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Heme-iron acquisition in fungi

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Heme is a bioavailable source of iron, for which different fungi have evolved several distinct acquisition mechanisms. In the iron-scarce animal host, in particular, microbial pathogens are able to utilize the large heme pool of hemoglobin. The opportunistic pathogenic fungus *Candida albicans* relies on a cascade of related extracellular soluble and cell wall-anchored hemophores to extract the heme from hemoglobin and to steer it across the cell wall to the plasma membrane, where it is endocytosed into the cell. Recent crystal structure determination of the soluble *C. albicans* hemophore Csa2 revealed a new protein fold with a unique heme-iron coordination, which suggests distinctive functional requirements for heme binding and transfer.

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Introduction

Iron is an indispensable element for almost all living organisms: thanks to its ability to assume several oxidation states, iron plays a catalytic role as cofactor in many essential enzymes involved in electron transfer and redox reactions, among other functions. However, in spite of the abundance of iron in the earth's crust, the low solubility of the oxidized, ferric iron ion (Fe^{3+}) results in poor bioavailability of iron in the presence of oxygen. Therefore, organisms use iron reduction systems and iron chelators to solubilize iron in oxidizing environments [1].

For pathogenic microorganisms, iron acquisition in the animal host is, despite its apparent abundance in this environment, even more challenging. This is because in the host organism, iron is sequestered in order to contain its catalytic activity which, when unrestrained, can use the reactive oxygen intermediates of the cellular metabolism to generate toxic hydroxyl radicals [2]. Cellular host iron is therefore bound to iron-binding proteins or stored in ferritin, while iron circulating in the blood is tightly bound to transferrin [3]. This sequestration of iron, together with the binding of iron in mucosal secretions by lactoferrin and lipocalin-2 [3,4] has the additional consequence of inhibiting proliferation of invading microorganisms, a phenomenon that has been called 'nutritional immunity' [5]. Upon inflammation, induction of the iron-regulatory protein hepcidin restricts serum iron levels even more, while hepcidin deficiency sensitizes the host to bacterial infection [6–8], further underscoring the importance of iron in host-pathogen interactions.

In order to overcome the free iron scarcity in the host environment, successful microbial pathogens have therefore evolved mechanisms for extracting iron from host proteins. Host iron can be extracted either in the form of elemental iron or, as is often the case, in the form of heme. Here, we describe recent developments in our understanding of the fungal heme-iron acquisition mechanisms.

Heme

A large proportion of biological iron is found in heme, a cofactor for numerous cellular proteins [9]. Heme consists of an iron atom coordinating a protoporphyrin IX ring. Like free iron, the different oxidation states of heme enable it to participate in redox reactions and electron transport. Heme also participates in other functions, such as oxygen transport; the majority of iron in the human body is found in hemoglobin heme [8]. In order to acquire iron in the host, many pathogenic bacteria and fungi have evolved mechanisms to utilize hemoglobin heme as iron source [10,11]. In addition, minor cuts and abrasions in mouth mucosal membranes, and normal blood loss through the gut mucosa [12,13], could provide sufficient hemoglobin at these and other mucosal surfaces to be utilized as iron source by commensal organisms as well. Furthermore, heme comprises an estimated one third of the iron in the Western diet, and consequently, free heme is also present in the gut environment [14]. Finally, since it is ubiquitous in all domains of life, free heme (typically in its oxidized ferric chloride form, or hemin) is found in decaying organic matter, to a sufficient extent that some soil organisms are heme auxotrophs [15]. Thus, heme uptake pathways can be expected to have evolved in commensal and saprophytic microorganisms as well as in pathogenic microorganisms.

In bacteria, several distinct transport systems were identified, some of which involve hemophores that can extract heme from hemoglobin. The captured heme is then

transferred through multiple surface proteins to a membrane transporter for internalization in Gram-positive bacteria, or transferred to outer membrane transporters in Gram-negative bacteria [16]. Although the elucidation of fungal heme-acquisition systems has lagged behind that of bacteria, it is now clear that heme acquisition in many fungi is likewise mediated by cell surface-bound and soluble hemophores, some of which can extract heme from hemoglobin, and which ultimately lead to heme internalization into the cytoplasm [17,18•,19•,20••].

Heme-iron acquisition in *Candida albicans*

The *C. albicans* heme-iron acquisition pathway

C. albicans is a human commensal ascomycete fungus, normally found on the mucosal surfaces of the digestive tract [21,22]. It is also the most common fungal opportunistic pathogen, responsible for an increasing number of systemic infections that are associated with high mortality rates [23]. Alongside a high-affinity elemental iron acquisition system [24,25], *C. albicans* has evolved a distinct pathway to scavenge iron from hemin and hemoglobin [26,27]. This pathway relies on endocytosis of external heme into the vacuole [28], followed by degradation of the heme to release the iron, via the heme oxygenase Hmx1 in the case of free hemin utilization [29,30], and via a mechanism that is still unclear in the case of hemoglobin heme. Before endocytosis, the heme is channeled through a cascade of extracellular GPI-anchored and secreted proteins that belong to a subclass of the Common in Fungal Extracellular Membrane (CFEM) protein family [17,20••,31•]. The related fungal pathogen *Candida parapsilosis* was shown to require CFEM proteins for efficient hemin acquisition as well [32].

The CFEM heme transfer cascade

The CFEM domain is a uniquely fungal protein domain defined by a distinctive pattern of eight cysteine residues with conserved interspacing [33]. Of the six CFEM proteins detectable in the *C. albicans* genome, five of them — Rbt5, Rbt51/Pga10, Pga7, Csa2, and Csa1/Wap1, the latter containing four CFEM domains — form a subset of related proteins. Three of these CFEM proteins (Rbt5, Pga7, and Csa2) were shown to bind heme, to extract it from the host hemoglobin, and to mediate its delivery to the fungal cell [17,20••,31•]. Deletion of *RBT5*, *PGA7* and *CSA2* caused reduced hemoglobin-iron utilization to various extents, from severe ($pga7^{-/-}$) to mild ($csa2^{-/-}$) [17,20••,31•]. Regarding *RBT51/PGA10*, while its deletion did not result in a detectable heme-iron utilization defect under standard laboratory conditions, its expression in *Saccharomyces cerevisiae* was able to confer some hemoglobin utilization to this organism, suggesting a potential role in *C. albicans* hemoglobin utilization as well [17].

The three CFEM proteins Rbt5, Pga7, and Csa2 are differentially localized on the *C. albicans* cell envelope. Rbt5 is an *O*-mannosylated, glycosylphosphatidylinositol (GPI)-anchored outer cell wall protein [17,31•]. It is highly induced in an animal infection model, and Rbt5-specific antibodies are found in patients recovering from candidemia, consistent with Rbt5 being exposed on the cell wall to the host's immune system [34,35]. Pga7 is also a GPI-anchored protein, but it is located more internally in the cell wall, as well as on the cell membrane [31•]. Csa2 is secreted to the medium [20••]. Heme can be efficiently transferred between the different CFEM proteins *in vitro*, and Pga7 and Rbt5 were shown by surface plasmon resonance to interact in the presence of heme [20••,31•]. Together with the different localization of the CFEM proteins on the cell surface, this suggests that the CFEM proteins form a transfer cascade for heme, extending from Csa2 in the medium, to Rbt5 on the cell wall, and on to Pga7 on the plasma membrane. This cascade might represent the first instance of a fungal *trans*-cell-wall transport system (Figure 1a).

Endocytic uptake of heme

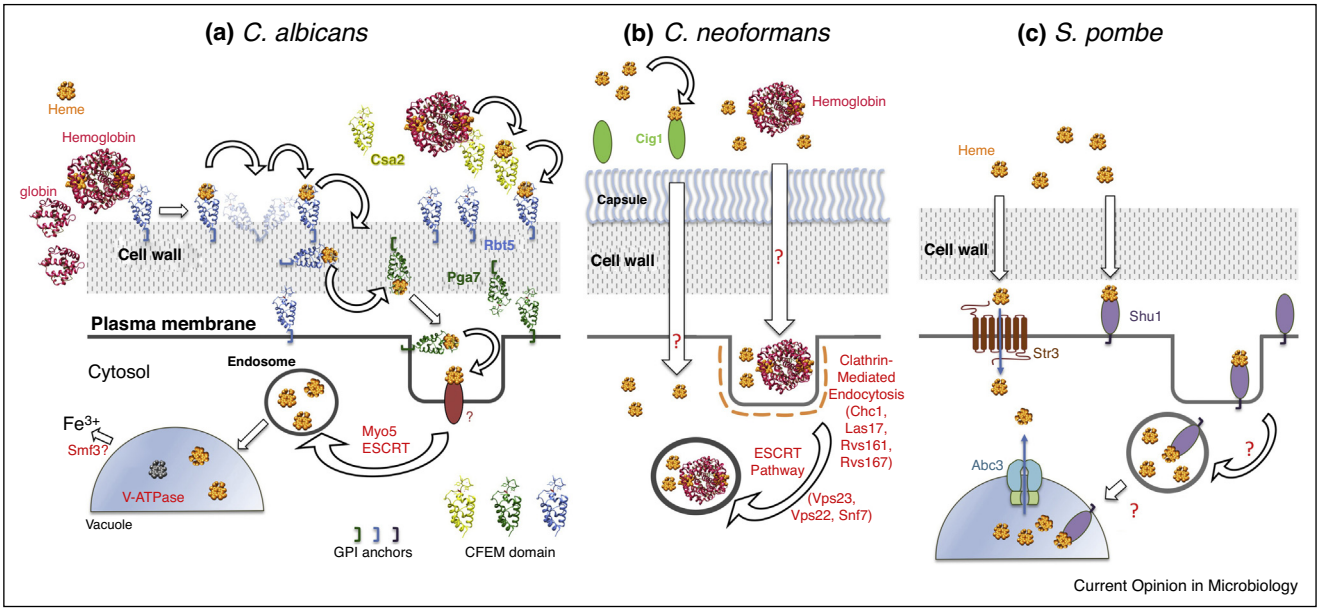
Beyond the extracellular CFEM protein cascade, *C. albicans* heme utilization depends on several specific ESCRT (endosomal sorting complex required for transport) proteins, as well as on Myo5, a type I myosin involved in endocytosis, and on an active vacuolar ATPase [28]. The scenario emerging from the genetic and biochemical data, together with the structural data described below, is that the heme is extracted extracellularly from hemoglobin, transferred across the cell wall by the CFEM protein cascade, delivered to the endocytic system at the plasma membrane, and transported to the endosome/vacuole compartment, where it is presumably degraded to release the iron (Figure 1a). A heme oxygenase homolog, Hmx1, was shown to be induced by high concentrations of free heme or hemoglobin, and to contribute to the utilization of free heme-iron and the catabolism of hemoglobin heme [29,30]. However, the Hmx1 homolog in *S. cerevisiae* is an endoplasmic reticulum membrane enzyme rather than a vacuolar enzyme, probably facing the cytosol rather than the lumen of the secretory pathway [36]. Furthermore, deletion of *C. albicans HMX1* does not appear to be required for hemoglobin-iron utilization at physiological concentrations (our unpublished results), suggesting that *C. albicans* may possess alternative heme-degrading enzymes in the vacuole.

The CFEM proteins

CFEM domain structure

Alignment of CFEM domain proteins reveals, in addition to the eight defining cysteine residues, an additional highly conserved proline before the first Cys, and a semi-conserved aspartic acid between the third and fourth Cys [33,37,38]. Structural analysis of the soluble hemophore Csa2 revealed that the CFEM domain adopts a novel 'Helical Basket' fold that has six α helices, of which the third helix is perpendicular to five antiparallel

Figure 1



Schematic depiction of the external heme uptake pathways in **(a)** *Candida albicans*, **(b)** *Cryptococcus neoformans*, and **(c)** *Schizosaccharomyces pombe*. See text for details.

helices (Figure 2a) [20••]. The helical basket fold is stabilized by four disulfide bonds formed by the eight cysteine residues (Figure 2a,b). The heme is bound to a flat platform on top of the CFEM domain, which includes a conserved aspartic acid residue (Asp80) that mediates heme-iron coordination. This portion of the CFEM domain is the most conserved among CFEM proteins that are related to Csa2 (Figure 2c). An N-terminal loop that folds on top of the heme (Figure 2a) is predicted to be flexible in the absence of heme [20••].

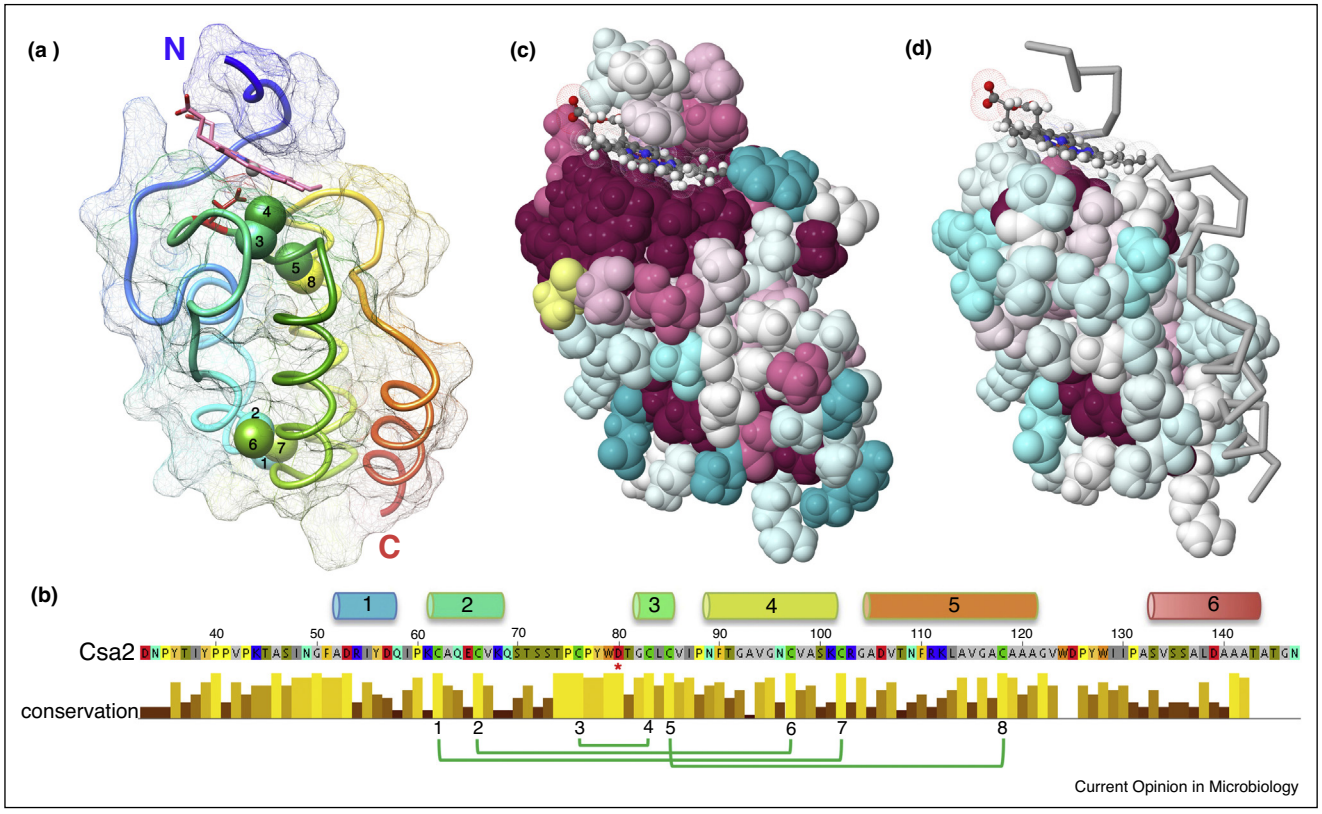
The aspartic acid-heme iron coordination in the holo-Csa2 structure is unique among the 650 different heme-proteins of known structure ([39] and www.rcsb.org). Substitution of this Asp with a canonical histidine as heme-iron ligand maintained heme binding and extraction from hemoglobin in Csa2, Rbt5, and Pga7, but these mutants were totally inactive *in vivo* [20••]. The distinctive Asp coordination confers redox sensitivity to heme binding by CFEM proteins: unlike the His-substituted mutants, which bind both ferrous (Fe^{2+}) and ferric (Fe^{3+}) heme, the wild-type proteins can only bind ferric heme [20••]. These observations raise the possibility that redox sensitivity is important for the heme transfer function of the CFEM proteins.

Transcriptional regulation of the CFEM genes in *C. albicans*

By microarray analysis, all five related CFEM protein genes (but not the sixth *C. albicans* CFEM gene, *SSR1*) are among the strongest-induced genes under iron limitation conditions, consistent with a role in iron acquisition [40,41]. CFEM protein genes, along with other iron acquisition genes, display another, less expected expression pattern, related to *C. albicans* morphogenesis. *C. albicans* is a dimorphic fungus, able to switch from a yeast to a hyphal morphology under specific environmental conditions, such as exposure to serum [42]. Interestingly, the five CFEM protein genes are also among the most consistently induced genes under various hyphal induction conditions [43,44] (Table 1). Since the CFEM proteins are not known to play a role in hyphal morphogenesis, one possibility is that this phenomenon represents an example of 'adaptive prediction' [45]. According to this theory, the usual temporal coincidence between two sets of signals, such as, for example, proximity to epithelial surfaces on the one hand, and free iron scarcity combined with heme-iron availability on the other hand, gets hard-wired into the microorganism's transcription program so that exposure to the first set of signals automatically elicits a transcriptional response typical to the second set of signals, even in their absence. This would enable anticipation of, and thus more rapid adaptation to, the changing iron source availability.

An alternative explanation for the induction of CFEM genes under hyphal induction conditions is that it could reflect an additional proposed role of the CFEM proteins, namely in biofilm formation [46,47], keeping in mind that hyphal morphogenesis and biofilm formation are intimately linked [48].

Figure 2



Structure and conservation of the CFEM domain. **(a)** Crystal structure of the Csa2 protein (PDB: 4Y7S). The α-carbon backbone chain of the protein is represented as a string, and the surface is shown as a mesh, whereas the heme is represented as a pink stick model. The portion of the structure below the heme consists of the CFEM domain, the portion above the heme is the N-terminal extension. The heme iron-coordinating Asp residue is shown in red. The CFEM cysteine sulfurs are shown as spheres, numbered sequentially as shown in **(b)**. (b) Representation of the primary structure of Csa2 with the location of the six α-helices indicated above the sequence. The relative conservation of each residue among 52 related proteins in 18 Saccharomycetales species is indicated below the sequence (alignment and representation using MAFFT [58]). The red asterisk indicates the heme iron-coordinating Asp residue. The numbers at the bottom indicate the eight CFEM cysteines, and the topology of their disulfide bond interactions is indicated by the green linkers. **(c)** The relative conservation of each residue among the 52 related Saccharomycetales CFEM proteins was mapped onto the Csa2 structure using CONSURF [59]. Red = most conserved, blue = least conserved, yellow = undetermined. The heme is shown as a ball-and-stick model. **(d)** The relative conservation of each residue among 786 representative fungal CFEM proteins was mapped onto the Csa2 structure as in (c). Note that the N-terminal extension and the 6th helix are depicted as backbone only, because they are missing in most CFEM proteins.

Table 1

Expression of CFEM protein genes under hyphal induction conditions and under iron starvation conditions

	Hyphal induction		Iron starvation	
Gene	log2 (fold change)	Rank	log2 (fold change)	Rank
RBT5	7.9	2	5.5	6
PGA7	6.6	6	3.6	31
CSA2	4.7	15	5.3	7
CSA1	3.0	67	6.0	4
RBT51	1.9	219	4.8	14
SSR1	<1	–	<1	–

The expression data for the hyphal induction conditions were extracted from Ref. [43]. They represent the average of eight induction conditions tested. The expression data for iron starvation were obtained from Ref. [41]. In both cases the rank order is from highest to lowest expression, out of a list of about 6000 *C. albicans* genes.

Are all CFEM proteins involved in heme acquisition?

While over 6000 CFEM protein sequences are listed in the Interpro database (www.ebi.ac.uk/interpro/), only a small minority were shown to be involved in heme-iron acquisition. Sequence conservation in a subset of 52 related CFEM proteins from Saccharomycetales, including the *C. albicans* and *C. parapsilosis* proteins shown or suspected to be involved in heme acquisition, were mapped onto the Csa2 structure (Figure 2c). This mapping shows that the heme-binding platform of the CFEM domain is clearly the most conserved part of the protein in this subset. In contrast, when conservation of over 700 representative CFEM protein sequences from throughout the fungal kingdom (RP15 subset from the Pfam CFEM proteome) is mapped onto the Csa2 structure, no distinct conserved domain is detectable beyond

the cysteines that define the CFEM consensus (Figure 2d), consistent with the assumption that the CFEM domain represents a common structural scaffold that can carry multiple alternative functions. In fact, some of the first characterized CFEM proteins have functions unrelated to heme acquisition [49]. Furthermore, analysis of the CFEM proteins in *Aspergillus fumigatus* [50] and in *Cryptococcus neoformans* [51•] did not detect any role in heme-iron utilization, and some CFEM proteins, such as Ccw14 from *S. cerevisiae*, are found in organisms that are not known to utilize heme as iron source. Ccw14 from *Candida glabrata* does however appear to have a role in cell wall structure [52], as do the *A. fumigatus* CFEM proteins [50].

Intriguingly, the *C. albicans* CFEM proteins involved in heme acquisition were also implicated in the maintenance of the cell wall structure and in the formation of biofilms [46,47]. The question whether the biofilm function of these CFEM proteins derives from their heme acquisition function, or whether it is distinct, has not been conclusively addressed yet. However, the fact that some of these CFEM proteins, such as Rbt5, can be very abundant [17,31•], combined with their mode of regulation (as described above) makes an independent cell wall structural role conceivable.

Heme iron acquisition in other fungi

Cryptococcus neoformans

C. neoformans is an environmental basidiomycete yeast that can infect immunocompromised and, more rarely, immunocompetent individuals. It is able to utilize hemin and hemoglobin as iron sources [51•,53]. Cig1, a secreted mannoprotein with possible heme-binding activity but lacking any discernible homology to the CFEM hemophores, contributes to optimal growth on hemin as iron source at physiological pH [18•]. Furthermore, similar to *C. albicans*, the ESCRT pathway is necessary for optimal hemin utilization [54]. This role is independent of the role of Cig1 in the pathway, because the double ESCRT – *cig1* mutants exhibit an additive heme utilization defect [54]. Lastly, hemin and hemoglobin utilization were recently shown to depend on the clathrin-mediated endocytosis pathway, as it is very defective in mutants of *CHC1* (clathrin heavy chain) and of *LAS17* (a homolog of the Wiscott–Aldrich Syndrome protein involved in clathrin-coated vesicle internalization), as well as of the N-BAR domain proteins involved in endocytosis, Rvs161 and Rvs167 [51•] (Figure 1b).

Schizosaccharomyces pombe

The fission yeast *S. pombe* is an environmental ascomycete that is able to efficiently import external hemin and utilize it as heme source, thanks to an iron starvation-induced plasma membrane heme receptor, Shu1, that mediates hemin internalization into the cell [19•]. Shu1 is a GPI-anchored protein, with limited sequence similarity to the CFEM proteins. Remarkably, upon exposure to hemin, Shu1, which lacks any detectable transmembrane domain, is internalized via an unknown mechanism into the vacuole, where it delivers its heme cargo [55•]. The imported heme is then transferred from the vacuole to the cytoplasm by a vacuolar transporter, Abc3, which mobilizes the heme via an inverted cysteine-proline motif [55•]. Interestingly, *S. pombe* contains an additional low-affinity plasma membrane heme transporter, Str3, which can mediate direct heme internalization into the cytosol in the absence of Shu1-Abc3 activity [56] (Figure 1c).

Paracoccidioides spp.

The dimorphic ascomycetes *Paracoccidioides brasiliensis* and *P. lutzii* are the etiological agents of paracoccidioidomycosis, a systemic fungal infection endemic to South America. These organisms, which can utilize hemoglobin, contain a GPI-anchored cell surface CFEM protein, PbRbt5, distantly related to the *Candida* spp. CFEM proteins [57]. PbRbt5 shows some evidence of hemin and hemoglobin binding; however, a knockdown of Pb*RBT5* did not affect hemoglobin utilization [57]. Alignment of the PbRbt5 sequence with the *C. albicans* CFEM protein sequences reveals the presence of the heme iron-coordinating Asp residue in the CFEM domain, but otherwise very little homology. Especially notable is the absence in PbRbt5 of the heme-interacting extension N-terminal to the CFEM domain. These observations raise the possibility that different CFEM proteins have evolved distinct modes of heme binding.

Conclusions

Fungi appear to have evolved at least three distinct pathways, identified in *C. albicans*, *C. neoformans*, and *S. pombe* respectively, for extracting and utilizing the ubiquitous heme molecule from the environment, underscoring the nutritional significance of this iron source. While significant progress has been made in our understanding of all three systems, the mechanisms are far from being completely elucidated. Some of the questions still outstanding are, for *C. neoformans*: how does Cig1 mediate delivery of heme to the cell? For *S. pombe*: how is Shu1 translocated from the plasma membrane to the vacuole in the presence of heme? For *C. albicans*: how is the heme transferred between CFEM proteins? What are the molecular mechanisms for delivery of the heme from the CFEM cascade to the plasma membrane, for internalization of the heme to the vacuole, and for extraction of the iron atom from hemoglobin heme?

The intricacies of the heme-iron acquisition pathways highlight the amount of resources that microorganisms must invest to acquire micronutrients. Given the importance of iron acquisition in the animal host, elucidation of these pathways might in addition enable, in the case of

pathogenic fungi, the development of novel classes of antifungal drugs.

Conflict of interest statement

Nothing declared.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Kosman DJ: **Iron metabolism in aerobes: managing ferric iron hydrolysis and ferrous iron autoxidation**. *Coord Chem Rev* 2013, **257**:210-217.

2. Graf E, Mahoneys JR, Bryant RG, Eaton JW: **Iron-catalyzed hydroxyl radical formation**. *J Biol Chem* 1984, **259**:3620-3624.

3. Aisen P, Listowsky I: **Iron transport and storage proteins**. *Annu Rev Biochem* 1980, **49**:357-393.

4. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, Akira S, Aderem A: **Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron**. *Nature* 2004, **432**:917-921.

5. Weinberg ED: **Nutritional immunity**. *JAMA* 1975, **231**:39.

6. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, Beaumont C, Kahn A, Vaulont S: **The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation**. *J Clin Invest* 2002, **110**:1037-1044.

7. Arezes J, Jung G, Gabayan V, Valore E, Ruchala P, Gulig PA, Ganz T, Nemeth E, Bulut Y: **Hepcidin-induced hypoferremia is a critical host defense mechanism against the siderophilic bacterium *Vibrio vulnificus***. *Cell Host Microbe* 2015, **17**:47-57.

8. Ganz T, Nemeth E: **Iron homeostasis in host defence and inflammation**. *Nat Rev Immunol* 2015, **15**:500-510.

9. Ponka P: **Cell biology of heme**. *Am J Med Sci* 1999, **318**:241-256.

10. Wandersman C, Delepelaire P: **Haemophore functions revisited**. *Mol Microbiol* 2012, **85**:618-631.

11. Cescau S, Cwerman H, Létoffé S, Delepelaire P, Wandersman C, Biville F: **Heme acquisition by hemophores**. *BioMetals* 2007, **20**:603-613.

12. Herzog P, Holtermüller K-H, Preiss J, Fischer J, Ewe K, Schreiber H-J, Berres M: **Fecal blood loss in patients with colonic polyps: a comparison of measurements with 51Chromium-labeled erythrocytes and with the haemoccult test**. *Gastroenterology* 1982, **83**:957-962.

13. Heinrich HC, Ičagić F: **Comparative studies on the "in vivo"-sensitivity of four commercial pseudoperoxidase-based faecal occult blood tests in relation to actual blood losses as calculated from measured whole body-^{59}Fe-elimination rates**. *Klin Wochenschr* 1980, **58**:1283-1297.

14. West AR, Oates PS: **Mechanisms of heme iron absorption: current questions and controversies**. *World J Gastroenterol* 2008, **14**:4101-4110.

15. Rao AU, Carta LK, Lesuisse E, Hamza I: **Lack of heme synthesis in a free-living eukaryote**. *Proc Natl Acad Sci U S A* 2005, **102**:4270-4275.

16. Choby JE, Skaar EP: **Heme synthesis and acquisition in bacterial pathogens**. *J Mol Biol* 2016, **428**:3408-3428.

17. Weissman Z, Kornitzer D: **A family of *Candida* cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization**. *Mol Microbiol* 2004, **53**:1209-1220.

18. • Cadieux B, Lian T, Hu G, Wang J, Biondo C, Teti G, Liu V, Murphy ME, Creagh AL, Kronstad JW: **The mannoprotein Cig1 supports iron acquisition from heme and virulence in the pathogenic fungus *Cryptococcus neoformans***. *J Infect Dis* 2013, **207**:1339-1347.
Identification of a putative hemophore involved in *C. neoformans* heme utilization.

19. • Mourer T, Jacques JF, Brault A, Bisaillon M, Labbe S: **Shu1 is a cell-surface protein involved in iron acquisition from heme in *Schizosaccharomyces pombe***. *J Biol Chem* 2015, **290**:10176-10190.
Identification of a new type of extracellular GPI-anchored heme receptor that mediates heme assimilation in *S. pombe.*

20. •• Nasser L, Weissman Z, Pinsky M, Amartely H, Dvir H, Kornitzer D: **Structural basis of haem-iron acquisition by fungal pathogens**. *Nat Microbiol* 2016, **1**:16156.
First crystal structure of a fungal hemophore. It reveals the structural basis of heme binding and transfer, including a unique and functionally important type of heme-iron coordination.

21. Nash AK, Auchtung TA, Wong MC, Smith DP, Gesell JR, Ross MC, Stewart CJ, Metcalf GA, Muzny DM, Gibbs RA *et al.*: **The gut mycobiome of the human microbiome project healthy cohort**. *Microbiome* 2017, **5**:153.

22. Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, Gillevet PM: **Characterization of the oral fungal microbiome (Mycobiome) in healthy individuals**. *PLoS Pathog* 2010, **6**:e1000713.

23. Bongomin F, Gago S, Oladele R, Denning D: **Global and multi-national prevalence of fungal diseases—estimate precision**. *J Fungi* 2017, **3**:57.

24. Ramanan N, Wang Y: **A high-affinity iron permease essential for *Candida albicans* virulence**. *Science* 2000, **288**:1062-1064.

25. Mamouei Z, Zeng G, Wang Y-M, Wang Y: ***Candida albicans* possess a highly versatile and dynamic high-affinity iron transport system important for its commensal-pathogenic lifestyle**. *Mol Microbiol* 2017, **106**:986-998.

26. Moors MA, Stull TL, Blank KJ, Buckley HR, Mosser DM: **A role for complement receptor-like molecules in iron acquisition by *Candida albicans***. *J Exp Med* 1992, **175**:1643-1651.

27. Weissman Z, Shemer R, Kornitzer D: **Deletion of the copper transporter Ca*CCC2* reveals two distinct pathways for iron acquisition in *Candida albicans***. *Mol Microbiol* 2002, **44**:1551-1560.

28. Weissman Z, Shemer R, Conibear E, Kornitzer D: **An endocytic mechanism for haemoglobin-iron acquisition in *Candida albicans***. *Mol Microbiol* 2008, **69**:201-217.

29. Santos R, Buisson N, Knight S, Dancis A, Camadro J-M, Lesuisse E: **Haemin uptake and use as an iron source by *Candida albicans*: role of Ca*HMX1*-encoded haem oxygenase**. *Microbiology* 2003, **149**:579-588.

30. Pendrak ML, Chao MP, Yan SS, Roberts DD: **Heme oxygenase in *Candida albicans* is regulated by hemoglobin and is necessary for metabolism of exogenous heme and hemoglobin to alpha-biliverdin**. *J Biol Chem* 2004, **279**:3426-3433.

31. • Kuznets G, Vigonsky E, Weissman Z, Lalli D, Gildor T, Kauffman SJ, Turano P, Becker J, Lewinson O, Kornitzer D: **A relay network of extracellular heme-binding proteins drives *C. albicans* iron acquisition from hemoglobin**. *PLoS Pathog* 2014, **10**:e1004407.
This paper shows that a family of extracellular hemophores are required for hemoglobin heme extraction and transport across the *C. albicans* cell envelope.

32. Ding C, Vidanes GM, Maguire SL, Guida A, Synnott JM, Andes DR, Butler G: **Conserved and divergent roles of Bcr1 and CFEM proteins in *Candida parapsilosis* and *Candida albicans***. *PLoS One* 2011, **6**:e28151.

33. Kulkarni RD, Kelkar HS, Dean RA: **An eight-cysteine-containing CFEM domain unique to a group of fungal membrane proteins**. *Trends Biochem Sci* 2003, **28**:118-121.

34. Amorim-Vaz S, Tran Vdu T, Pradervand S, Pagni M, Coste AT, Sanglard D: **RNA enrichment method for quantitative transcriptional analysis of pathogens in vivo applied to the fungus *Candida albicans***. *mBio* 2015, **6**:e00942-15.

35. Mochon AB, Jin Y, Kayala MA, Wingard JR, Clancy CJ, Nguyen MH, Felgner P, Baldi P, Liu H: **Serological profiling of a *Candida albicans* protein microarray reveals permanent host-pathogen interplay and stage-specific responses during candidemia**. *PLoS Pathog* 2010, **6**:e1000827.

36. Protchenko O, Philpott CC: **Regulation of intracellular heme levels by *HMX1*, a homologue of heme oxygenase, in *Saccharomyces cerevisiae***. *J Biol Chem* 2003, **278**:36582-36587.

37. Zhang ZN, Wu QY, Zhang GZ, Zhu YY, Murphy RW, Liu Z, Zou CG: **Systematic analyses reveal uniqueness and origin of the CFEM domain in fungi**. *Sci Rep* 2015, **5**:13032.

38. Dvir H, Kornitzer D: **CFEM protein Csa2**. *Encyclopedia of Inorganic and Bioinorganic Chemistry*. John Wiley & Sons, Ltd.; 2018:1-8.

39. Reedy CJ, Elvekrog MM, Gibney BR: **Development of a heme protein structure electrochemical function database**. *Nucleic Acids Res* 2007, **36**:D307-D313.

40. Lan CY, Rodarte G, Murillo LA, Jones T, Davis RW, Dungan J, Newport G, Agabian N: **Regulatory networks affected by iron availability in *Candida albicans***. *Mol Microbiol* 2004, **53**:1451-1469.

41. Chen C, Pande K, French SD, Tuch BB, Noble SM: **An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis**. *Cell Host Microbe* 2011, **10**:118-135.

42. Sudbery PE: **Growth of *Candida albicans* hyphae**. *Nat Rev Microbiol* 2011, **9**:737-748.

43. Azadmanesh J, Gowen AM, Creger PE, Schafer ND, Blankenship JR: **Filamentation involves two overlapping, but distinct, programs of filamentation in the pathogenic fungus *Candida albicans***. *G3 Genes Genomes Genet* 2017, **7**:3797-3808.

44. Braun BR, Head WS, Wang MX, Johnson AD: **Identification and characterization of TUP1-regulated genes in *Candida albicans***. *Genetics* 2000, **156**:31-44.

45. Brunke S, Hube B: **Adaptive prediction as a strategy in microbial infections**. *PLoS Pathog* 2014, **10**:e1004356.

46. Perez A, Ramage G, Blanes R, Murgui A, Casanova M, Martinez JP: **Some biological features of *Candida albicans* mutants for genes coding fungal proteins containing the CFEM domain**. *FEMS Yeast Res* 2011, **11**:273-284.

47. Srikantha T, Daniels KJ, Pujol C, Kim E, Soll DR: **Identification of genes upregulated by the transcription factor Bcr1 that are involved in impermeability, impenetrability, and drug resistance of *Candida albicans* a/α biofilms**. *Eukaryot Cell* 2013, **12**:875-888.

48. Nobile CJ, Johnson AD: ***Candida albicans* biofilms and human disease**. *Annu Rev Microbiol* 2015, **69**:71-92.

49. Kulkarni RD, Thon MR, Pan H, Dean RA: **Novel G-protein-coupled receptor-like proteins in the plant pathogenic fungus *Magnaporthe grisea***. *Genome Biol* 2005, **6**:R24.

50. Vaknin Y, Shadkchan Y, Levdansky E, Morozov M, Romano J, Osherov N: **The three *Aspergillus fumigatus* CFEM-domain GPI-anchored proteins (CfmA-C) affect cell-wall stability but do not play a role in fungal virulence**. *Fungal Genet Biol* 2014, **63**:55-64.

51. • Bairwa G, Caza M, Horianopoulos L, Hu G, Kronstad J: **Role of clathrin-mediated endocytosis in the use of heme and hemoglobin by the fungal pathogen *Cryptococcus neoformans***. *Cell Microbiol* 2019, **21**:e12961.
The authors use a toxic heme homolog to genetically identify genes required for *C. neoformans* heme uptake, among which are components of the clathrin-mediated endocytosis pathway.

52. Srivastava VK, Suneetha KJ, Kaur R: **A systematic analysis reveals an essential role for high-affinity iron uptake system, hemolysin and CFEM domain-containing protein in iron homeostasis and virulence in *Candida glabrata***. *Biochem J* 2014, **403**:103-114 http://dx.doi.org/10.1042/BJ20140598.

53. Jung WH, Sham A, Lian T, Singh A, Kosman DJ, Kronstad JW: **Iron source preference and regulation of iron uptake in *Cryptococcus neoformans***. *PLoS Pathog* 2008, **4**:e45.

54. Hu G, Caza M, Cadieux B, Bakkeren E, Do E, Jung WH, Kronstad JW: **The endosomal sorting complex required for transport machinery influences haem uptake and capsule elaboration in *Cryptococcus neoformans***. *Mol Microbiol* 2015, **96**:973-992.

55. • Mourer T, Normant V, Labbe S: **Heme assimilation in *Schizosaccharomyces pombe* requires cell-surface-anchored protein Shu1 and vacuolar transporter Abc3**. *J Biol Chem* 2017, **292**:4898-4912.
Demonstration that the *S. pombe* heme acquisition system involves the relocalization of the heme receptor from the plasma membrane to the vacuole and the transit of heme through the vacuole.

56. Normant V, Mourer T, Labbé S: **The major facilitator transporter Str3 is required for low-affinity heme acquisition in *Schizosaccharomyces pombe***. *J Biol Chem* 2018, **293**:6349-6362.

57. Bailao EF, Parente JA, Pigosso LL, Castro KP, Fonseca FL, Silva-Bailao MG, Bao SN, Bailao AM, Rodrigues ML, Hernandez O *et al.*: **Hemoglobin uptake by *Paracoccidioides* spp. is receptor-mediated**. *PLoS Negl Trop Dis* 2014, **8**:e2856.

58. Katoh K, Kuma K, Toh H, Miyata T: **MAFFT version 5: improvement in accuracy of multiple sequence alignment**. *Nucleic Acids Res* 2005, **33**:511-518.

59. Ashkenazy H, Abadi S, Martz E, Chay O, Mayrose I, Pupko T, Ben-Tal N: **ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules**. *Nucleic Acids Res* 2016, **44**:W344-W350.