fe and mobilization of Fe stores from the vacuole. Two genes within this Fe-responsive regulon, *CTH1* and *CTH2*, encode mRNA-destabilizing proteins that implement mechanisms of cellular Fe sparing that coordinate a cell-wide metabolic adaptation to Fe deficiency (Puig et al. 2008). *S. cerevisiae* possesses two mechanisms of high affinity Fe uptake. The first relies on the re-oxidation of ferrous Fe by the Fet3 multicopper oxidase and the internalization of the metal ion through the Ftr1 Fe permease. Ftr1/Fet3 assembly in the Golgi is a pre-requisite for targeted plasma membrane localization of the complex and its function. Because Fet3 requires bound Cu for enzymatic activity, the P-type ATPase Ccc2 Cu transporter is also regulated by Aft1 to ensure adequate Cu delivery into the secretory compartment. Mobilization of vacuolar Fe stores is mediated by an orthologous ferroxidase/permease complex, Fet5/Fth1, as well as by the low affinity Smf3 divalent metal transporter. The second mechanism of high-affinity Fe uptake uses low molecular weight $Fe^{3+}$ chelators, termed siderophores, which are recognized by specific cell surface transporters that mediate their internalization. *S. cerevisiae* encodes 4 transporters (Arn1-Arn4) with overlapping as well as distinct specificities for siderophores of fungal and bacterial origin (Philpott and Protchenko 2008). Siderophore-Fe is released intracellularly although the mechanisms by which this is achieved are still poorly understood. **b** In *Cryptococcus* species, the Fe regulon is repressed under Fe satiety by a GATA-type transcriptional regulator, *Cir1* which dissociates from the promoter of the majority of target genes under Fe deficiency. This de-represses the Fe regulon leading to the upregulation of genes encoding components of high-affinity environmental Fe assimilation and mobilization from intracellular stores. The Cfo1/Cft1 ferroxidase/Fe permease complex is orthologous to Fet3/Ftr1 in *S. cerevisiae* and accumulates at the cell surface, as does at least one siderophore transporter, Sit1, with similarity to *S. cerevisiae* Arn3/Sit1. Copper transport into the secretory compartment by Ccc2 is not only essential for Cfo1/Fet5 ferroxidase function but also for the activity of the diphenol oxidase, laccase, that catalyzes the formation of melanin. In addition to the cytoprotective activities of melanin, it has been proposed that the ferrous oxidase activity of this enzyme may also contribute towards Fe mobilization into cells (Jung and Kronstad 2008). A third mechanism of extracellular Fe solubilization employs the secretion of reductants that include 3-hydroxyanthranilic acid and α-ketoglutarate (Nyhus et al. 1997)

well as its mobilization from intracellular storage and Fe-sparing mechanisms are remarkably similar (Vergara and Thiele 2008; Kaplan and Kaplan 2009).

## Iron uptake in fungi

The essential requirement for Fe and its low bioavailability in the environment is clearly reflected by the multiple mechanisms employed by microbes to promote active uptake of this nutrient. Armed with a remarkable arsenal of cell surface proteins capable of binding or retrieving Fe from host proteins, microbes can successfully colonize the mammalian host in which Fe withholding makes the availability of this nutrient forbidding for the sustenance of microbial life. If under aqueous aerobic conditions, Fe is already largely oxidized and present as Fe (hydr)oxide species that exhibit minimal solubility at neutral pH (Boukhalfa and Crumbliss 2002), within the human host, Fe-binding proteins further reduce serum Fe concentrations to approximately $10^{-24}$ M (Fischbach et al. 2006). This poses a challenge for microorganisms for which the minimum intracellular concentration of Fe required to sustain growth and proliferation is estimated to be approximately $10^{-6}$ M. Although several microbes that constitute the mammalian microbiota are capable of direct utilization of host Fe-binding proteins, such as Tf, Lf and heme, the most ubiquitous mechanism of high affinity Fe acquisition is through reductive elemental Fe uptake and the utilization of low molecular weight organic $Fe^{3+}$ chelators known as siderophores (Miethke and Marahiel 2007) (Fig. 2).

## Reductive Fe uptake

Reductive Fe uptake utilizes cell-surface metalloreductases to reduce almost all sources of ferric Fe (heme standing as the sole exception), whether in the form of (hydr)oxides, organic chelates or host-bound Fe-laden Tf or Lf. The ferrous Fe is then transported into the cytoplasm, either through high affinity Fe transporters or low-affinity divalent metal transporters, such as Smf1 found in *S. cerevisiae*, *C. glabrata* and *C. albicans*, and orthologous to mammalian NRAMP metal transporters (Philpott and Protchenko 2008). High-affinity Fe uptake into the cell is mediated by a protein complex composed of a multicopper ferroxidase-permease pair. *S. cerevisiae* Fet3 is assembled in the Golgi with four Cu ions into a complex with the Fe permease, Ftr1, and cotranslocated to the plasma membrane (Kosman 2003). Four ferrous ions, reduced through the activity of the membrane-bound Fre1 and

Fre2 ferric reductases, are oxidised by Fet3 and subsequently transported by the Ftr1 permease with the concomitant Fet3-catalyzed reduction of molecular oxygen to water. As such, reductive Fe assimilation does not occur in the absence of oxygen, Cu and heme as a co-factor for the b-type ferrireductases at the plasma membrane. Components of the reductive iron uptake machinery have been identified in several fungal pathogens. Ferric reductases exist in *Histoplasma capsulatum* (Timmerman and Woods 2001), *C. neoformans* (Jacobson et al. 1998), *A. fumigatus* (Schrettl et al. 2004) and *C. albicans* (Ramanan and Wang 2000; Knight et al. 2002). The multicopper oxidase in *C. albicans*, *FET99*, is orthologous to Fet3 and its partner permease CaFtr1 is likewise induced by Fe deprivation (Ramanan and Wang 2000; Knight et al. 2002). Reduction of host Fe-binding proteins such as holo-Tf is mediated to a large extent by this mechanism and, as such, *ftr1*Δ mutants are avirulent in a mouse model of systemic *Candida* dissemination (Knight et al. 2005). As a siderophore non-producing fungus, *C. albicans* most likely relies on reductive assimilation of circulating $Fe^{3+}$ sources through Fre-mediated reduction to $Fe^{2+}$, for which Tf has low affinity.

Similarly, the *C. neoformans cft1*Δ mutant, defective in reductive Fe assimilation, shows a severe attenuation in virulence (Jung et al. 2009). For *C. neoformans*, Fe and Cu are intrinsic to virulence and regulate melanin and capsule formation, two critical virulence factors in this organism (Jung and Kronstad 2008; Kim et al. 2008). In particular, the capsule plays a determinant role in masking *Cryptococcus* from components of the host immune system and protecting the cell against a plethora of anti-microbial proteins and peptides (Seider et al. 2010).

Reliance on reductive Fe assimilation, however, varies among fungal pathogens. Inspection of the *A. fumigatus* genome indicates the presence of a high affinity Fe permease-encoding gene, *ftrA* and a ferroxidase, *fetC* (Schrettl et al. 2004), but in contrast to *C. albicans* and *C. neoformans*, the ferric reduction activity of *A. fumigatus freA* was not sufficient to fully compensate for the absence of siderophore biosynthetic capacity under low Fe conditions in vitro (Schrettl et al. 2004; Hissen and Moore 2005). In support of these findings, siderophore biosynthesis but not reductive Fe assimilation is essential for *A. fumigatus* virulence in a mouse model of invasive aspergillosis (Schrettl et al. 2004).

## Siderophore utilization

The high affinity non-reductive uptake of Fe employed by bacteria and fungi alike is mediated through the utilization of siderophores, low molecular weight organic ferric ion chelators. Siderophores are synthesized and secreted by most bacteria and fungi as well as some higher plants and constitute a widespread mechanism of high affinity microbial scavenging of environmental or host-bound Fe (Miethke and Marahiel 2007). The importance of siderophore utilization is underscored by the fact that bacteria and fungi have evolved transporters that allow them to utilize siderophores they do not produce (xenosiderophores). The concept of siderophore utilization, in which a valuable metabolic product is released into the extracellular environment where it can be hijacked by other cells of the same or different species, is an altruistic behaviour and constitutes an example of a 'whole group' cooperative trait whose metabolic cost to the individual is outweighed by the benefits to the local group of organisms (Griffin et al. 2004). Expression of specific siderophore-chelate transporters or their reduction at the cell membrane by ferric reductases, especially in filamentous fungi and yeast, represents not only a highly effective and specific mechanism of Fe scavenging (siderophores exhibiting the highest stability constants of up to $10^{50}$ M for enterobactin) but, when internalized, also overcome the toxicity associated with the solubilized Fe ion. By generating extremely negative redox potentials at the metal center, solubilized siderophore-Fe is maintained outside of the range for redox catalysis, effectively preventing the generation of ROS (Crichton and Pierre 2001; Boukhalfa and Crumbliss 2002).

## Specificity of siderophore transporters

Irrespective of their ability to produce siderophores, virtually all fungi have the capacity to compete with fungal and even bacterial siderophore-producers for the transport and utilization of siderophore-bound Fe (Miethke and Marahiel 2007). Siderophore transport in *S. cerevisiae* is mediated by a subfamily of membrane permeases of the major facilitator

superfamily (Fig. 2a) (Philpott and Protchenko 2008). With 12–14 predicted transmembrane domains, these secondary transporters display varying degrees of siderophore specificity, with Arn2/Taf1 recognizing almost exclusively triacetylfusarine C (TAFC) and fusigen and Arn4/Enb1 specifically mediating the transport of enterobactin, a bacterial siderophore produced by members of the *Enterobacteriaceae* and *Streptomycetaceae* (Raymond et al. 2003). A wide range of hydroxamate-type siderophores that include ferrichromes, coprogen and to a lesser extent, TAFC are recognized by Arn1 and Arn3/Sit1, the latter also mediating the uptake of bacterial ferrioxamine (Yun et al. 2000; Yun et al. 2000). Sharing a high degree of homology with *S. cerevisiae* siderophore transporters, deletion of *C. albicans SIT1* (Siderophore Transporter 1) compromises the utilization of ferrichromes, and to a lesser extent of TAFC but not ferrioxamine that appears to either be assimilated through the reductive system or by another siderophore transporter. Interestingly, two open reading frames with some homology to Sit1 have been identified in *C. albicans* although siderophore-transport activity has not yet been described (Heymann et al. 2002). Given the critical role of hyphal formation in *C. albicans* virulence, it would be interesting to evaluate whether siderophore transporters are expressed in hyphae. In vitro, Sit1 activity was shown to be required for the invasion and penetration of human epithelial cells in an in vitro model of oral candidiasis, however, *SIT1* deletion did not attenuate *C. albicans* virulence in a mouse model of *Candida* bloodstream infection. In the sterile environment of mammalian serum, the presence of siderophore Fe is unlikely to contribute significantly as a source of Fe. In contrast, a functional high affinity reductive uptake in addition to the ability of *C. albicans* to efficiently transport and degrade heme (via the Rbt5/Rbt51 heme receptors and the intracellular Hmx1 heme oxygenase, respectively) is well suited for the utilization of local host Fe sources (Santos et al. 2003; Weissman et al. 2008). Evaluation of the role of xenosiderophore utilization in the survival of siderophore non-producers likely requires novel in vivo assays recapitulating the polymicrobial flora of the mammalian mucosal membranes, gut and skin.

The observation that the hyphal-specific invasin-like adhesin, Als3, can bind ferritin and mobilize bound Fe for cellular utilization may further reflect the success of *C. albicans* as a significant cause of fungemia and represents the first report for direct microbial utilization of ferritin-bound Fe (Almeida et al. 2008).

In *C. neoformans*, Fe-regulated Sit1 mediates the utilization of ferrioxamine but not ferrichrome (Tangen et al. 2007). However, the ability of the *sit1*Δ mutant to grow in the presence of ferrichrome argues for the existence of an as yet uncharacterized siderophore transporter. The deletion of *SIT1* alone does not lead to attenuation in a mouse model of cryptococcosis, reminiscent of that observed in *C. albicans*. However, the variable intra- and inter-species dependence on distinct mechanisms of Fe uptake for virulence is expected to differ also as a function of infection site. *C. neoformans* infection is primarily confined to the lungs (Lin and Heitman 2006). This infection route and the importance of siderophore utilization towards virulence are particularly relevant within the context of Cystic Fibrosis (CF), one of the most common lethal genetic disorders in Caucasians (Rowe et al. 2005). CF patients carry mutations in the CF transmembrane conductance regulator (CFTR), a chloride ion transporter, whose activity is particularly important in maintaining adequate fluidity on the surface of lung epithelial cells. CF patients suffer from increased mucous viscosity that impairs microbial clearance from the lung epithelium, leading to high susceptibility to and chronic respiratory infection. The mixed nature and greater microbial colonization that arises in CF lung epithelia can be predicted to impose harsh intra- and inter-species competition for Fe sources and to the copious release and utilization of ferrated-siderophores (Mossialos and Amoutzias 2009). Siderophore production has been documented in several pathogenic fungi including *Aspergillus* species, the major CF-associated fungal pathogen, as well as in *Histoplasma capsulatum* and *Fusarium graminearum*. However, it is absent in *C. neoformans*, in *Candida* species as well as from *Saccharomyces cerevisiae*. *Aspergillus nidulans* synthesizes at least three siderophores: TAFC, fusigen and ferricrocin. TAFC and fusigen are secreted and ferricrocin is mostly retained intracellularly as a source of iron storage (Oberegger et al. 2001). *A. nidulans* can also take up heterologous siderophores including ferrirubin, produced by *Aspergillus ochraceus*, and bacterial

enterobactin. *Aspergillus fumigatus* synthesizes several hydroxamate siderophores, TAFC and ferricrocin being the most abundantly produced when *A. fumigatus* is cultured in serum-containing media (Hissen et al. 2004). Studies have shown that these siderophores are required to desferrate human serum holo-Tf in vitro and in vivo (Hissen et al. 2004; Hissen and Moore 2005). Furthermore, growth of the *A. fumigatus sidA*Δ mutant, defective in siderophore biosynthesis, in human serum is inhibited unless endogenous siderophores are supplied.

Were it not for a dedicated mammalian counter-strategy that involves two lipocalin superfamily proteins (Lcn1 and Lcn2) (Clifton et al. 2009) and given the proficiency with which siderophores strip Fe from virtually all host sources, microbial Fe stealth via siderophore utilization would essentially limit the efficacy of host Fe withholding. Initiation of the acute phase response leads to the increased expression and plasma concentration of Lcn2 (siderocalin, neutrophil gelatinase-associated lipocalin, 24p3) (Meheus et al. 1993). Lcn2 is a bacteriostatic protein constitutively present in neutrophil granules that contributes to host Fe sequestration by binding the bacterial siderophores enterobactin (Goetz et al. 2002), carboxymycobactin (Holmes et al. 2005) and bacillibactin (Abergel et al. 2006), efficiently outcompeting bacteria and fungi that utilize these siderophores, such as *A. nidulans* that uses the MirA transporter to uptake enterobactin (Haas et al. 2003). The mechanism for lipocalin/siderophore recognition relies on hybrid electrostatic/cation-$\pi$ interactions within the positively charged binding pocket. Siderophores composed of hydroxamate or citrate moieties, such as bacterial aerobactin and those produced by most fungi, are electrostatically neutral and do not interact with lipocalin within the binding calyx (Goetz et al. 2002). Thus, although these siderophores have considerably lower affinity for ferric Fe than the catechol-type siderophores, they escape Lcn2 sequestration. Interestingly, several species of bacteria including *Enterobacteriaceae*, *Salmonella* and *Yersinia* express a receptor for fungal hydroxamate siderophores underscoring the complexity and dynamics of mixed microbial colonization. Lcn1, expressed predominantly in tears and respiratory secretions, binds a broad range of fungal hydroxamate siderophores, albeit with lower affinity than that of Lcn2 for enterobactin (Fluckinger et al. 2004). Together, these two proteins mediate an important anti-microbial defence mechanism by sequestering microbial siderophores.

## Human disorders of iron overload

In the face of the multiple and efficacious armament exploited by fungi in the acquisition of this highly sequestered essential nutrient, what are the effects of Fe overload on the susceptibility, severity and outcome of infectious disease? Iron overload is characterized by dysregulated Fe absorption or storage in excess of body requirements that leads to increased Tf saturation as well as Fe deposition with damage to vital organs presumably due to its toxicity as a generator of reactive oxygen intermediates. Pathophysiological Fe loading can occur not only as a result of genetic imposition, such as is the case with Hereditary hemochromatosis, but also as a result of the treatment of conditions requiring multiple blood transfusions, including $\beta$-thalassemia (Fig. 3).

Hereditary hemochromatosis (HH), is the most common single gene disorder in individuals of Northern European descent, with an incidence in the United States of 1 in 200–300 individuals (Durupt et al. 2000). Hereditary hemochromatosis, associated with high serum Tf saturation and parenchymal Fe accumulation, encompasses a set of disorders that result from mutations in a relatively restricted number of genes, including *HFE*, *TFR2* (Tf receptor 2), *HJV* (hemojuvelin), *HAMP* (hepcidin) and *FPN* (ferroportin). The common theme for the outcome of these genetic lesions is either altered hepcidin expression, or in the case of the autosomal dominant Fpn mutations, insensitivity of the transporter to hepcidin activity (Hentze et al. 2010). However, despite the cumulative manifestations of Fe overload observed in these individuals, the clinical presentation associated with these two scenarios is quite distinct. Hepcidin-insensitive Fpn mutations recapitulate classical HH associated with decreased *HAMP* gene expression. In contrast, Fpn Fe-transport deficient mutations initially result in borderline anemia, high macrophage Fe stores and low Tf saturation (Girelli et al. 2008). Of particular relevance, transport-deficient Fpn mutations have been shown to compromise the ability of macrophages to clear phagocytosed microorganisms. In support of this, Paradkar and colleagues demonstrated that the Fe

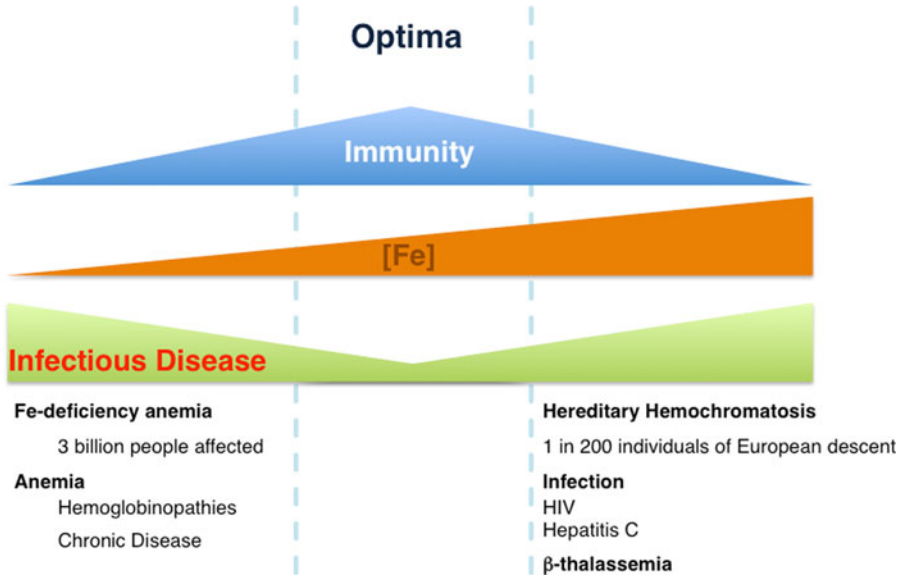



**Fig. 3** Iron status as a modifier of human fungal infection. In healthy individuals, optimal immune function relies on the homeostatic regulation of Fe concentrations within a physiological range that ensures Fe sufficiency for cellular immune function but is restrictive for microbial proliferation. Fe overload diseases such as hereditary hemochromatosis (HH) and secondary Fe loading, as a result of transfusion-dependent $\beta$-thallasemia as well as viral infection, are conditions that dial Fe homeostasis away from the optima required for cell-mediated immunity. The different etiologies of HH mutations with regards to macrophage Fe levels and the clinical evidence supporting Fe overload in increased susceptibility to infectious disease predict that a subset of genetically-imposed Fe overload conditions, as well as those resulting from viral infection, compromise macrophage immune function. On the other side of the spectrum, Fe deficiency, whether due to dietary insufficiency, genetically-imposed hemoglobinopathies or chronic inflammation, whilst decreasing circulating Fe levels, also impact negatively on immunity at the very least at the level of macrophage activation and oxidative burst, both Fe-dependent processes

export deficient *flatiron* mouse, in which a mutation in Fpn prevents its correct localization to the cell surface, supports the growth of the intracellular pathogen, *Chlamydia psittaci* (Paradkar et al. 2009). Finally, clinical evidence spanning the last 60 years shows that HH patients often develop several bacterial infections, notably *Vibrio vulnificus* primary septicemia (Barton and Acton 2009; Neupane and Kim 2009) as well as fungemia associated most often with *A. fumigatus* (Khan et al. 2007).

Remarkably, infection with a number of viruses appears to be correlated to hyperferremia and increased susceptibility to secondary infections by opportunistic pathogens (Drakesmith and Prentice 2008). Hepatitis C virus (HCV) and HIV-1 manipulate host cellular Fe homeostasis to drive cellular metabolism of infected cells that, correspondingly, enhances viral replication, assembly and propagation (Drakesmith and Prentice 2008). Notably, HIV-infected macrophages frequently display elevated Fe with a positive correlation observed between the degree of Fe loading and mortality (de Monye et al. 1999; Xu et al. 2010). The increased Fe levels in macrophages is predicted to further compromise microbial Fe-withholding to adventitious pathogens such as *M. tuberculosis*, *H. capsulatum*, *Candida* species and *C. neoformans*, the most common opportunistic pathogens of AIDS patients (Barluzzi et al. 2002; Ranganathan and Hemalatha 2006). With an estimated 200 million people infected with these two viruses worldwide, secondary Fe overload might constitute a very significant niche for the increase of microbial burden and mortality in these individuals due to opportunistic fungal infectious disease (Fig. 3).

## Concluding remarks

The mammalian host is a nutrient-rich environment for the few fungal species that have acquired the ability to grow at elevated temperatures, thus escaping the innate mechanism of thermal exclusion

(Bergman and Casadevall 2010). The absolute dependence of both host and microbe on Fe utilization imposes host mechanisms that decrease its bioavailability. As a result of this selective nutritional pressure, microorganisms have evolved often multiple and diverse strategies for Fe acquisition. The increasing clinical evidence for increased morbidity and mortality due to infectious disease in patients with increased body Fe support host Fe regulation as a modulator of the stalemate between pathogenicity and microbiostasis.

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