MINIREVIEW

Candida albicans iron acquisition within the host

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Abstract

As a commensal and opportunistic pathogen, Candida albicans possesses a range of determinants that contribute to survival, persistence and virulence. Among this repertoire of fitness and virulence attributes are iron acquisition factors and pathways, which allow fungal cells to gain this essential mineral in the iron-poor environment of the host. The aim of this review is to present the strategies used by C. albicans to exploit host iron reservoirs and their impact on C. albicans pathogenicity. Because iron in the human host is mostly linked to host proteins, pathogens such as C. albicans must possess mechanisms to gain iron from these proteins. Here, we introduce the most important groups of human proteins, including haemoglobin, transferrin, lactoferrin and ferritin, which contain iron and that are potential iron sources for invading microorganisms. We then summarize and discuss the known and proposed strategies by which C. albicans exploits or may exploit iron from host proteins and compare these with strategies from other pathogenic microorganisms.

Keywords

Candida albicans; virulence; iron uptake; host iron sources.

Introduction

In most humans, the polymorphic yeast Candida albicans coexists as a harmless commensal within the normal microbial flora. However, this fungus may cause disease when the equilibrium of the microbial flora is disturbed (e.g. during long-term antibiotic treatment), when the immune system of the host is compromised and/or when epithelial barriers are damaged (e.g. after surgery or application of an intravenous catheter). Candida albicans can cause two main forms of infections: superficial infections of the skin or mucosa, and invasive candidiasis, where the fungus can disseminate throughout the blood system and infect virtually every organ (Calderone, 2002). Candida albicans yeast cells are able to undergo morphological transitions and form either hyphal or pseudohyphal filaments. The ability to switch between yeast and filamentous forms is generally recognized as an important virulence attribute (Sudbery et al., 2004; Kumamoto & Vinces, 2005). However, C. albicans possesses a range of further determinants that contribute to survival, persistence and virulence. Furthermore, this repertoire of fitness and virulence factors within the host is stage and tissue specifically regulated rather than constitutively expressed. In agreement with this view, it seems as though particular virulence determinants are of pathogenic significance only in certain host niches (Hube, 2004, 2006; Brown et al., 2007).

Iron, as an essential cofactor for several proteins, is required for numerous biochemical processes including cellular respiration and metabolism, oxygen transport, drug metabolism and DNA synthesis (Welch et al., 2001). In the microbial world, there is constant competition for iron, both between microorganisms within microbial communities and between microorganisms and their hosts during both commensal carriage and infection. Because iron is an essential element for both the host and C. albicans, iron uptake during infection is considered a virulence attribute and even colonization and proliferation are possible only if sufficient iron is accessible to the fungus (reviewed in Sutak et al., 2008). Therefore, it is not surprising that the iron content of the host influences the susceptibility to C. albicans infections. For example, pretreatment of endothelial cells with the iron chelator phenanthroline reduces damage by C. albicans (Fratti et al., 1998). Moreover, invasion of C. albicans into phenanthroline-treated cells is reduced in comparison with nontreated cells. Similar results...
were obtained through the pretreatment of oral epithelial cells with the iron chelator bathophenanthrolineisulphonic acid (BPS) (Almeida et al., 2008). In contrast, loading the epithelial cells with iron increased damage by C. albicans, although the ability of fungal hyphae to invade host cells remained unchanged. In a mouse model of systemic candidiasis, an intravenous injection with colloidal iron (60 mg kg⁻¹ body weight) for 3 consecutive days before intravenous inoculation of C. albicans yeast cells (10⁷ cells) significantly increased the mortality rate of mice: within 28 days of infection, 40% of mice without iron administration died, while 80% mortality was observed among iron-loaded animals (Abe et al., 1985). Similar correlations between host iron content and pathogenicity have been observed for the pathogenic fungi Cryptococcus neoformans (Barluzzi et al., 2002) and Aspergillus fumigatus (Kontoyiannis et al., 2007) and bacteria such as Mycobacterium tuberculosis, Salmonella species and Yersinia species (Schaible & Kaufmann, 2004; Doherty, 2007). Finally, the anti-Candida activity of ciclopirox olamine, a potent antifungal agent, is proposed to be caused by iron chelation (Niewerth et al., 2003; Lee et al., 2005; Sigle et al., 2005).

Iron availability within the host influences C. albicans pathogenicity. Hence, the aim of this review is to present the strategies used by this fungus to exploit host iron reservoirs and their impact on C. albicans pathogenicity. Because iron in a human host is not freely available, but mostly linked to host proteins, pathogens such as C. albicans must possess mechanisms to gain iron from these proteins. In the following section, we will briefly introduce the most important groups of human iron-containing proteins that are potential iron sources for invading microorganisms. We will then summarize and discuss the known and proposed strategies of how C. albicans exploits or may exploit iron from host proteins.

**Iron within the host**

**Haemoglobin**

In vertebrates, iron is almost totally sequestered in association with specific proteins (summarized in Fig. 1), which prevents the iron-dependent catalysis of free radical cascades.

![Diagram of iron acquisition strategies](https://academic.oup.com/femsyr/article-abstract/9/7/1000/511914)

**Fig. 1.** *Candida albicans* strategies for iron exploitation within the host. To acquire iron, *C. albicans* possesses three high-affinity iron acquisition systems: (1) a reductive system responsible for iron exploitation from transferrin, ferritin or from the environment; (2) a siderophore uptake system responsible for iron acquisition from a range of siderophores produced by other organisms; and (3) a haem-iron uptake and degradation system capable of acquiring iron from haemoglobin and probably from haem-proteins.
and the generation of toxic components and also restricts its availability to pathogens. In humans, iron is present at around 40–50 mg kg⁻¹ body weight (Crichton & Charloteaux-Wauters, 1987), with 66% of the total body iron circulating in the blood coupled to haemoglobin (Evans et al., 1999).

Haemoglobin, the oxygen-binding protein of red blood cells, transports oxygen from the lungs to tissues and carbon dioxide back to the lungs. It is a compact globular protein of approximately 64 kDa comprised of two pairs of polypeptide chains, termed α and β, which assume the structure of an α2β2 tetramer (reviewed in Marengo-Rowe, 2006). Each polypeptide chain nestles one haem prosthetic group. Haem can bind oxygen only when its central iron ion is in the ferrous form (Fe²⁺); oxidation of the iron ion from the ferrous to the ferric form (Fe³⁺) yields methaemoglobin, which cannot bind oxygen. Thus, each haemoglobin molecule possesses four haem groups containing one ferrous ion per prosthetic group (Evans et al., 1999).

**The transferrin family**

The transferrin family constitutes another class of iron protein. This family consists of a group of monomeric glycoproteins of approximately 90 kDa with a high affinity for ferric iron (K₅₇ ~10⁻²⁵ M) (Aisen et al., 1978). In humans, there are two major members of the transferrin family.

The first member is itself named transferrin. Found in serum, transferrin can bind ferric iron and transport it from sites of iron absorption and storage to sites of utilization (Fletcher & Huehns, 1968). The majority of transferrin is synthesized in the liver and, despite being the most important iron transport molecule, only about 3 mg iron (approximately 0.1% of the total body iron) is bound to transferrin (Evans et al., 1999). In healthy human beings, around 30% of transferrin is saturated with iron (Han, 2005), leaving the free iron concentration of serum at around 10⁻¹⁸ M (Bullen et al., 1978). Transferrin possesses two iron-binding sites and bicarbonate is required for its structure is recovered at pH 7.4 (Dominguez-Vera, 2004).

Transferrin binding sites and bicarbonate is required for its structure is recovered at pH 7.4 (Dominguez-Vera, 2004). The proposed mechanism by which transferrin releases iron to other host cells is via binding to the transferrin receptor coupled with acidification to pH 5.6 (Bali & Aisen, 1992).

Similar to transferrin, lactoferrin, the second member of the transferrin family, possesses two ferric iron-binding sites and requires bicarbonate for iron binding (Jolles et al., 1976). Lactoferrin is present in body fluids such as milk, saliva, tears and serum, and is released from neutrophils upon degranulation (Vorland, 1999). Independent of its iron-binding ability, lactoferrin contains a defensin-like peptide and has microbicidal activity against microorganisms such as *C. albicans*, *Streptococcus mutans*, *Vibrio cholerae* and enterobacteria (Yamauchi et al., 1993; Vorland et al., 1999; Tanida et al., 2001; Ueta et al., 2001; Ward et al., 2002).

**Ferritin**

The iron storage protein ferritin is found in virtually all human cells; however, the liver, spleen and bone marrow contain the highest levels of this protein. In healthy human adults, about 30% of total body iron is present in ferritin (Fleming & Wood, 1995). While transferrin is of central importance in sequestering extracellular iron, ferritin plays a key role in maintaining intracellular iron homeostasis by capturing free iron and preventing the toxic reactions catalysed by iron (Torti & Torti, 2002). Consequently, deletion of the ferritin H subunit gene is lethal in mice (Ferreira et al., 2000). Although the vast majority of the ferritin in the human body is intracellular, low levels (20–300 μg L⁻¹) can be found circulating in blood (Harrison & Adams, 2002) and serum ferritin is an important index of body iron, although its function remains unknown (Lipschitz et al., 1974; Torti & Torti, 1994).

Structurally, ferritin is composed of 24 subunits forming a protein nanocage (spherical shape) of 12 nm outer diameter and an inner cavity about 8 nm in diameter and is capable of accommodating up to around 4500 ferric iron ions (Harrison et al., 1991). Human ferritin is composed of two types of subunits: H (21 kDa) and L (20 kDa) (Arosio et al., 1978). Depending on the tissue type, the ratio of H to L subunits can vary from predominantly L in the liver and spleen to predominantly H in the heart and kidney (Arosio et al., 1976). The H subunit possesses ferroxidase activity and thus influences the uptake of iron by ferritin (Sun & Chasteen, 1992). The L subunit plays a role in iron-core nucleation and confers stability to assembled ferritin molecules (Levi et al., 1992). Furthermore, the quaternary structure of ferritin dissociates *in vitro* at pH < 2.0 and the structure is recovered at pH 7.4 (Dominguez-Vera, 2004).

**Other iron-containing proteins and the labile iron pool**

Additionally, iron can be found intracellularly as part of haem proteins (e.g. catalase, cytochrome and myoglobin), nonhaem proteins (e.g. ribonucleotide reductase) and proteins with iron–sulphur clusters (e.g. aconitase and succinate dehydrogenase) (Furuyama et al., 2007; Furuyama & Vargas, 2007; Rouault & Tong, 2008). Finally, linking the stages of iron acquisition from transferrin, storage within ferritin and metabolic utilization, there exists a transitory pool of chelatable and redox-active iron that serves as a
crossroad of cellular iron metabolism, known as the labile iron pool (Kakhlon & Cabantchik, 2002).

**Candida albicans** strategies for host iron exploitation

**Siderophore uptake**

When faced with an iron-poor environment, certain bacteria and fungi can synthesize low-molecular-weight (<1 kDa) iron-binding proteins known as siderophores. These high-affinity iron chelators are able to scavenge environmental iron, making it available to microorganisms (Andrews et al., 2003; Haas, 2003). Because the affinity to iron of some microbial siderophores is even higher than the affinity of host iron-binding proteins, these siderophores are able to capture iron from host proteins. For example, the purified siderophore desferri-exochelin, secreted by *M. tuberculosis*, rapidly removes iron from transferrin and lactoferrin, and at a slower rate from ferritin (Gobin & M. tuberculosis, 1996), indicating that ferritin is an extremely stable molecule, not easily accessible to pathogenic microorganisms. The filamentous fungus *A. fumigatus* produces two siderophores (triacetylfusarinine C and ferricrocin), both capable of removing iron from transferrin, and these iron acquisition mechanisms have been shown to be important for the survival of *A. fumigatus* in human serum (Hissen et al., 2004).

Although *C. albicans* siderophore production had been demonstrated by biochemical assays in earlier studies (Holzberg & Artis, 1983; Ismail et al., 1985), genes encoding factors of a possible siderophore biosynthetic pathway have not been discovered in the *C. albicans* genome (Haas, 2003; Lan et al., 2004). However, it is clear that *C. albicans* and other yeasts can use iron from siderophores produced by other organisms, a strategy with examples in several bacteria known as ‘iron parasitism’ (Heymann et al., 2002; Hu et al., 2002; Lesuisse et al., 2002; Kosman, 2003; Wandersman & Delepelaire, 2004).

While the baker’s yeast *Saccharomyces cerevisiae* possesses four different siderophore transporters within the cell membrane (Kosman, 2003), each of them specific for transporting different siderophore types, *C. albicans* has only one: Sit1/Arn1 (Heymann et al., 2002; Hu et al., 2002). This transporter can mediate the uptake of a range of siderophores from other organisms and from other iron complexes (Ardon et al., 2001; Heymann et al., 2002; Lesuisse et al., 2002; Bernier et al., 2005). Through Sit1/Arn1, siderophores are internalized via an endocytic pathway, which has not yet been fully characterized (Hu et al., 2002; Weissman et al., 2008). A mutant lacking Sit1/Arn1 is attenuated in its ability to cause damage in the reconstituted human epithelium (RHE) model of infection (Heymann et al., 2002), but has wild-type virulence in a mouse model of systemic infection (Hu et al., 2002). However, it remains unknown how Sit1/Arn1 may influence iron uptake or damage during RHE infections because *C. albicans* is unable to produce its own siderophores and other siderophores or siderophore-producing cells were not included in this infection system.

Genes encoding proteins similar to the transcriptional repressor of siderophore uptake/biosynthesis and regulator of reductive iron assimilation in the maize fungal pathogen *Ustilago maydis* (Urbs1) (An et al., 1997; Eichhorn et al., 2006) have been identified in other fungal species. The orthologue of Urbs1 in *A. fumigatus* (SreA) is also a repressor of siderophore biosynthesis (and a regulator of numerous other iron-responsive genes) in this organism and a mutant lacking SreA displayed defective growth under iron-rich conditions, but was not attenuated in a mouse infection model, in agreement with the view that iron is limited within the host (Schrettl et al., 2008).

Based on its homology to Urbs1 of *U. maydis*, Lan et al. (2004) identified SFU1 (suppressor of ferric uptake) in the genome of *C. albicans*. Comparisons between transcript profiles of *C. albicans* wild-type and Δsfu1 mutant strains revealed 139 potential target genes of Sfu1, many of which are iron-responsive. However, the role of this putative transcription factor in *C. albicans* virulence has not been determined.

Based on its homology to URBS1, a central iron-responsive transcriptional regulator (Cir1) was recently identified in *C. neoformans* (Jung et al., 2006). Similar to transcriptional regulators of iron uptake systems in bacteria (such as Fur1; see Andrews et al., 2003), Cir1 not only regulates the expression of genes involved in iron uptake but also multiple other activities such as calcium homeostasis, the ability to grow at 37 °C, glucan synthesis, sterol biosynthesis and two key virulence factors: capsule formation and melanin production (Jung et al., 2006). With such multiple functions, a mutant lacking *CIR1* was, as expected, avirulent in a mouse inhalation model of cryptococcosis.

**Haemoglobin uptake and degradation**

The ability to utilize haemoglobin as an iron source was the first described strategy of *C. albicans* iron exploitation from a host protein (Moors et al., 1992). The first step in haemoglobin iron utilization by *C. albicans* in vivo is probably via binding to erythrocytes. *Candida albicans* hyphae are able to rosette erythrocytes via complement-receptor-like molecules (Moors et al., 1992). For erythrocyte lysis, *C. albicans* possesses a haemolytic factor, probably a mannoprotein attached to the fungal cell surface (Watanabe et al., 1999). Although extracellular hydrolases may be involved, the mechanism by which *C. albicans* causes...
haemolysis and the molecular basis of this mechanism remains unknown. Following its release, the uptake of haemoglobin is mediated by specific haemoglobin-receptors on the surface of *C. albicans* (Fig. 1). Weissman & Kornitzer (2004) first identified the haemoglobin-receptor gene family, two of which (*RBT5* and *RBT51/PGA10*) have been characterized in vitro as possessing haemoglobin-binding properties. The haemoglobin-receptor gene family comprises the genes *RBT5, RBT51, WAP1/CSA1, CSA2* and *PGA7* (Weissman & Kornitzer, 2004). All five proteins contain the so-called CFEM domain, characterized by a sequence of eight spaced cysteine residues (Kulkarni *et al*., 2003) (Table 1). This domain has been found in many fungal membrane proteins, mostly proteins of unknown function (Kulkarni *et al*., 2003). Tanaka *et al.* (1997) demonstrated that *C. albicans* hyphae bound haemoglobin stronger than the yeast form suggesting that the expression of haemoglobin-receptors may be coregulated with hyphal formation. Indeed, both *RBT5* and *WAP1* have a hyphal-specific expression pattern (Braun *et al*., 2000). However, the growth experiments for haemoglobin iron utilization were based on *C. albicans* yeast and not hyphal cells (Weissman & Kornitzer, 2004). Additionally, it has been shown that haemoglobin is bound to Rbt5 and internalized via an endocytic pathway by yeast cells (Weissman *et al*., 2008), a pathway distinct from the siderophore internalization pathway (Froissard *et al*., 2007). One possible explanation for these controversial results may be found in the transcriptional analysis comparing *C. albicans* cells under conditions with and without iron. Lan *et al.* (2004) showed that *RBT5* expression was 184.7-fold upregulated under low-iron conditions (10 µM) in comparison with high-iron conditions (100 µM). Furthermore, the binding assay performed by Tanaka and colleagues used iron-replete yeast cells, in contrast to Weissman and colleagues. It is therefore possible that, under iron limitation, the expression of *RBT5* is morphology independent. A similar morphology-independent expression of a hyphal-associated gene was also recently reported for the ferritin receptor *Als3* (Sosinska *et al*., 2008).

Strikingly, an *rbt5Δ* mutant displays wild-type virulence in a mouse model of systemic infection and during rabbit corneal infection (Braun *et al*., 2000). This is probably due to the compensatory mechanisms of other iron acquisition systems and/or due to the fact that other members of the haemoglobin receptors family may compensate for the absence of Rbt5 (Weissman & Kornitzer, 2004).

Once haemoglobin is internalized inside vacuoles by the endocytic pathway, it must to be hydrolysed or denatured – probably through vacuolar proteases and/or the acidic vacuolar pH – to release haem (Weissman *et al*., 2008). To exploit iron from haem, *C. albicans* possesses a haem oxygenase (Santos *et al*., 2003). The subcellular localization of haem oxygenase-mediated haem degradation in *C. albicans*, however, remains unclear. *HMX1* – a gene with no other homologues in the *C. albicans* genome – encodes an active haem oxygenase, which degrades haemoglobin to α-biliverdin (Pendrak *et al*., 2004a). The role of *HMX1* in *C. albicans* virulence has not yet been determined. Interestingly, its expression is positively regulated by iron deprivation, by haem and by a temperature shift from 30 to 37 °C (Santos *et al*., 2003). Furthermore, a Δ*hmx1* mutant was unable to grow under iron restriction caused by the iron chelator ferrozine. However, a role for haem oxygenase activity in other iron acquisition pathways of *C. albicans* such as the reductive pathway has not yet been described (Pendrak *et al*., 2004a).

In addition to its role as an iron source, a secondary function of haemoglobin for *C. albicans* during candidiasis has been proposed. The binding of haemoglobin to the surface of *C. albicans* (discussed above) triggers the expression of putative surface receptors that enable the fungus to bind to fibronectin, laminin and fibrinogen (Yan *et al*., 1998). Based on these findings, it has been suggested that the binding to haemoglobin can stimulate *C. albicans* binding to additional proteins of the host cell surface and the extracellular matrix. When free haemoglobin is found in blood, this mechanism may help the fungus to rapidly escape from the bloodstream to the host tissues. Moreover,

<table>
<thead>
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<th>Orf19*</th>
<th>Gene name*</th>
<th>Size (amino acids)*</th>
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<th>GPI-anchored (predicted)1</th>
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</tbody>
</table>

The protein sequence of Rbt5 was obtained from the CGD and BLAST searches were also performed using CGD.

*Information obtained from CGD (http://www.candidagenome.org/).

1In silico protein analysis was performed with the online software ‘SMART’ (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1).

1GPI-anchor prediction was performed using the online software ‘BIG-PI PREDICTOR’ (http://mendel.imp.ac.at/gpi/gpi_server.html).

GPI, glycosylphosphatidylinositol.

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Table 1. Predicted members of the haemoglobin-receptor gene family
such a haemoglobin-recognition system may allow the fungus to sense such events as entry into the bloodstream or disruption of endothelial barriers during tissue invasion (Pendrak et al., 2004b).

**Transferrin and lactoferrin as potential iron sources for C. albicans**

Specific, receptor-mediated binding of transferrin and lactoferrin – in order to exploit bound iron – has been demonstrated for Neisseria species. These receptors are located in the bacterial outer membrane and are induced upon iron starvation. Following binding to the receptors, iron is stripped from transferrin or lactoferrin by an as yet undetermined mechanism and the apoproteins (iron-free proteins) are released extracellularly rather than being internalized and degraded (reviewed in Perkins-Balding et al., 2004). One proposed mechanism for triggering the release of iron from transferrin – and probably lactoferrin – is acidification. For example, under iron limitation, Staphylococcus aureus increases the production of lactate, resulting in acidification of the surrounding environment and, consequently, the release of iron from transferrin (Friedman et al., 2006).

Apo-transferrin is known to inhibit C. albicans growth through iron chelation (Han, 2005; Lee & Han, 2006). In contrast, Knight and colleagues demonstrated that C. albicans can use transferrin as an iron source in vitro. The authors speculated that monoferric transferrin (transferrin associated with only one iron molecule) could not be used as an iron source by C. albicans. Furthermore, it was shown that binding is necessary for iron utilization from transferrin, although the transferrin receptor remains unknown (Knight et al., 2005).

Lactoferrin has not been described as an iron source for C. albicans. In contrast, lactoferrin has been shown to be a potent inhibitor of C. albicans growth in several studies (Andrè et al., 2008; Lahoz et al., 2008; Venkatesh & Rong, 2008).

**Ferritin as a potential iron source for C. albicans**

Ferritin is an extremely stable protein and highly resistant to microbial attack. Nevertheless, a number of studies have suggested that certain microbial pathogens can use ferritin as an iron source during *in vitro* growth. For example, Yersinia pestis can grow on agar containing haemin, myoglobin, haemoglobin or ferritin (Sikkema & Brubaker, 1989). Listeria monocytogenes and Burkholderia cepacia can grow in a liquid medium with ferritin as the sole source of iron (Jin et al., 2006; Whitby et al., 2006). However, the molecular mechanisms of iron acquisition from ferritin by these organisms remain unknown and it is not clear whether ferritin from host cells can be used by any of these species.

*Neisseria meningitidis* was the first microorganism shown experimentally to exploit ferritin as an iron source during interaction with host cells (Larson et al., 2004). However, this bacterium is not able to directly utilize iron from ferritin, but rather induces degradation of cytosolic ferritin by manipulating the host cellular machinery and utilizes the resultant free cytosolic iron.

Recently, we have demonstrated that *C. albicans* is able to bind ferritin on the external layer of hyphal, but not yeast cell walls (Almeida et al., 2008; Fig. 1). Moreover, it has been shown that a single member of the Als-protein family (Als3) was essential for binding of ferritin to hyphae and that this binding promoted efficient exploitation of ferritin as an iron source. The Als protein family of *C. albicans* encodes large cell surface glycosylphosphatidylinositol-anchored glycoproteins that were originally implicated in the process of adhesion to host surfaces by the group of Hoyer (Hoyer, 2001). Expression of ALS3 was shown to be hyphal-specific (Hoyer et al., 1998) and was observed *in vivo* during oral and systemic infections (Thewes et al., 2007; Zakikhany et al., 2007).

Several lines of evidence support the role of Als3 as a ferritin receptor: (1) Hyphae, but not yeast cells, are able to bind ferritin and Als3 is known to be a hyphal-associated protein. However, the binding of ferritin was not dependent on the hyphal morphology *per se*, as a mutant lacking Hgc1 (Zheng et al., 2004) did not produce true hyphae, but still expressed ALS3 and bound ferritin. (2) Mutants lacking transcription factors known to regulate ALS3 expression (Tec1, Bcr1) (Schweizer et al., 2000; Nobile & Mitchell, 2005) had a reduced ability to bind ferritin. In agreement with this, a mutant that was unable to form hyphae (∆ras1) and that did not express ALS3 also displayed reduced binding of ferritin and reduced growth on ferritin plates. (3) A mutant lacking ALS3 was dramatically reduced in its ability to bind ferritin and displayed poor growth on ferritin plates. An *als3Δ*+ALS3 reintegrant strain had a restored ability to bind ferritin and a partially restored ability to grow on ferritin plates, although not to wild-type levels, possibly due to a gene dosage effect. (4) Strikingly, an *S. cerevisiae* strain expressing Als3 (but not other members of the ALS family) was able to bind ferritin. Moreover, Sosinska and colleagues observed that hypoxic conditions and iron restriction in a vagina-simulative medium affected the cell morphology and the cell wall proteome of *C. albicans*. Surprisingly, one of the proteins found in the cell wall of yeast cells under these iron-limited conditions was Als3 (Sosinska et al., 2008). This result demonstrates hyphal-independent expression of Als3, possibly via iron starvation, providing further evidence for the role of Als3 in iron acquisition.

The discovery that the *als3Δ* mutant completely lost its ability to bind ferritin was surprising, considering that ALS3
belongs to a gene family with several similar members. Although not every member of the Als family was investigated for ferritin binding, the fact that the C. albicans mutant lacking Als3 and S. cerevisiae strains expressing Als1 or Als5 (two other members of the C. albicans Als family) were unable to bind ferritin (while a S. cerevisiae strain expressing Als3 showed ferritin binding) suggests a unique property of Als3 (Almeida et al., 2008). Additionally, als3Δ mutant cells were unable to damage endothelial cells and oral epithelial cells and were strongly attenuated in their ability to damage epithelial tissue in an in vitro model of oral infections (RHE) (Zhao et al., 2004; Phan et al., 2007; Almeida et al., 2008).

Following binding, the iron content of ferritin must be released. A probable mechanism for this process is active acidification. Similar to S. aureus (as mentioned above), C. albicans was only able to use ferritin as an iron source under conditions that allowed acid production and under low buffer capacity of the medium, suggesting that iron acquisition from ferritin by C. albicans is pH mediated (Almeida et al., 2008).

The reductive pathway mediates iron uptake from the environment and from two iron-containing host proteins

To utilize iron from transferrin, from ferritin or from the environment, C. albicans uses the reductive pathway (Fig. 1). This system is located in the plasma membrane and consists of three activities. Two surface ferric reductases, which are able to reduce insoluble extracellular ferric (Fe³⁺) ions into soluble ferrous (Fe²⁺) ions, have been described: Cfl1/Fre1 (Yamada-Okabe et al., 1996; Hammacott et al., 2000) and Cfl95/Fre10/Rbt2 (Braun et al., 2000; Knight et al., 2002, 2005; Knight & Dancis, 2006). In addition, a further 13 homologous genes, encoding putative ferric reductases, were identified in the C. albicans genome (Baek et al., 2008). Our own search of the Candida Genome Database (CGD) identified 17 genes with homology to FRE10 (Table 2). For functional enzymatic activity, ferric reductases must possess a ferric reductase domain and an FAD- and/or an NAD-binding domain, responsible for electron donation (De Luca & Wood, 2000). However, not all predicted proteins listed in Table 2 possess these domains. For example, orf19.1844 and orf19.6139 are annotated in the CGD (http://www.candidagenome.org/) as genes encoding ferric reductases (FRE43 and CFL93, respectively), but the predicted proteins possess neither ferric reductase, FAD-binding nor NAD-binding domains (see Table 2). The importance of most of these putative ferric reductase genes for viability or pathogenicity is unknown. Only RBT2 has been disrupted and an rbt2Δ mutant showed wild-type virulence during rabbit corneal infection (Braun et al., 2000). Although few of these putative ferric reductases have been analysed in detail, at the transcriptional level, their expression has been shown to be regulated by iron availability (Bensen et al., 2004; Lan et al., 2004; Lee et al., 2005), supporting the prediction that these genes indeed encode ferric reductases.

The second component of the reductive pathway consists of multicopper oxidase(s). Reduced ferrous iron generated

<table>
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<th>Gene name*</th>
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<th>Transmembrane-domains (number)¹</th>
<th>Ferric reductase domain¹</th>
<th>FAD-binding domain¹</th>
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The protein sequence of Fre10 was obtained from CGD and BLASTP searches were also performed using CGD.

*Information obtained from CGD.

¹In silico protein analysis was performed using the online software ‘SMART’.
The protein sequence of Fet3 was obtained from CGD and a BLASTP search was also performed in CGD.

“Information obtained from CGD.

1In silico protein analysis was performed using the online software ‘SMART’.

Table 3. Multicopper oxidases encoded by Candida albicans

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<tr>
<th>Orf19*</th>
<th>Gene name*</th>
<th>Size (amino acids)*</th>
<th>Chromosome*</th>
<th>Signal peptide1</th>
<th>Transmembrane-domains (number)1</th>
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The protein sequence of Fet3 was obtained from CGD and a BLASTP search was also performed in CGD.

“Information obtained from CGD.

1In silico protein analysis was performed using the online software ‘SMART’.

Table 4. Iron permeases encoded by Candida albicans

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The protein sequence of Ftr1 was obtained from CGD and a BLASTP search was also performed in CGD.

“Information obtained from CGD.

1In silico protein analysis was performed with the online software ‘SMART’.

by surface reductase activity is toxic due to the spontaneous generation of free radicals. To prevent this production of toxic free radicals, Fe2⁺ is oxidized to Fe³⁺ by multicopper oxidase activity (De Luca & Wood, 2000; Kosman, 2003). The C. albicans genome contains five putative multicopper oxidase genes (CGD), two of which – FET3 and FET99 – have been characterized. Candida albicans FET3, but not FET99, was able to complement FET3 deletion in S. cerevisiae (Eck et al., 1999; Knight et al., 2002). A C. albicans strain lacking FET3 is defective in in vitro high-affinity iron uptake, but displayed wild-type virulence in a mouse model of systemic infection, probably due to the activity of other multicopper oxidases during infection (Eck et al., 1999). In our own in silico analysis, four of the five predicted proteins possess three Cu-oxidase domains (required for oxidase activity) (Kosman, 2003), while the predicted sequence of Fet3 contained no Cu-oxidase domain (Table 3). Because copper is required for oxidase activity, the intracellular copper transporter Ccc2 is essential for the function of the reductive pathway and for ferritin iron exploitation (Weissman et al., 2002; Almeida et al., 2008). However, in a mouse model of systemic candidiasis, a ccc2Δ mutant displayed only a moderate reduction in virulence at a low cell inoculum (10⁶) and was fully virulent at a higher (10⁷) inoculum (Weissman et al., 2002). This suggests that the function of Ccc2 can be bypassed by other activities in vivo.

The third component of the reductive pathway consists of iron permeases. These enzymes form a protein complex with multicopper oxidases and transport Fe³⁺ into the cell. The high-affinity iron permease gene FTR1 is induced and is essential for growth upon iron deprivation and is essential for iron acquisition from ferritin and transferrin (Ramanan & Wang, 2000; Knight et al., 2005; Almeida et al., 2008). Fungal cells lacking FTR1 lost their ability to damage oral epithelial cells and were completely avirulent in a mouse model of systemic infection (Ramanan & Wang, 2000; Almeida et al., 2008). These data demonstrate that Ftr1 is an essential component of the reductive pathway both in vitro and in vivo, is involved in iron uptake in low-iron environments (such as within the host) and in iron acquisition from at least two different host proteins, making this permease crucial for C. albicans virulence. This, combined with the fact that FTR1 is fungal-specific, with no sequence similarity to any human gene, makes Ftr1 an attractive target for drug development.

A further three homologous genes, putatively encoding iron permeases, can be found in the C. albicans genome. Both FTH1 and FTH2 encode putative high-affinity iron transporters for intravacuolar iron storage (Urbanowski & Piper, 1999). Ftr2 shares 87% protein identity with Ftr1, but FTR2 is induced when higher levels of iron are available and has no impact on fungal virulence (Ramanan & Wang, 2000). All four permeases possess the iron-binding motif Arg-Glu-Gly-Leu-Glu (REGLE), which is essential for permease activity (Fang & Wang, 2002) (Table 4).

The role of the pH in iron acquisition

The RIM101 pathway of C. albicans is required for pH sensing and is essential for an appropriate transcriptional

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response to changes in the environmental pH. The transcription factor Rim101 and the putative pH sensor Dfg16 are important components of this pathway (Bensen et al., 2004; Thewes et al., 2007). Because iron is less accessible at alkaline pH, the RIM101 pathway is responsible for the upregulation of genes involved in the reductive pathway under alkaline conditions. Accordingly, both Rim101 and Dfg16 are required for growth in iron-limited media at alkaline but not acidic pH (Bensen et al., 2004; Thewes et al., 2007). Moreover, both rim101Δ and dfg16Δ mutants have decreased ability to acquire iron from ferritin at physiological pH, but display no growth defect at acidic pH (our own unpublished data). Lastly, it has recently been shown that the transcription factor Rim101 directly regulates the expression of two ferric reductases in C. albicans (FRE2 and FRP1) (Baek et al., 2008; Liang et al., 2009) and also regulates the expression of the ferritin receptor Als3 (Nobile et al., 2008).

Therefore, it can be concluded that sensing the environmental pH and coordinated upregulation of genes involved in iron acquisition are necessary for iron exploitation from ferritin and probably from other iron sources as well.

Other iron sources: probable exploitation by C. albicans

After cell invasion and tissue destruction, C. albicans may be able to use the cytoplasmic free iron (labile iron pool), iron from proteins containing iron–sulphur clusters and other nonhaem proteins via the reductive pathway. The latter may be facilitated by the activity of secreted proteases that can degrade such proteins. Moreover, haem proteins may be bound via Rbt5 and haem iron exploited via Hmx1. However, the use of such potential iron sources during the interaction with host cells remains to be investigated.

Conclusions and outlook

As described above, C. albicans is extremely well equipped to exploit host iron sources during commensal growth and during both superficial and systemic infection. Candida albicans is able to acquire iron within the host from siderophores produced by other members of the microbial flora, from erythrocyte’s haemoglobin, from extracellular transferrin and from intracellular ferritin (Fig. 1). So far, only a mutant lacking the high-affinity permease Ftr1 showed reduced virulence in in vivo infection models, while the lack of other selected components of the iron uptake systems (as far as investigated) had no dramatic impact on fungal pathogenesis. This demonstrates that, instead of being dependent on a single iron source, C. albicans is able to use several iron sources during interactions with the host. This is in contrast to another important human pathogenic fungus, A. fumigatus, which depends on the production and uptake of siderophores (Schrettl et al., 2007). In order to use the multiple different iron sources available within the host, C. albicans possesses three independent high-affinity iron uptake systems (Fig. 1). The fact that the iron permease Ftr1 is required for iron acquisition in environments with a low iron content and from at least two host iron proteins perhaps explains why this permease is so crucial for virulence as deletion of FTR1 prevents iron acquisition from multiple iron sources. Future research towards the development of a molecule capable of specifically blocking the permease activity of Ftr1 could provide a novel therapeutic for the treatment of candidiasis.

However, it is currently unclear which iron uptake systems are necessary for different stages of infection or within different tissues. A strategy using double or triple mutants lacking genes of the key components of the various uptake systems such as the iron source receptors (e.g. Sit1, Als3 and Rbt5) could provide some clues about the preferential iron source/sources used during a specific stage of infection.

The iron uptake pathways of C. albicans are not self-contained systems, but are integrated into other pathogenic patterns. For example, using the hyphal-associated adhesin and invasin Als3 for gaining iron from ferritin, C. albicans possesses a unique iron exploitation strategy that links hyphal formation, adhesion, invasion and iron acquisition. In fact, the transcriptional regulation of Als3 is independent of external iron sources and seems to be strictly linked to hyphal formation under most investigated conditions (Green et al., 2004, 2005). However, hyphal formation itself is associated with adhesion, proteolytic activity, cellular invasion and damage (Sundstrom, 2002; Naglik et al., 2004; Phan et al., 2007; Thewes et al., 2007; Zakikhany et al., 2007), and it is the hyphal morphology that predominantly reaches the intracellular compartments of epithelial cells where ferritin is located.

In spite of the multiple iron exploitation strategies of C. albicans, it is likely that the fungus must survive periods where no iron sources are available within the different niches of the human body. For such periods, intracellular iron storage would be essential. In A. fumigatus, the siderophores involved in intracellular iron storage are required for germ tube formation, asexual sporulation, resistance to oxidative stress, catalase A activity and virulence (Schrettl et al., 2007). It was previously reported that C. albicans cell division was totally blocked only after five passages (of 5 days incubation each) in an iron-limited (LIM) medium supplemented with BPS (an extracellular iron chelator) (Eck et al., 1999). The authors proposed intracellular iron storage as the mechanism by which C. albicans can continue to divide in the absence of extracellular iron. However, the molecular mechanisms by which C. albicans is able to store iron intracellularly have yet to be investigated. A better
understanding of iron storage in *C. albicans* may shed light on intracellular iron regulation and its involvement in the virulence of this important human pathogen.

Finally, acidification of the surrounding environment is important for iron acquisition from ferritin by *C. albicans*. As acidification may also be beneficial for a number of other properties [such as the activity of aspartic proteases (Naglik et al., 2003)], it would be of importance to show whether such acidification occurs *in vivo* and its role in iron acquisition from other sources.

In summary, *C. albicans* demonstrates remarkable flexibility in gaining access to and utilizing iron. This is perhaps not surprising, given the metabolic resourcefulness of this fungus regarding other nutrients: *C. albicans* is known to be capable of growing on a wide and diverse range of carbon and nitrogen sources, possesses high-affinity phosphate uptake systems and can rapidly adapt to changes in the physical environment. Sophisticated iron acquisition strategies represent another weapon in the arsenal of this opportunistic pathogen for life within its human host.

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**References**


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