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Connecting iron regulation and mitochondrial function in *Cryptococcus neoformans*

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Iron acquisition is essential for the proliferation of microorganisms, and human pathogens such as the fungus *Cryptococcus neoformans* must use sophisticated uptake mechanisms to overcome host iron sequestration. Iron is of particular interest for *C. neoformans* because its availability is an important cue for the elaboration of virulence factors. In fungi, extracellular iron is taken up through high affinity, low affinity, siderophore-mediated, and heme uptake pathways, and the details of these mechanisms are under active investigation in *C. neoformans*. Following uptake, iron is transported to intracellular organelles including mitochondria where it is used in heme biosynthesis and the synthesis of iron–sulfur (Fe–S) cluster precursors. One Fe–S cluster binding protein of note is the monothiol glutaredoxin Grx4 which has emerged as a master regulator of iron sensing in *C. neoformans* and other fungi through its influence on the expression of proteins for iron uptake or use. The activity of Grx4 likely occurs through interactions with Fe–S clusters and transcription factors known to control expression of the iron-related functions. Although the extent to which Grx4 controls the iron regulatory network is still being investigated in *C. neoformans*, it is remarkable that it also influences the expression of many genes encoding mitochondrial functions. Coupled with recent studies linking mitochondrial morphology and electron transport to virulence factor elaboration, there is an emerging appreciation of mitochondria as central players in cryptococcal disease.

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Introduction

Invasive fungal infections are an underappreciated threat to human health [1–3]. Fungal diseases are increasing in frequency, and there is a clear need for a detailed, mechanistic understanding of fungal pathogenesis to support improved diagnostics, the discovery and deployment of additional antifungal drugs, and the development of effective vaccines [1–3]. One of the most prevalent fungal diseases is caused by the basidiomycete yeast *Cryptococcus neoformans* [3,4,5••]. The bulk of cryptococcal disease occurs in immunocompromized individuals suffering from HIV/AIDS, and it is estimated that this fungus causes ~300 000 cases of meningoencephalitis per year, resulting in ~200 000 deaths globally [3,4,5••]. Cryptococcal meningitis is thought to be responsible for ~15% of all AIDS related deaths, with the greatest occurrence in Sub-Saharan Africa [3,4,5••].

Iron acquisition is a critical aspect of microbial pathogenesis and, as such, represents a potentially fruitful target for antifungal therapy. This is because fungi and other pathogens must overcome nutritional immunity (e.g. iron withholding) to proliferate and cause disease in mammalian hosts [6,7]. Additionally, pathogens interpret the availability of iron and other nutrients to regulate the deployment of virulence factors. In the case of *C. neoformans*, iron levels control elaboration of the polysaccharide capsule that makes a major contribution to the virulence of the fungus [8,9]. The characterization of iron acquisition functions and their potential as drug targets are being actively investigated for *C. neoformans* and many other pathogens [10–21]. For example, siderophores conjugated to antibiotics and other drugs show promise for the treatment of microbial infections [10–14]. Additionally, non-iron metallo-protoporphyrins, which are toxic analogs of heme, are inhibitory for bacterial and fungal pathogens, as are chelators and other molecules that interfere with iron acquisition [15–19]. Extracellular proteins for iron acquisition are also vaccine candidates as demonstrated for bacterial and fungal pathogens [20,21].

In this review, we summarize aspects of iron acquisition in *C. neoformans* to set the stage for a discussion of recent studies on connections between iron and mitochondria, as well as iron-related regulatory networks that control the expression of mitochondrial processes. We focus on mitochondria because they play a central role in iron homeostasis by containing abundant iron-dependent proteins (e.g. for respiration) and the machinery for two key iron-

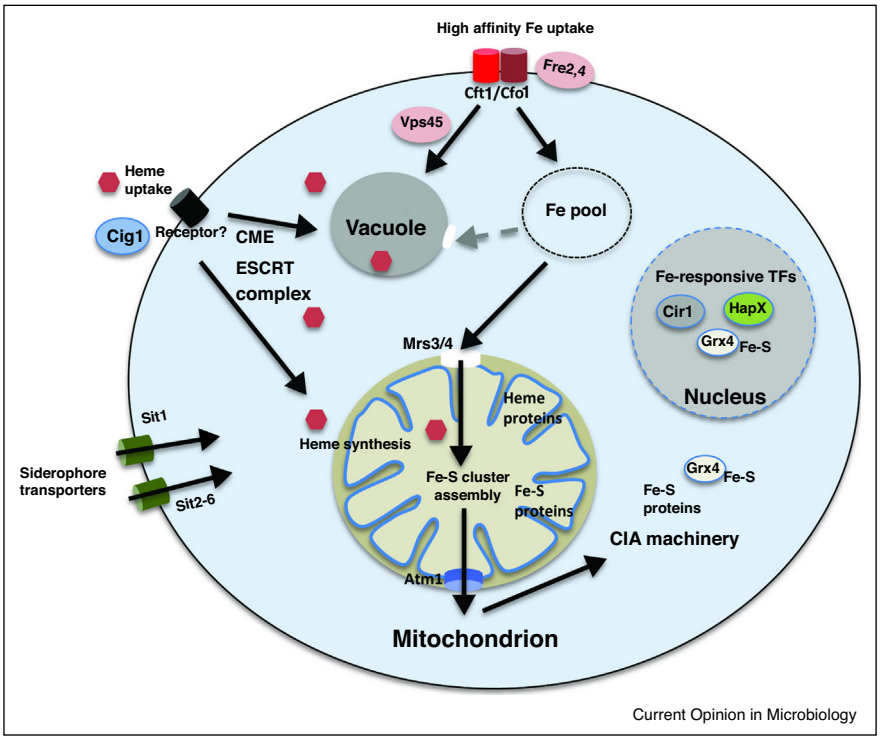
related processes: heme biosynthesis and the biogenesis of iron–sulfur (Fe–S) proteins [22,23]. We also highlight the emerging role of mitochondria in fungal virulence and note their importance as potential targets for antifungal therapy [24,25,26••,27••]. We limit our discussion to *C. neoformans*, but refer readers to a number of recent reviews that more generally consider metal uptake and regulation for fungal pathogens [9,28,29,30•,31•,32••].

An overview of mechanisms of iron acquisition in *C. neoformans* and connections to virulence

Fungi potentially acquire iron by four mechanisms: 1) low affinity transport of ferrous iron; 2) use of a ferroxidase-iron permease complex for high affinity uptake; 3) production and uptake of siderophores and; 4) acquisition of iron from heme and hemoglobin [9,28,29,30•,31•,32••]. The components for these processes in *C. neoformans* have been reviewed recently and are summarized in Figure 1 [9,28,29,30•]. In brief, physiological evidence indicates the presence of a low-affinity iron uptake system, but the underlying mechanisms and components have not yet been characterized [33]. High-affinity iron uptake involves the ferroxidase Cfo1 and the permease Cft1, and these proteins are required for acquisition from inorganic and host iron (e.g. transferrin) iron sources, as well as for virulence and proliferation in the central nervous system of mice [reviewed in Refs. 9,28,29,30•]. Interestingly, mice infected with *cfo1* or *cft1* mutants eventually succumb to disease suggesting the presence of additional uptake mechanisms [28,29,30•]. Ferric reductases believed to reduce iron at the cell surface also support subsequent iron uptake by the Cft1–Cfo1 high affinity system [28,29,30•].

C. neoformans is able to take up siderophores produced by other microbes, although the fungus is not known to synthesize its own siderophores. One highly conserved siderophore transporter, Sit1, participates in the uptake of ferrioxamine B, but does not contribute to virulence in a mouse model of cryptococcosis [28,29,30•]. Heme is also an important iron source for *C. neoformans* and a number of functions for uptake have been identified. Earlier work identified a contribution of the ESCRT pathway as well as a putative hemophore, Cig1, and studies in the last year revealed contributions to heme trafficking for clathrin

Figure 1



Mechanisms of iron acquisition and mitochondrial iron trafficking for *C. neoformans.*
Iron is taken up by a high affinity uptake system consisting of ferric reductases (e.g. Fre2 and Fre4), the iron permease Cft1 and the ferroxidase Cfo1 [9,28,29,30•]. *C. neoformans* also possesses six potential siderophore transporters (Sit1-6) and at least one system for heme uptake involving the candidate hemophore Cig1 and trafficking functions such as clathrin-mediated endocytosis (CME), endosomal sorting complex required for transport (ESCRT) and the regulator of vesicle fusion Vps45 [9,28,29,30•,34•,35•]. Iron transport and processing at the mitochondrion are illustrated with known components shown for import (Mrs3/4) and export (Atm1) [41,44•,45••]. The monothiol glutaredoxin Grx4 is also shown as a putative sensor of iron-sulfur (Fe–S) clusters and an interacting partner with transcription factors in the nucleus (Cir1 and HapX) [51••].

mediated endocytosis and the Sec1/Munc18 protein Vps45 that regulates vesicle trafficking and fusion [28,29,30•,34•,35•]. In general, loss of these functions results in attenuated virulence in mice [34•,35•].

As mentioned, iron is not only important for *C. neoformans* as a limiting nutrient during proliferation in the host, but its availability also influences capsule size [8,9]. This observation prompted a number of studies to identify regulatory factors that sense iron availability and ultimately control uptake, iron use, and virulence. The first factor identified was Cir1, a GATA transcription factor that not only regulates the expression of iron acquisition functions (e.g. *CFT1* and *CFO1*) but also the major virulence-associated traits of growth at 37°C, capsule formation, and production of the melanin pigment that contributes to defense against the host immune system [36]. Subsequently, components of the CCAAT complex including HapX were identified as regulators of iron acquisition and iron use [37]. Interestingly, HapX has regulatory interactions with Cir1 and with other transcription factors including Rim101, a regulator of the pH response, and Mig1, a regulator of mitochondrial functions [38,39,40•]. For example, HapX positively influences the expression of *RIM101*, and Rim101 is important for growth in low iron medium and the expression of iron uptake genes such as *CFO1*, *CFT1*, and *SIT1* [37–39]. A *rim101* mutant also has increased *HAPX* expression *in vivo* [39].

Mitochondrial processing of iron

Mitochondria are a major site of iron processing and use in cells [22,23]. Once in the cell, iron enters mitochondria through a conserved inner mitochondrial membrane transporter identified as Mrs3/4 in *Saccharomyces cerevisiae* [22,23], and also characterized in *C. neoformans* [41]. Within mitochondria, iron serves as a cofactor for a number of conserved proteins with critical functions in the electron transport chain, the TCA cycle, fatty acid oxidation, and lipoate and biotin biosynthesis [22,23]. However, before it can participate as a cofactor, imported ferric iron must be processed and incorporated into iron-containing compounds including heme and Fe–S clusters. The biogenesis of Fe–S clusters has been characterized in some detail in *S. cerevisiae*, and a subset of the core proteins is listed in Table 1 [22,23]. The Frr3 and Frr4 proteins thought to participate in Fe–S cluster biogenesis in *C. neoformans* were identified through complementation of mutants that displayed constitutive iron reduction [42]. *FRR3* turned out to encode an ortholog of the Isu1 and Isu2 scaffold proteins for Fe–S cluster assembly in *S. cerevisiae*, and *FRR4* encodes an ortholog of the iron binding protein, frataxin that is also required for Fe–S cluster biogenesis in yeast [22,23,42]. Another Fe–S scaffold protein in yeast, Nfu1, has an ortholog in *C. neoformans* that is required for iron, copper, and manganese homeostasis [43]. There are several other proteins involved in Fe–S cluster biogenesis in *S. cerevisiae* including the cysteine desulfurase (Nfs1), scaffold proteins (Isa1, Isa2), and chaperones (Ssq1, Jac1, Mge1; reviewed in Refs. [22,23]). Orthologs are predicted for many of these proteins in *C. neoformans*, although only a few have been studied to date (Table 1).

Fe–S cluster precursors can be exported via the mitochondrial ABC-transporter Atm1 and incorporated into cytosolic and nuclear proteins [22,23,44•,45••]. For *C. neoformans*, a common theme emerges when mutants lacking the machinery for mitochondrial iron import and Fe–S cluster precursor export are examined. The total iron content in the mutants is higher than levels in wild-type cells in both the *atm1Δ* and the *mrs3/4Δ* mutants [41,44•] suggesting that the inability to process iron in the mitochondria and return it to the cytosol in usable forms causes a dysregulation in iron uptake. This phenomenon is likely related to the role of Fe–S clusters in coordinating iron regulatory networks (see below). Importantly, recent work also implicated Atm1 in the

Table 1

Genes encoding mitochondrial iron uptake and Fe–S cluster biogenesis proteins in *S. cerevisiae*, and candidate orthologs in *C. neoformans*

S. cerevisiae	*C. neoformans* gene ID	Description	Reference
Nfs1	CNAG_01442[a]	Cysteine desulferase	–
Isu1/2	Frr3 (CNAG_04039)	Scaffold protein	[42]
Isa1	CNAG_02131[a]	Scaffold protein	–
Isa2	CNAG_00491[a]	Scaffold protein	–
Nfu1	Nfu1 (CNAG_3395)	Scaffold protein	[43]
Ssq1	CNAG_05199[a]	Hsp70-type chaperone	–
Jac1	CNAG_04288[a]	J-domain co-chaperone	–
Mge1	CNAG_01881[a]	Nucleotide exchange factor co-chaperone	–
Atm1	Atm1 (CNAG_04358)	Mitochondrial ABC-type transporter	[44•,45••]
Yfh1	Frr4 (CNAG_05011)	Frataxin	[42]
Mrs3/4	Mrs3 (CNAG_02522)[a]	Mitochondrial carrier family	[41]

[a] Predicted *C. neoformans* ortholog from strain H99 based on sequence similarity.

response of *C. neoformans* to copper toxicity [45••]. Specifically, copper induces the expression of *ATM1* by a mechanism that is dependent on the copper regulator Cuf1, and depletion of Atm1 results in enhanced sensitivity to copper stress and decreased fitness upon phagocytosis by macrophages [45••]. This work highlights the importance of Fe–S cluster-containing proteins and Fe–S protein biogenesis machinery in fungal susceptibility to the copper toxicity that is a component of mammalian defense [45••].

Proteins regulating iron homeostasis and mitochondrial function

Sensing Fe–S clusters is also a key aspect of the regulation of iron homeostasis in fungi, and detailed information on the participation of monothiol glutaredoxins is available for the model yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* (reviewed in Ref. [46]). Monothiol glutaredoxins with CGFS active sites are typically small Fe–S coordinating proteins which function in Fe–S cluster biogenesis and the regulation of transcription factor activity [46]. For example, monothiol glutaredoxins influence the transcriptional activators Aft1/Aft2 that normally induce transcription of the iron regulon in response to low iron conditions in *S. cerevisiae*. Aft1 and Aft2 are known to respond to Fe–S clusters [47], and specifically Fe–S clusters of mitochondrial origin [48], and the glutaredoxins Grx3 and Grx4 attenuate the activity of Aft1 and Aft2 [49]. *S*imilarly, a monothiol glutaredoxin, Grx4, is a key regulator of transcription factors involved in iron uptake and homeostasis in *S. pombe* [46,50••]. In particular, Grx4 binds to and influences the activity of Fep1, a transcriptional repressor of iron-uptake functions, and Php4, a repressor of iron-utilizing functions [46,50••].

Recent work also revealed that the monothiol glutaredoxin Grx4 is a central player in iron homeostasis and regulation in *C. neoformans* [51••]. The iron regulatory network in *C. neoformans* has similarities with that of *S. pombe*, although many of the details are still being uncovered and the extent of Grx4's influence has only recently emerged [51••]. In *C. neoformans*, one of the major iron-responsive transcription factors, Cir1, shares some sequence similarity to Fep1. However, Cir1 is a larger polypeptide (952 amino acids versus 564 for Fep1) and it appears to function both as a transcriptional repressor and activator based on transcript abundance of genes encoding iron uptake and other functions [36]. As mentioned above, Cir1 is also a major regulator of virulence factor elaboration including growth at host temperature, capsule formation and the deposition of melanin in the cell wall [36]. Interestingly, HapX in *C. neoformans* has partial sequence similarity to the iron-responsive transcription factor Php4 from *S. pombe*; again, however, HapX is a much larger polypeptide (718 amino acids versus 295 for Php4) [37]. A microarray study to compare the *hapXΔ* deletion mutant to the wild-type strain under low iron conditions also revealed HapX to be a transcriptional repressor of iron-uptake functions [37].

Not surprisingly, a *grx4* deletion mutant of *C. neoformans* showed similar influences on transcript abundance when compared with the *cir1Δ* and *hapxΔ* mutants [36,37,51••]. For example, there was increased expression of genes encoding iron transporters, heme biosynthesis proteins, Fe–S cluster containing proteins, and Fe–S cluster biogenesis proteins in the absence of Grx4 [51••]. Grx4 also physically interacts with Cir1 as detected by a yeast two-hybrid analysis, and it is speculated that the protein also interacts with HapX through Fe–S clusters [51••]. Interestingly, the *grx4Δ* deletion mutant is also hypersensitive to many agents that impact mitochondrial function including inducers of oxidative stress and inhibitors of electron transport chain complexes (many components of which contain Fe–S clusters) [51••]. The connections between mitochondria and iron through Grx4 (and Cir1 and HapX) are consistent with observations in *S. pombe*. However, Grx4 in *C. neoformans* appears to have an influence on the expression of a greater number of functions, perhaps through interactions with other transcription factors that may connect iron and mitochondrial function [51••]. For example, the transcript levels for the transcription factors Mig1 and Rim101 are both influenced by HapX and it is tempting to speculate that Grx4 contributes to the activity of HapX by providing a sensing mechanism for Fe–S clusters (and iron availability) [37,40•,51••]. HapX has a negative influence on *MIG1* expression while Mig1 has a positive influence on *HAPX* expression under low iron conditions [40•]. Furthermore, deletion of *MIG1* altered the transcript levels for proteins involved in the electron transport chain and TCA cycle, and increased sensitivity to oxidative stress, the antifungal drug fluconazole, and inhibitors of the electron transport chain [40•]. Interestingly, deletion of *MIG1* or *HAPX* individually did not impair survival in macrophages, but a double mutant had decreased survival [40•]. Similarly, the double mutant was only slightly less virulent that the wild-type strain upon inoculation in mice (and this was mostly due to the *hapXΔ* mutation), but the burden of the mutant was reduced in the kidney, lungs, liver, and spleen (but not the brain). Notably, HapX orthologs in other human fungal pathogens, including *Candida albicans* and *Aspergillus fumigatus*, are also iron responsive, associated with mitochondrial function, and important for virulence [52,53]. Overall, these results reinforce connections between iron-related transcription factors and mitochondrial function, but also suggest only minor roles in virulence for these factors.

Mitochondria and cryptococcal virulence

As highlighted by recent reviews, mitochondria are emerging as important contributors to the virulence of fungal pathogens, and as promising targets for antifungal therapy [24,25,26••,27••]. Recent studies emphasize these

connections for *C. neoformans* [35•,54•,55•]. For example, dynamin-related proteins (DRPs) that mediate mitochondrial fusion and fission have been characterized with regard to roles in morphology, resistance to oxidative and nitrosative stress, and virulence [54•]. In particular, mutants lacking the fusion DRP Fzo1 had a virulence defect in a mouse inhalation model in that cells were cleared from the lungs within a week of infection (in contrast to the proliferation observed for wild-type cells). In contrast, deletion of genes from mitochondrial fission (*MDV1*, *DNM1*, and *FIS1*) did not influence virulence in the assay for lung proliferation. The *fzo1Δ* mutants were able to proliferate in culture at 37°C and produce a wild-type level of capsule, but they had defects in producing the virulence factor melanin, and they were sensitive to oxidative and nitrosative stress and inhibitors of electron transport (rotenone and potassium cyanide). Interestingly, the mutants were also impaired in their ability to survive intracellularly in macrophages, and this phenotype could be rescued by scavengers of reactive oxygen species. Inhibitors for electron transport were also recently used to demonstrate a connection between mitochondrial function and elaboration of the polysaccharide capsule [55•]. Specifically, treatment with antimycin A, salicylhydroxamic acid, or rotenone impaired capsule growth, and enlargement of the capsule also correlated with an increase in mitochondrial membrane potential. These observations were used to suggest that capsule growth is a stress response and that additional studies are needed on the energetics of capsule formation. Finally, we note that recent work on the Sec1/Munc18 protein Vps45, as mentioned above, also plays an extensive role in iron/heme acquisition, is associated with mitochondria, and influences mitochondrial membrane permeability [35•]. A *vps45Δ* mutant also shows sensitivity to oxidative stress and inhibitors of the electron transport chain, and is impaired for virulence in mice and survival in macrophages thus providing another connection between iron, mitochondria, and virulence. The importance of mitochondria to virulence is underscored by the characterization of hypervirulent isolates of a related species *Cryptococcus gattii* which were found to have increased expression of mitochondrial-associated genes resulting in enhanced proliferation in phagocytes [56]. Furthermore, a subset of these displayed a tubular mitochondrial morphology after phagocytosis which helped promote the growth of neighboring cells [57]. Overall, these findings highlight mitochondria as key central players in virulence through studies on organellar morphology and on specific proteins contributing to trafficking and mitochondrial function.

Conclusions

Our understanding of the components and regulation of iron uptake pathways in *C. neoformans* is expanding and emerging themes from recent work highlight the importance of mitochondria as a target organelle for iron delivery and processing, and as contributors to the virulence of the fungus. In particular, mitochondria appear to be crucial for sensing that iron requirements have been met through the assembly and availability of Fe–S clusters, ultimately influencing the expression of iron uptake functions, and linking cellular energetics to virulence-related traits such as resistance to oxidative stress. We have focused on iron uptake and regulation, and connections with mitochondria in this review. However, it is important to highlight, as indicated is a number of excellent recent reviews that mitochondrial functions are also relevant as potential targets for antifungal drugs and as contributors to drug resistance [24,25,26••,27••]. Of course, mitochondria are complex organelles with a multitude of connections with key cellular processes. It will, therefore, be challenging but informative to further characterize the molecular connections between iron sensing, mitochondria and the regulation of virulence factors in *C. neoformans*. The potential pay off is that new opportunities may arise to target mitochondria for novel antifungal therapies.

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. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC: **Hidden killers: human fungal infections**. *Sci Transl Med* 2012, **4**:165rv.

2. Brown GD, Denning DW, Levitz SM: **Tackling human fungal infections**. *Science* 2012, **336**:647.

3. Limper AH, Adenis A, Le T, Harrison TS: **Fungal infections in HIV/AIDS**. *Lancet Infect Dis* 2017, **17**:e334-e343.

4. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM: **Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS**. *AIDS* 2009, **23**:525-530.

5. •• Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A, Boulware DR: **Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis**. *Lancet Infect Dis* 2017, **17**:873-881.

In a widely cited update of the global burden of HIV-associated cryptococcal meningitis, the authors estimate the prevalence of the cryptococcosis and reveal a substantial level of disease, particularly in sub-Saharan Africa. Remarkably, the analysis indicated that cryptococcal meningitis was responsible for 15% of AIDS-associated deaths worldwide.

6. Hood MI, Skaar EP: **Nutritional immunity: transition metals at the pathogen-host interface**. *Nat Rev Microbiol* 2012, **10**:525-537.

7. Palmer LD, Skaar EP: **Transition metals and virulence in bacteria**. *Annu Rev Genet* 2016, **50**:67-91.

8. Vartivarian SE, Anaissie EJ, Cowart RE, Sprigg HA, Tingler MJ, Jacobson ES: **Regulation of cryptococcal capsular polysaccharide by iron**. *J Infect Dis* 1993, **167**:186-190.

9. Kronstad JW, Hu G, Jung WH: **An encapsulation of iron homeostasis and virulence in *Cryptococcus neoformans***. *Trends Microbiol* 2013, **21**:457-465.

10. Lamb AL: **Breaking a pathogen's iron will: inhibiting siderophore production as an antimicrobial strategy**. *Biochim Biophys Acta* 2015, **1854**:1054-1070.

11. Balhara M, Chaudhary R, Ruhil S, Singh B, Dahiya N, Parmar VS, Jaiwal PK, Chhillar AK: **Siderophores; iron scavengers: the novel & promising targets for pathogen specific antifungal therapy**. *Expert Opin Ther Targets* 2016, **20**:1477-1489.

12. Foley TL, Simeonov A: **Targeting iron assimilation to develop new antibacterials**. *Expert Opin Drug Discov* 2012, **7**:831-847.

13. Möllmann U, Heinisch L, Bauernfeind A, Köhler T, Ankel-Fuchs D: **Siderophores as drug delivery agents: application of the "Trojan Horse" strategy**. *Biometals* 2009, **22**:615-624.

14. Bernier G, Girijavallabhan V, Murray A, Niyaz N, Ding P, Miller MJ, Malouin F: **Desketoneoenactin-siderophore conjugates for Candida: evidence of iron transport-dependent species selectivity**. *Antimicrob Agents Chemother* 2005, **49**:241-248.

15. Kelson AB, Carnevali M, Truong-Le V: **Gallium-based anti-infectives: targeting microbial iron-uptake mechanisms**. *Curr Opin Pharmacol* 2013, **13**:707-716.

16. Stojiljkovic I, Evavold BD, Kumar V: **Antimicrobial properties of porphyrins**. *Expert Opin Investig Drugs* 2001, **10**:309-320.

17. Newman SL, Gootee L, Stroobant V, van der Goot H, Boelaert JR: **Inhibition of growth of *Histoplasma capsulatum* yeast cells in human macrophages by the iron chelator VUF 8514 and comparison of VUF 8514 with deferoxamine**. *Antimicrob Agents Chemother* 1995, **39**:1824-1829.

18. Spellberg B, Ibrahim AS, Chin-Hong PV, Kontoyiannis DP, Morris MI, Perfect JR, Fredricks D, Brass EP: **The Deferasirox-AmBisome Therapy for Mucormycosis (DEFEAT Mucor) study: a randomized, double-blinded, placebo-controlled trial**. *J Antimicrob Chemother* 2012, **67**:715-722.

19. Harrison TS, Griffin GE, Levitz SM: **Conditional lethality of the diprotic weak bases chloroquine and quinacrine against *Cryptococcus neoformans***. *J Infect Dis* 2000, **182**:283-289.

20. Alteri CJ, Hagan EC, Sivick KE, Smith SN, Mobley HL: **Mucosal immunization with iron receptor antigens protects against urinary tract infection**. *PLoS Pathog* 2009, **5**:e1000586.

21. Brena S, Cabezas-Olcoz J, Moragues MD, Fernández de Larrinoa I, Domínguez A, Quindós G, Pontón J: **Fungicidal monoclonal antibody C7 interferes with iron acquisition in *Candida albicans***. *Antimicrob Agents Chemother* 2011, **55**:3156-3163.

22. Mühlenhoff U, Hoffmann B, Richter N, Rietzschel N, Spantgar F, Stehling O, Uzarska MA, Lill R: **Compartmentalization of iron between mitochondria and the cytosol and its regulation**. *Eur J Cell Biol* 2015, **94**:292-308.

23. Braymer JJ, Lill R: **Iron-sulfur cluster biogenesis and trafficking in mitochondria**. *J Biol Chem* 2017, **292**:12754-12763.

24. Shingu-Vazquez M, Traven A: **Mitochondria and fungal pathogenesis: drug tolerance, virulence, and potential for antifungal therapy**. *Eukaryot Cell* 2011, **10**:1376-1383.

25. Calderone R, Li D, Traven A: **System-level impact of mitochondria on fungal virulence: to metabolism and beyond**. *FEMS Yeast Res* 2015, **15**:1-11.

26. •• Verma S, Shakya VPS, Idnurm A: **Exploring and exploiting the connection between mitochondria and the virulence of human pathogenic fungi**. *Virulence* 2018, **9**:426-446.
This timely review provides a comprehensive survey of connections between mitochondria and virulence in fungal pathogens of humans. Key aspects include mitochondrial morphology, genetics, role in drug resistance and interactions with other organelles.

27. •• Li D, Calderone R: **Exploiting mitochondria as targets for the development of new antifungals**. *Virulence* 2017, **8**:159-168.
The authors present a compelling analysis and discussion of mitochondrial proteins in fungi that may serve as targets for antifungal drug discovery. Opportunities to target complexes I and III in the electron transport chain are highlighted.

28. Jung WH, Do E: **Iron acquisition in the human fungal pathogen *Cryptococcus neoformans***. *Curr Opin Microbiol* 2013, **16**:686-691.

29. Ding C, Festa RA, Sun TS, Wang ZY: **Iron and copper as virulence modulators in human fungal pathogens**. *Mol Microbiol* 2014, **93**:10-23.

30. • Bairwa G, Jung WH, Kronstad JW: **Iron acquisition in fungal pathogens of humans**. *Metallomics* 2017, **9**:215-227.
The authors summarize and compare mechanisms of iron acquisition by fungal pathogens of humans, and highlight opportunities to augment antifungal therapy by targeting iron uptake.

31. • Blatzer M, Latgé JP: **Metal-homeostasis in the pathobiology of the opportunistic human fungal pathogen *Aspergillus fumigatus***. *Curr Opin Microbiol* 2017, **40**:152-159.
Metal homeostasis has been well studied in the human pathogenic fungus *Aspergillus fumigatus* and this timely review summarizes key aspects for iron, copper, zinc, calcium, manganese, and magnesium. Importantly, the review highlights the detailed knowledge that has accumulated for siderophore biosynthesis and precursor supply involving various cellular compartments including mitochondria.

32. •• Gerwien F, Skrahina V, Kasper L, Hube B, Brunke S: **Metals in fungal virulence**. *FEMS Microbiol Rev* 2018, **42** http://dx.doi.org/10.1093/femsre/fux050.
This thorough review provides timely information on metal regulatory networks, acquisition functions and detoxification mechanisms in fungi with an emphasis on fungal pathogens.

33. Jacobson ES, Goodner AP, Nyhus KJ: **Ferrous iron uptake in *Cryptococcus neoformans***. *Infect Immun* 1998, **66**:4169-4175.

34. • 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* 2018, **6**:e12961.
The authors report a novel screen with non-iron metalloprotophoryin to identify potential heme uptake functions in *C. neoformans*. The work revealed a role for clathrin-mediated endocytosis in heme acquisition and in virulence.

35. • Caza M, Hu G, Nielson ED, Cho M, Jung WH, Kronstad JW: **The Sec1/Munc18 (SM) protein Vps45 is involved in iron uptake, mitochondrial function and virulence in the pathogenic fungus *Cryptococcus neoformans***. *PLoS Pathog* 2018, **14**:e1007220.
This report presents a detailed analysis of the role of Vps45, a regulator of vesicle trafficking and fusion, in iron acquisition. Importantly, the authors found that Vps45 connected iron acquisition to mitochondrial functions, cell wall integrity, susceptibility to antifungal drugs and virulence.

36. Jung WH, Sham A, White R, Kronstad JW: **Iron regulation of the major virulence factors in the AIDS-associated pathogen *Cryptococcus neoformans***. *PLoS Biol* 2006, **4**:e410.

37. Jung WH, Saikia S, Hu G, Wang J, Fung CK, D'Souza C, White R, Kronstad JW: **HapX positively and negatively regulates the transcriptional response to iron deprivation in *Cryptococcus neoformans***. *PLoS Pathog* 2010, **6**:e1001209.

38. O'Meara TR, Norton D, Price MS, Hay C, Clements MF, Nichols CB, Alspaugh JA: **Interaction of *Cryptococcus neoformans* Rim101 and protein kinase A regulates capsule**. *PLoS Pathog* 2010, **6**:e1000776.

39. O'Meara TR, Xu W, Selvig KM, O'Meara MJ, Mitchell AP, Alspaugh JA: **The *Cryptococcus neoformans* Rim101 transcription factor directly regulates genes required for adaptation to the host**. *Mol Cell Biol* 2014, **34**:673-684.

40. • Caza M, Hu G, Price M, Perfect JR, Kronstad JW: **The zinc finger protein mig1 regulates mitochondrial function and azole drug susceptibility in the pathogenic fungus Cryptococcus neoformans**. *mSphere* 2016, **1** pii: e00080-15.

This study provides a detailed analysis of the role of the Mig1 transcription factor and highlights the interaction of Mig1 with the HapX transcriptional regulator of functions that use iron in *C. neoformans.*

41. Nyhus KJ, Ozaki LS, Jacobson ES: **Role of mitochondrial carrier protein Mrs3/4 in iron acquisition and oxidative stress resistance of *Cryptococcus neoformans***. *Med Mycol* 2002, **40**:581-591.

42. Jacobson ES, Troy AJ, Nyhus KJ: **Mitochondrial functioning of constitutive iron uptake mutations in *Cryptococcus neoformans***. *Mycopathologia* 2005, **159**:1-6.

43. Kim J, Park M, Do E, Hee Jung W: **Mitochondrial protein Nfu1 influences homeostasis of essential metals in the human fungal pathogen *Cryptococcus neoformans***. *Mycobiology* 2014, **42**:427-431.

44. • Do E, Park S, Li M-H, Wang J-M, Ding C, Kronstad JW: **The mitochondrial ABC transporter Atm1 plays a role in iron metabolism and virulence in the human fungal pathogen *Cryptococcus neoformans***. *Med Mycol* 2018, **56**:458-468.
Characterization of a mutant lacking the *ATM1* gene in *C. neoformans* established an impact of the ortholog of the mitochondrial transporter of Fe–S precursor on iron metabolism and virulence.

45. •• Garcia-Santamarina S, Uzarska MA, Festa RA, Lill R, Thiele DJ: ***Cryptococcus neoformans* iron-sulfur protein biogenesis machinery is a novel layer of protection against Cu stress**. *mBio* 2017, **8**:e01742-17.
The authors investigated the mechanism of copper toxicity in the context of cryptococcal virulence. They discovered that Fe–S protein assembly machinery and Fe–S containing proteins are important targets of copper toxicity and that the expression of Atm1, a mitochondrial transporter of an Fe–S precursor, is induced by copper.

46. Li H, Outten CE: **Monothiol CGFS glutaredoxins and BolA-like proteins: [2Fe-2S] binding partners in iron homeostasis**. *Biochemistry* 2012, **51**:4377-4389.

47. Chen OS, Crisp RJ, Valachovic M, Bard M, Winge DR, Kaplan J: **Transcription of the yeast iron regulon does not respond directly to iron but rather to iron-sulfur cluster biosynthesis**. *J Biol Chem* 2004, **279**:29513-29518.

48. Rutherford JC, Ojeda L, Balk J, Mühlenhoff U, Lill R, Winge DR: **Activation of the iron regulon by the yeast Aft1/Aft2 transcription factors depends on mitochondrial but not cytosolic iron-sulfur protein biogenesis**. *J Biol Chem* 2005, **280**:10135-10140.

49. Ojeda L, Keller G, Muhlenhoff U, Rutherford JC, Lill R, Winge DR: **Role of glutaredoxin-3 and glutaredoxin-4 in the iron regulation of the Aft1 transcriptional activator in *Saccharomyces cerevisiae***. *J Biol Chem* 2006, **281**:17661-17669.

50. •• Dlouhy AC, Beaudoin J, Labbé S, Outten CE: ***Schizosaccharomyces pombe* Grx4 regulates the transcriptional repressor Php4 via [2Fe-2S] cluster binding**. *Metallomics* 2017, **9**:1096-1105.
This study presents elegant biochemical evidence for Fe–S binding by the monothiol glutaredoxin Grx4 in *Schizosaccharomyces pombe* and documents the Grx4 regulation of the transcription factor Php4 via bridging of an Fe–S cluster.

51. •• Attarian R, Hu G, Sánchez-León E, Caza M, Croll D, Do E, Bach H, Missall T, Lodge J, Jung WH, Kronstad JW: **The monothiol glutaredoxin Grx4 regulates iron homeostasis and virulence in *Cryptococcus neoformans***. *mBio* 2018, **9** pii: e02377-18.
The authors identified the monothiol glutaredoxin Grx4 in *C. neoformans* and established that the protein interacts with the transcription factor Cir1, a major regulator of iron uptake and virulence factor expression. Grx4 is required for virulence, and it regulates a large number of iron-related genes including many encoding mitochondrial functions.

52. Hsu PC, Yang CY, Lan CY: ***Candida albicans* Hap43 is a repressor induced under low-iron conditions and is essential for iron-responsive transcriptional regulation and virulence**. *Eukaryot Cell* 2011, **10**:207-225.

53. Schrettl M, Beckmann N, Varga J, Heinekamp T, Jacobsen ID, Jöchl C, Moussa TA, Wang S, Gsaller F, Blatzer M *et al.*: **HapX-mediated adaption to iron starvation is crucial for virulence of *Aspergillus fumigatus***. *PLoS Pathog* 2010, **6**:e1001124.

54. • Chang AL, Doering TL: **Maintenance of mitochondrial morphology in *Cryptococcus neoformans* is critical for stress resistance and virulence**. *mbio* 2018, **9** pii: e01375-18.
This study uncovers a role for the dynamin-related protein Fzo1 in virulence via an influence on the production of the virulence factor melanin at host temperature and the response to oxidative stress during interactions with phagocytes.

55. • Trevijano-Contador N, Rossi SA, Alves E, Landín-Ferreiroa S, Zaragoza O: **Capsule enlargement in *Cryptococcus neoformans* is dependent on mitochondrial activity**. *Front Microbiol* 2017, **8**:1423.
The authors uncovered a role for the electron transport chain in capsule elaboration by treating cells with the inhibitors antimycin A, salicylhydroxamic acid and rotenone.

56. Ma H, Hagen F, Stekel DJ, Johnston SA, Sionov E, Falk R, Polacheck I, Boekhout T, May RC: **The fatal fungal outbreak on Vancouver Island is characterized by enhanced intracellular parasitism driven by mitochondrial regulation**. *Proc Natl Acad Sci U S A* 2009, **106**:12980-12985.

57. Voelz K, Johnston SA, Smith LM, Hall RA, Idnurm A, May RC: **'Division of labour' in response to host oxidative burst drives a fatal *Cryptococcus gattii* outbreak**. *Nat Commun* 2014, **5**:5194.