Iron homeostasis—Achilles’ heel of Aspergillus fumigatus?
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The opportunistic fungal pathogen Aspergillus fumigatus adapts to iron limitation by upregulation of iron uptake mechanisms including siderophore biosynthesis and downregulation of iron-consuming pathways to spare iron. These metabolic changes depend mainly on the transcription factor HapX. Consistent with the crucial role of iron in pathophysiology, genetic inactivation of either HapX or the siderophore system attenuates virulence of A. fumigatus in a murine model of aspergillosis. The differences in iron handling between mammals and fungi might serve to improve therapy and diagnosis of fungal infections.

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Current Opinion in Microbiology 2011, 14:400–405
This review comes from a themed issue on Host-microbe interactions: Fungi
Edited by Scott Filler
Available online 1st July 2011
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DOI 10.1016/j.mib.2011.06.002

Introduction
Owing to its capacity to mediate electron transfer and acid–base reactions, iron is an essential nutrient for all eukaryotes and nearly all prokaryotes [1]. Either alone, or incorporated into iron–sulfur clusters or heme, this metal is an indispensable cofactor for a variety of cellular processes including respiration, amino acid metabolism, and biosynthesis of DNA and sterols. However, excess iron has the potential to catalyze the formation of cell-damaging reactive oxygen species [2]. Inversely, detoxification of oxidative stress depends on iron as, for example, catalases and peroxidases require heme as cofactor, which further underlines the complex intertwining of iron metabolism and oxidative stress. Despite its high abundance in the Earth’s crust, the bioavailability of iron is very limited owing to its oxidation into insoluble ferric hydroxides by atmospheric oxygen. Furthermore, the mammalian innate immune system restricts iron availability to microbial invaders via a variety of mechanisms such as iron scavenging by transferrin or lactoferrin [3,4]. To overcome iron limitation and to avoid iron excess, all organisms and in particular pathogens have developed tightly regulated mechanisms to balance acquisition, storage and consumption of iron. In addition, iron starvation serves as a stimulus of iron-independent virulence determinants in pathogens. Consequently, the control of access to iron is one of the central battlefields on which the outcome of infection is decided.

Aspergillus fumigatus is a ubiquitous saprophytic fungus, which has become the most common air-borne fungal pathogen of humans [5]. Clinical manifestations range from allergic reactions to life-threatening invasive disease, termed aspergillosis, particularly in immuno-compromised patients. This review summarizes the current knowledge on iron homeostasis-maintaining mechanisms of A. fumigatus and their role in virulence.

Iron acquisition and storage
Control of iron uptake is considered the major iron homeostatic mechanism in A. fumigatus and other fungi because iron excretion systems have not been identified to date [6]. In contrast to various bacterial and some fungal pathogens [7,8], A. fumigatus lacks specific uptake systems for host iron sources such as heme, ferritin, or transferrin [9]. Instead iron supply is ensured by low-affinity ferrous iron acquisition as well as two high-affinity iron uptake systems, reductive iron assimilation (RIA) and siderophore-assisted iron uptake [9] (Figure 1a). At the molecular level, low-affinity iron uptake has been characterized in Saccharomyces cerevisiae but not in any other fungal species. The involved permeases transport not only ferrous iron, but also other metals such as copper and zinc [1*]. RIA starts with reduction of ferric iron sources to the more soluble ferrous iron by plasma membrane-localized ferrireductases [10]. Subsequently, the ferrous iron is re-oxidized and imported by a protein complex consisting of the ferroxidase FetC and the iron permease FtrA. Siderophores are low molecular mass, ferric iron-specific chelators. A. fumigatus excretes two different siderophores, fusaricin C (FsC) and triacyltetrasaricin C (TAFc), to mobilize extracellular iron (Figure 1b). The ferri-forms of FsC and TAFc are taken up by siderophore-iron transporters (SIT). SIT constitute a subfamily of the major facilitator protein superfamly acting most probably as proton symporters energized by the plasma membrane potential [11,12]. SIT-mediated iron uptake appears to be universally conserved in the fungal kingdom, even in species not producing siderophores such as S. cerevisiae, Candida spp. and Cryptococcus neoformans [6,12–14]. Possible reasons are the solubility and therefore high energy-status of siderophore-chelated iron and the putative role of ‘stealing’ siderophores in microbial warfare. For intracellular release of iron, TAFc and FsC are hydrolyzed, partly by the esterase EstB [15].
Siderophore-mediated iron uptake is conserved in most bacterial and some plant species. An exclusive feature of fungi, however, is the presence of intracellular siderophores. *A. fumigatus* possesses two different intracellular siderophores (Figure 1b), hyphal fericrocin (FC) and conidial hydroxyferricrocin (HFC), for distribution and storage of iron [16,17]. Additionally, *A. fumigatus* probably employs vacuolar iron storage, as indicated by the iron-inducible expression of CccA [18], the ortholog of the vacuolar iron importer Ccc1p of *S. cerevisiae* [1,2]. In contrast to bacteria, plants and animals, however, fungi lack ferritin-mediated iron storage and detoxification.

**Siderophore biosynthesis**

FsC is a cyclic tripeptide consisting of three N5-anhydromevalonyl-N5-hydroxyornithine residues linked by ester bonds; TAFc is the N5-acetylated FsC, FC is a cyclic hexapeptide with the structure Gly-Ser-Gly-(N5-acetyl-N5-hydroxyornithine)3 and HFC is hydroxylated FC [6]. The siderophore biosynthetic pathway characterized by reverse genetics is shown in Figure 1c. The first committed step in the biosynthesis of all four siderophores is hydroxylation of ornithine catalyzed by the ornithine monoxygenase SidA. Subsequently, the pathways for biosynthesis of extracellular and intracellular siderophores split owing to the transfer of different acyl-groups to N5-hydroxyornithine: an acetyl group is transferred via an unknown enzyme to form intracellular siderophores and anhydromevalonyl is transferred by transacylase SidF to form extracellular siderophores. Assembly of FsC and FC is catalyzed by two different non-ribosomal peptide synthetases (NRPS), SidD and SidC, respectively. TAFC and HFC are formed by SidG-mediated N2-acetylation of FsC and hydroxylation of FC, respectively. Additionally, the 4′-phosphopantetheinyl transferase PptA is essential for siderophore biosynthesis because NRPS, along with polyketide synthetases and the lysine-biosynthetic α-aminoadipate reductase requires activation by this enzyme [19,20].

**Biological functions of siderophores**

Genetic elimination of extracellular siderophores (ΔsidF and ΔsidD mutants) decreases growth, conidiation and oxidative stress resistance under iron limited, but not iron sufficient conditions where other iron acquisition systems can compensate for the lack of siderophores [16].
Elimination of intracellular siderophores (ΔsidC mutant) reduces conidiation and blocks sexual development (as shown in A. nidulans) owing to the role of FC in intracellular iron transport from substrate-contacting hyphae into aerial hyphae [16*,17*,21]. The reduced intracellular iron supply causes conidial iron shortage, which impairs iron-dependent enzymes such as catalase A and consequently decreases conidial resistance to oxidative stress [16*,17*]. Moreover, such conidia show delayed germination during iron starvation owing to the lack of conidial iron storage [16*]. Ablation of the entire siderophore system (ΔsidA mutant) combines the defects caused by inactivation of either extracellular or intracellular siderophores and renders A. fumigatus extremely sensitive to iron starvation [9,16*].

Both extracellular and intracellular siderophores contribute to pathogenic growth because elimination of the entire siderophore system (ΔsidA mutant) results in absolute avirulence of A. fumigatus in a murine model of invasive pulmonary aspergillosis [9,22], while deficiency in either extracellular (ΔsidF or ΔsidD mutants) or intracellular siderophores (ΔsidC mutants) causes partial attenuation of virulence [16*]. Blocking TAFC production, while concomitantly increasing Fsc production (ΔsidG mutant) affects neither growth nor virulence, indicating that the structural differences between these two siderophores do not play a role in these processes [16*]. Consistent with a role in iron acquisition during infection, the A. fumigatus siderophores are able to remove iron from host proteins, such as transferrin [23,24].

Genetic inactivation of RIA (ΔftrA mutant) does not affect virulence of A. fumigatus [9]. Nevertheless, several lines of evidence indicate that RIA also plays a role during infection: (i) elimination of extracellular siderophores causes only partial attenuation of virulence, (ii) genome-wide expression profiling revealed induction of both the siderophore system and RIA during initiation of murine infection [25], and (iii) mutants lacking both RIA and the siderophore system (ΔftrAΔsidA double mutant) are unable to grow unless supplemented with siderophores or extremely high iron concentrations to fuel low-affinity iron uptake [9]. Of note, RIA has been shown to be crucial for virulence of fungi that do not produce siderophores, such as C. albicans and C. neoformans [26,27].

Restoration of conidial HFC content by supplementation with FC during conidiation partially cures the virulence defect of ΔsidA conidia [16*]. This demonstrates a crucial role of the conidial siderophore during initiation of infection, most probably owing to its importance for germination and oxidative stress resistance.

Defects in the siderophore system decrease intracellular growth and survival of A. fumigatus after phagocytosis by murine alveolar macrophages, which represent the first line of defense in the lung during pulmonary aspergillosis [28]. Consistent with these findings, siderophore-deficiency alters the immune response of murine macrophages against infection with A. fumigatus [29]. Similarly, the siderophore system is also important for virulence of Histoplasma capsulatum, a dimorphic fungal pathogen that replicates in the yeast form within macrophages [30]. Taken together, these data demonstrate that the siderophore system is crucial not only for extracellular but also for intracellular growth.

The evolutionary conserved role of iron in fungal virulence is underlined by the indispensable role of siderophores in various other experimental models of aspergillosis, that is, a murine cutaneous model, Drosophila melanogaster, and Galleria mellonella [31–33], as well as various phytopathogenic ascomycetes [34].

**Regulation of iron homeostasis and its role in virulence**

In A. fumigatus, iron starvation causes extensive transcriptional remodeling that is mediated by the two central transcription factors, SreA and HapX (Figure 2) [18,35*]. The DNA-binding GATA-factor SreA represses RIA and the siderophore system during iron sufficiency in order to avoid toxic effects. The bZip-transcription factor HapX physically interacts with the DNA-binding CCAAT-binding complex (as shown in A. nidulans [36]) and represses
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The attenuated virulence caused by defects in siderophore biosynthesis or HapX strongly suggests that A. fumigatus faces iron limitation during mammalian infection. This concept is supported by the finding that increased bone marrow iron stores represent an independent risk factor for invasive aspergillosis [44]. Moreover, human protection against A. fumigatus includes growth inhibition by polymorphonuclear leukocytes via lactoferrin-mediated iron depletion and possibly siderocalin-mediated scavenging of siderophores [45,46].

Aspergillosis is difficult to diagnose and treat, which is reflected by the high mortality rates that continue to be associated with this disease [5]. The essentiality of iron for fungal virulence and the differences in iron acquisition mechanisms between mammals and fungi such as Aspergillus spp. might help to improve therapy and diagnosis of fungal infections. Specifically, the fungal siderophore biosynthetic pathway represents a promising target for selective therapeutic intervention. Furthermore, SIT constitutes one of few protein families that are unique to fungi and are not present in prokaryotes or other eukaryotes [47]. Thus, this protein family is a promising drug target, and it could also be used to target drug delivery specifically to the fungus by a Trojan horse approach [48], in which antifungal agents are covalently attached to siderophores and selectively imported by the infecting fungus. The potential of iron chelation therapy is indicated by the synergistic effect of iron chelators and antifungal drugs that has been demonstrated both in vitro and in a murine aspergillosis model [49,50]. Moreover, the recently demonstrated PET (Positron Emission Tomography) imaging of invasive pulmonary aspergillosis in a rat model, based on fungal accumulation of TAFC-chelated 68Gallium (Figure 3), underlines the potential of utilizing siderophores for the diagnosis of fungal infections [51].

The understanding of the role of iron in fungal infections clearly has advanced enormously in recent years. Nevertheless, its potential application in treatment and diagnosis of fungal infections still requires deeper insights.

Conflicts of interest

No conflicting interests.

Acknowledgements

Work in the authors' laboratory is supported by Austrian Science Foundation Grants FWF P-21643-B11 and I-282-B09 (to HH). We apologize to the authors whose work could not be cited because of space limitations.

Figure 3

PET imaging of invasive pulmonary aspergillosis in a rat model using 68Ga-TAFC. Images demonstrating accumulation of 68Ga in infected lungs were taken one hour after injection of 68Ga-TAFC into the femoral vein. Kidneys are labeled in infected and non-infected animals owing to renal excretion of 68Ga-TAFC. This figure was reprinted by permission of the Society of Nuclear Medicine from [51].


References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


This review comprehensively covers iron uptake, storage and regulation in the fungal model organism S. cerevisiae.


This review explains the importance of redox exchange in cellular handling of iron – the basis for reductive iron assimilation.


This study, together with Ref. [8], characterized the role of siderophores in acquisition and intracellular handling of iron, physiology and virulence in A. fumigatus using siderophore-deficient mutants.


This paper demonstrates the role of siderophores in intracellular iron distribution. Ferricrocin represent the first intracellular iron transporter characterized in any species.


This study describes the role of HapX in transcriptional and metabolic adaptation to iron limitation and virulence. It demonstrates that HapX represses iron-consuming pathways and activates production of siderophores and ribotoxin.


