The Mannoprotein Cig1 Supports Iron Acquisition From Heme and Virulence in the Pathogenic Fungus Cryptococcus neoformans

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Iron acquisition is critical for virulence of the human pathogenic fungus Cryptococcus neoformans. The cryptococcal transcript for the extracellular mannoprotein Cig1 is highly regulated by iron and abundant in iron-starved cells, suggesting a role in iron acquisition. Indeed, loss of Cig1 resulted in delayed growth on heme at physiological pH. Expression of CIG1 is regulated by the pH-responsive transcription factor Rim101, and loss of Rim101 also impaired growth on heme. A cig1Δ mutant was less susceptible than the wild-type strain to noniron metalloporphyrins, further indicating a role for Cig1 in heme uptake. Recombinant Cig1 exhibited the absorbance spectrum of a heme-binding protein upon heme titration, and Cig1 may therefore function as a hemophore at the cell surface. Cig1 contributed to virulence in a mouse model of cryptococcosis but only in a mutant that also lacked the high-affinity iron uptake system. Overall, Cig1-mediated heme uptake is a potential therapeutic target in C. neoformans.

Keywords. Cryptococcus neoformans; extracellular mannoprotein; heme uptake; fungal pathogenesis; Rim101; pH; fungal hemophore.

The pathogenic fungus Cryptococcus neoformans is the most common cause of fungal meningoencephalitis, especially in immunocompromised individuals, with a worldwide burden of disease estimated at 1 million cases per year, resulting in approximately 625,000 deaths [1,2]. Iron availability is an important aspect of cryptococcal disease because the metal influences both growth and elaboration of major virulence factors, such as the polysaccharide capsule [3]. C. neoformans has evolved multiple strategies to acquire iron, including reductive, high-affinity uptake and the use of xenosiderophores [4, 5]. C. neoformans also uses hemoglobin as a source of iron [6,7]. Heme is an abundant source of iron for many bacterial pathogens, as well as for some fungi (eg, Histoplasma capsulatum and Candida albicans), that infect humans. Bacteria use heme transporters and receptors, as well as extracellular heme-binding proteins (hemophores) [6,7]. In H. capsulatum, heme uptake is mediated by unidentified cell surface receptors, while 2 heme-binding proteins, Rbt5 and Rbt51, are receptors for heme and hemoglobin in C. albicans [8–10]. Previous work to identify iron uptake systems in C. neoformans characterized the transcriptome upon growth in different iron sources and identified robust iron regulation of the transcript of the CIG1 gene [3, 11–13]. The CIG1 transcript is highly abundant in iron-starved cells and encodes a secreted mannoprotein [14,15]. CIG1 transcription is also highly regulated by...
GAL7p::PKA1 Regulated BL-21 strain expressing recombinant Cig1::GST E. coli
GAL7p::CIG1 in H99 Regulated LIM alone or supplemented with 10 µM hemin (Sigma), FeCl3
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starvation, the cells were harvested, washed, and inoculated in LIM alone or supplemented with 10 µM hemin (Sigma), FeCl3
(Sigma), or ferrioxamine (Sigma) to a final concentration of 5 \times 10^5 cells/mL. Cultures were incubated at 30°C, and growth
was monitored by measuring the optical density at 600 nm,
using a DU530 Life Science UV/Vis spectrophotometer (Beckman Instruments).

Quantitative Real-Time Polymerase Chain Reaction
To examine gene expression, cells were grown in LIM with or
without 10 µM heme for 6 hours at 30°C. Total RNA was
extracted, and complementary DNA was synthesized as de-
scribed elsewhere [13]. The relative gene expression was quan-
tified using the 7500 system (Applied Biosystems) on the basis of the 2^{−ΔΔCT} method [20]. Primers were designed using
Primer Express software 3.0 (Applied Biosystems). The control
18S ribosomal RNA was used for normalization. Statistical
analysis was performed by the Student t test.

Uptake of Noniron Metalloporphyrins (MPs)
Cells were grown overnight in YPD (ie, without starvation for
iron), washed, and serially diluted in low-iron water. YPD or
LIM plates with or without 10 µM heme or 100 µM FeCl3
were spread with 200 µL of gallium-protoporphyrin (Ga-
PPIX; Frontier Scientific), manganese-protoporphyrin (Mn-
PPIX; Frontier Scientific), or GaCl3 (Sigma) prior to spotting
the diluted cells. The plates were incubated for 2 days at 30°C.

Absorption Spectrophotometry
Heme groups have characteristic absorbance bands that
depend on the ligation and conformational state of the chro-
morphore, allowing detection of heme-binding proteins. Titra-
tions of purified recombinant Cig1–glutathione S-transferase
(GST) protein with heme were monitored by absorption spec-
troscopy, as previously described [21]. Briefly, increasing
amounts of heme dissolved in 0.1 M NaOH and diluted in 50
mM Tris and 100 mM NaCl buffer (pH 7.5) were added to 1
mL of 5 µM Cig1-GST in buffer. The preparation was incubat-
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before measuring the absorbance spectra. Heme added to
buffer without protein served as a reference. Spectra were mea-
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(Agilent Technologies) with an optical path length of 1 cm in
a quartz cell. Details about purification of the Cig1-GST
protein, gel electrophoresis, Western blot analysis, and isother-
mal titration calorimetry (ITC) experiments are provided in
the Supplementary Materials.

Virulence Assays
The virulence of cryptococcal strains was examined using
female A/Jcr mice (4–6 weeks old) from the Jackson

 MATERIALS AND METHODS

Strains and Growth Assays
Strains and primers used in the study are listed in Table 1 and
Supplementary Table 1, respectively. Details about in silico
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Table 1. Strains Used in This Study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Source</th>
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<tbody>
<tr>
<td>H99</td>
<td>C. neoformans wild-type strain</td>
<td>J. Heitman</td>
</tr>
<tr>
<td>cig1Δ</td>
<td>cig1 deletion mutant</td>
<td>Present study</td>
</tr>
<tr>
<td>cig1Δ + CIG1</td>
<td>CIG1 complemented strain</td>
<td>Present study</td>
</tr>
<tr>
<td>cfo1Δ</td>
<td>cfo1 deletion mutant</td>
<td>[17]</td>
</tr>
<tr>
<td>cig1Δ cfo1Δ</td>
<td>cig1 cfo1 double deletion mutant</td>
<td>Present study</td>
</tr>
<tr>
<td>cig1Δ cfo1Δ + CIG1</td>
<td>CIG1 complemented in cig1 cfo1 double deletion mutant</td>
<td>Present study</td>
</tr>
<tr>
<td>rim101Δ</td>
<td>rim101 deletion mutant</td>
<td>[16]</td>
</tr>
<tr>
<td>rim101Δ + RIM101</td>
<td>RIM101 complemented strain</td>
<td>[16]</td>
</tr>
<tr>
<td>GAL7p::CIG1 in rim101Δ</td>
<td>Regulated CIG1 in rim101 deletion mutant background</td>
<td>Present study</td>
</tr>
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<td>GAL7p::CIG1 in H99</td>
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</tr>
<tr>
<td>Cig1::GST</td>
<td>E. coli BL-21 strain expressing recombinant GST-tagged Cig1</td>
<td>Present study</td>
</tr>
<tr>
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<td>[18]</td>
</tr>
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Abbreviations: C. neoformans, Cryptococcus neoformans; E. coli, Escherichia coli; GST, glutathione S-transferase.
Laboratory (Sacramento, CA). The iron-starved fungal cells were washed and resuspended in phosphate-buffered saline (PBS; Invitrogen, Canada). The mice were anesthetized intraperitoneally with ketamine (80 mg/kg of body weight) and xylazine (5.5 mg/kg) in PBS and were inoculated intranasally with 50 μL of the cell suspensions (5 × 10⁴ cells). The health of the mice was monitored daily, after inoculation. Statistical analysis of survival differences was performed by log-rank tests. The fungal loads in the lungs and brains of 3 mice from each group were determined as previously described [11]. Statistical analysis was performed using an unpaired, 2-tailed t test. The protocol for the virulence assays (protocol A08-0586) was approved by the University of British Columbia Committee on Animal Care.

RESULTS

Cig1 Is Required for Iron Acquisition From Heme
We initially examined the role of Cig1 in iron use by constructing a cig1Δ mutant and a complemented strain containing a reintroduced copy of CIG1 (cig1Δ::CIG1). We then compared the growth of these strains and the wild-type parent in various iron sources. Iron-depleted medium did not support the growth of any of the strains, and the cig1Δ mutant grew as well as the wild-type and cig1Δ::CIG1 strains in FeCl₃ and ferrioxamine (Figure 1A–C). However, when grown in heme, the cig1Δ mutant demonstrated a longer lag phase (approximately 60 hours), compared with the wild-type and cig1Δ::CIG1 strains (approximately 36 hours; Figure 1D). The cig1Δ mutant eventually initiated exponential growth and achieved a similar cell density at stationary phase. These results indicate that Cig1 is required for efficient use of heme iron but has no apparent role for other iron sources under the conditions tested. The eventual growth of the mutant also suggests that C. neoformans has an additional mechanism(s) to adapt to heme in the absence of Cig1.

High-Affinity Iron Uptake Does Not Contribute to the Cig1-Dependent Use of Heme
C. neoformans uses a high-affinity system to acquire iron from inorganic sources and transferrin [11, 17]. To determine whether this system contributes to Cig1-dependent growth on heme, we used a cfo1Δ mutant lacking the ferroxidase for high-affinity uptake [17] and a cig1Δ cfo1Δ double mutant. The cfo1Δ mutant did not grow in FeCl₃ but grew as well as the wild-type strain in heme (Figures 1B and 1D), confirming that reductive high-affinity uptake was not involved in heme iron acquisition [17]. The cig1Δ cfo1Δ mutant showed a similar growth defect as the cig1Δ mutant in heme. The wild-type strain’s level of growth on heme was restored when CIG1 was reintroduced into the cig1Δ cfo1Δ strain, confirming that the phenotype was due to loss of CIG1 and not CFO1 (Figure 1D). Together, these results indicate that high-affinity iron uptake does not contribute to the role of Cig1 in growth on heme.

Cig1 Is Only Required for Growth in Heme at Physiological pH
To examine the role of Cig1 in more detail, we tested the growth of the strains in the presence of heme at different pH because iron is more available under acidic conditions [22]. Although the cig1Δ and cig1Δ cfo1Δ mutants exhibited a growth defect at pH 7.2, the strains grew as well as the wild-type strain at pH 5.6 (Figures 1D and 1E). We also examined the transcript levels of CIG1 in cells grown at acidic and physiological pH. The expression of CIG1 was downregulated at pH 5.6, compared with pH 7.2, independent of heme (Figure 1F). These results suggest that Cig1 plays an active role in iron acquisition from heme at physiological pH and that a Cig1-independent system exists to acquire iron from heme at low pH.

Rim101 Also Contributes to Iron Acquisition From Heme
Given that the pH influenced both the expression of CIG1 and the contribution of Cig1 to growth on heme, we next examined the contribution of Rim101, a conserved master regulator of the pH response in fungi [23, 24]. Rim101 positively regulated the expression of CIG1, and a rim101Δ mutant had a growth defect at alkaline pH [16]. Analysis of growth on heme revealed that the rim101Δ mutant showed an extended lag phase and eventual growth to the wild-type cell density, a phenotype similar to that of the cig1Δ mutant (Figure 2A). Complementation of the rim101Δ mutation restored the wild-type level of growth on heme.

We also examined the contribution of Rim101 to growth on heme independently of CIG1 by replacing the promoter of CIG1 with that of the GAL7 gene (galactose inducible and glucose repressible) in the wild-type and rim101Δ strains. As expected, GAL7p::CIG1 in the wild-type strain exhibited a similar growth defect to cig1Δ on heme and glucose (Figure 2A) but a wild-type growth pattern in galactose (Figure 2B). In contrast, GAL7p::CIG1 in rim101Δ remained in lag phase significantly longer than either rim101Δ or cig1Δ when grown in glucose (Figure 2A). This additive growth defect suggests that Rim101 plays a role in iron acquisition from heme that is independent of its regulation of CIG1. Surprisingly, growth in galactose extended the lag phase of the wild-type and cig1Δ::CIG1 strains and particularly exacerbated the growth defect of the cig1Δ mutant (Figure 2B). The use of galactose as the carbon source also eliminated the growth defect on heme for both the rim101Δ and GAL7p::CIG1 rim101Δ strains relative to the wild-type strain. These results further support the presence of a Cig1-independent mechanism for heme use and suggest an influence of carbon source; that is, galactose may promote the expression of a system to...
use heme iron that compensates for the loss of Rim101. Examination of CIG1 transcript levels confirmed the expected carbon source regulation of the GAL7p::CIG1 construct and also revealed that growth in galactose reduced expression of CIG1 (Figure 2C). This may explain the extended lag phase in the wild-type and complemented strains on galactose and heme.

Loss of Cig1 Reduces Susceptibility to Noniron MPs

We next used noniron MPs (toxic heme analogs) to investigate the role of Cig1 in heme uptake. Noniron MPs such as Ga-PPIX and Mn-PPIX share structural similarities with heme and therefore gain entry into cells via heme uptake pathways, as shown in bacteria [25]. Noniron MPs may stimulate production of reactive oxygen radicals leading to toxicity and displace heme in essential metabolic functions [25]. The growth of the strains was compared in the presence of Ga-PPIX or Mn-PPIX. When grown on YPD or LIM with added FeCl₃, the wild-type, cig1Δ, rim101Δ, and complemented strains all grew well in the absence or presence of Ga-PPIX, indicating that the heme uptake system was not essential in these conditions (Figure 3). Interestingly, the cfo1Δ mutant did not grow on YPD in the presence of Ga-PPIX or Mn-PPIX. When grown on YPD or LIM with added FeCl₃, the wild-type, cig1Δ, rim101Δ, and complemented strains all grew well in the absence of Ga-PPIX, indicating that the heme uptake system was not essential in these conditions (Figure 3). Interestingly, the cfo1Δ mutant did not grow on YPD in the presence of Ga-PPIX, whereas the cig1Δ cfo1Δ mutant grew well, presumably because heme uptake systems are upregulated in absence of the high-affinity iron uptake system. Reintroduction of CIG1 into the double mutant also resulted in susceptibility to Ga-PPIX, although not to the level of that for the cfo1Δ mutant (possibly because

Figure 1. Cig1 is required for growth in heme at physiological pH. Growth curves of iron-starved cells in low-iron medium (LIM; A); LIM plus 10 µM FeCl₃ (B); LIM plus 10 µM ferrioxamine (C); LIM plus 10 µM heme, pH 7.2 (D); and LIM plus 10 µM heme, pH 5.6 (E). Growth was monitored by measuring the optical density at 600 nm. F. Expression of CIG1 in wild-type (WT) cells grown in LIM, with or without 10 µM heme, at pH 5.6 or 7.2. All experiments were repeated 3 times, and the data are plotted as average ± SD. Statistical analysis was performed using the Student t test. *P < .05.
of partial complementation). Additionally, the \(\text{cfo1}\Delta\) mutants did not grow well on FeCl\(_3\), whether Ga-PPIX was absent or present. When heme was provided as the iron source, the wild-type, \(\text{cfo1}\Delta\), and complemented strains were unable to grow in the presence of Ga-PPIX, whereas the \(\text{cig1}\Delta\) and \(\text{rim101}\Delta\) mutants grew well. The toxicity of Ga-PPIX for the wild-type strain was eliminated by adding excess heme (Supplementary Figure 2A). Interestingly, the \(\text{cig1}\Delta\text{cfo1}\Delta\) mutant had difficulty growing on heme plates in the presence or absence of Ga-PPIX; this phenotype is likely due to a contribution of Cfo1 to an additional mechanism of heme use and/or to high-affinity acquisition of any free iron contaminating the heme. Growth in the presence of Mn-PPIX also yielded similar results to Ga-PPIX, although a 10-fold higher concentration of Mn-PPIX was required. Finally, all strains grew similarly well in the presence or absence of GaCl\(_3\), thus supporting the idea that toxicity was due to uptake of Ga-PPIX rather than gallium. Taken together, these results suggest that Cig1 is involved in the uptake of noniron MPs, resulting in toxicity for the cells, and that Cig1 also plays a role in heme uptake.

**Recombinant Cig1 Binds to Heme**

The Cig1 polypeptide contains a putative signal peptide but no GPI anchor, and Biondo et al identified Cig1 in culture supernatant [14]. The extracellular localization and role in heme iron acquisition suggest that Cig1 could directly interact with heme as a receptor or hemophore [26]. We therefore examined the ability of Cig1 to bind heme, using recombinant Cig1-GST protein purified from *Escherichia coli*. Titration of Cig1-GST with heme revealed a distinct absorption spectrum defined by a maximum Soret peak at 407 nm, as well as \(\beta\) and \(\alpha\) peaks at 570 nm and 600 nm, respectively, indicative of a heme-binding protein (Figure 4A). Although some Cig1-GST degradation products were detected after purification, heme binding was not observed with GST alone (Supplementary Figure 2B and 2C). Heme-binding saturation was not detected for Cig1, even when a 10-molar ratio excess of heme was mixed with the protein (Figure 4B). Therefore, the complexity of the interaction between Cig1 and heme did not allow a dissociation constant \(K_d\) to be determined. Similarly, when heme was added to Cig1-GST in an ITC assay, weak binding was observed, but saturation was not detected, thus preventing determination of a \(K_d\) (Supplementary Figure 2D). Overall, the data suggest that Cig1 is a heme-binding protein, although the interaction appears to be weak or transient.

**Loss of Cig1 Attenuates Virulence**

Virulence was examined in a mouse inhalation model of cryptococcosis, and the \(\text{cig1}\Delta\) mutant was found to be as virulent as the wild-type strain; that is, 100% of the mice exhibited symptoms of cryptococcosis by day 23 after infection (Figure 5A). We hypothesize that disease was observed because the \(\text{cig1}\Delta\) mutant may retain the ability to use other...
iron sources besides heme (eg, transferrin) during growth in mice and that *C. neoformans* has multiple mechanisms of heme use. As previously reported, the *cfo1Δ* mutant was attenuated for virulence but still caused disease in mice [17]. Interestingly, the *cig1Δcfo1Δ* mutant was more severely attenuated for virulence than the *cfo1Δ* mutant, with 2 of the 10 mice not showing symptoms by 60 days after inoculation (Figure 5A). Reintroduction of *CIG1* into the double mutant restored virulence, thus supporting a contribution of Cig1, although the severity of disease was slightly less than that caused by *cfo1Δ* (possibly because of partial complementation). Overall, these results indicate that Cig1 contributes to virulence but that its role is masked by additional iron uptake systems. An analysis of the distribution of fungal cells in the lungs and brains of infected mice indicated no difference between the wild-type and *cig1Δ* strains (Figure 5B and 5C).

**DISCUSSION**

We have identified a role for the extracellular mannoprotein Cig1 in a novel heme uptake mechanism, as demonstrated by the delayed growth of the *cig1Δ* mutant on heme, the reduced susceptibility of the mutant to noniron MPs that require a heme uptake system for toxicity, and heme binding by recombinant Cig1-GST. The activity of Cig1 is interesting in the context of heme use by pathogenic bacteria. Heme uptake in these pathogens involves cell surface receptors that bind heme with high affinity. Some bacteria also secrete heme-binding proteins (hemophores) to scavenge free heme or to extract heme from host protein and return it to cell-surface receptors [26, 27]. Heme is then either transferred between binding domains of multiple surface proteins to a membrane transporter for internalization, in the case of gram-positive bacteria, or transferred directly to the membrane transporter, in the case of gram-negative bacteria [6, 28]. We hypothesize that Cig1 may fulfill a similar role as a hemophore for *C. neoformans*.

The pathogenic fungi *C. albicans* and *H. capsulatum* are also capable of using heme and/or hemoglobin as an iron source [8, 29]. Rbt5 and Rbt51 were identified in *C. albicans* as receptors for heme and hemoglobin and were localized at the plasma membrane [9]. Like Cig1, Rbt5 and Rbt51 are both mannoproteins, the expression of *RBT5* is induced by iron starvation, and deletion of *RBT5* reduces the ability of *C. albicans* to use iron from heme. Deletion of *RBT5* did not reveal a role in virulence, although the CaFtr1 iron permease of the
high-affinity iron uptake system contributes to disease [30, 31]. We speculate that Cig1 may also facilitate delivery of heme to the cell surface of *C. neoformans* for subsequent internalization.

Many heme-binding proteins contain 1 or multiple conserved heme-binding domains, such as the NEAT domains found in bacteria or, possibly, the CFEM domain found in Rbt5 and Rbt51 from *C. albicans* [9, 32, 33]. No conserved heme-binding domains were detected for Cig1, although heme binding was detected by spectrophotometric titration and ITC, using recombinant Cig1-GST protein. The absorbance spectra showed distinct peaks characteristic of a heme-binding protein. We were unable to determine the $K_d$, and similar cases have been reported in the literature. For example, a heme-binding protein in rabbits has been reported to bind a 25–35 molar excess of heme [34]. Similarly, the IsdX2 protein from *Bacillus anthracis* required a 20:1 ratio of heme to...

**Figure 4.** Cig1 binds to heme. *A*, Absorbance spectra of Cig1–glutathione S-transferase (GST) and heme were measured after addition of increasing amounts of heme (0–60 µM) to purified Cig1-GST (5 µM). The difference between the absorbance (Δ absorbance) of the Cig1–heme complex and free heme at different heme concentrations is plotted against the measured wavelengths. The left arrow shows the Soret peak, and the right arrow shows the β and α peaks. *B*, Heme-binding curve of Cig1-GST. The difference between the absorbance of the Cig1–heme complex and free heme at 407 nm is plotted against the increasing concentration of heme. Both assays were repeated 3 times, and representative graphs are shown.

**Figure 5.** *CIG1* is required for virulence in the absence of *CFO1*. *A*, Ten female A/JCr mice were inoculated intranasally with each of the *Cryptococcus neoformans* strains indicated. The survival of the mice was monitored daily up to 60 days. Statistical analysis was performed by log-rank tests. The difference in survival between the wild-type (WT) strain and *cfo1Δ* and *cig1Δ cfo1Δ* strains was significant (*P* < .0001). Additionally, the difference between *cfo1Δ* and *cig1Δ cfo1Δ* strains was significant (*P* < .0001). The fungal burden in the lungs (*B*) and brain (*C*) of 3 mice from each group was determined by counting the colony forming units (CFU). The organs were collected at the end point and homogenized, and the cells were plated onto yeast extract peptone dextrose agar plus 35 µg/mL chloramphenicol. Statistical analysis was performed using an unpaired, 2-tailed *t* test. *P* < .05.
protein to reach saturation, and multiple inflection points were detected [35]. It is possible that the lack of saturation for Cig1 is caused by weak or nonspecific binding, that heme binds to Cig1 at multiple sites on the protein, or that the protein contains residual heme on purification. Additionally, the activity of Cig1-GST obtained from E. coli may be different than that of the native protein because of the absence of posttranslational modifications and/or the presence of the GST tag. Another possibility is that the Cig1-GST protein induced heme aggregation or showed nonspecific binding because the recombinant protein was not properly folded. Further work is therefore needed to investigate heme binding for Cig1 purified from C. neoformans.

Several findings indicate that additional Cig1-independent mechanisms exist for iron acquisition from heme in C. neoformans. First, deletion of CIG1 resulted in an extended lag phase on heme but eventual growth to a density comparable to that of the wild-type strain. Although heme likely contains some contaminating inorganic iron that could be acquired by the high-affinity system [11, 17], we found that the cig1Δ cof1Δ mutant behaved similarly to the cig1Δ mutant. This rules out a substantial contribution of free iron to the eventual growth of the mutants. A second finding was the absence of a growth defect of the cig1Δ mutant and downregulation of CIG1 expression at acidic pH. This pH effect implicated the fungal transcription factor Rim101 because of its known regulation of pH-responsive genes and genes encoding functions for iron acquisition [16, 36, 37]. The expression of CIG1 was downregulated in the rim101Δ mutant of C. neoformans [16], and we additionally found that deletion of RIM101 resulted in a growth defect on heme similar to that of the cig1Δ mutant. Although Rim101 likely influences heme uptake by regulating CIG1, it also has an independent contribution to growth on heme, as demonstrated by the exacerbated growth defect of a strain lacking both Cig1 and Rim101. We also observed that rim101Δ did not have a growth defect when grown on heme in the presence of galactose. Therefore, carbon source appears to influence iron acquisition from heme. Certainly this was the case for Cig1, because transcript levels were reduced on galactose versus glucose media. Finally, support for other mechanisms of heme use comes from the Cig1-dependent toxicity of noniron MPs. The toxicity of Ga-PPIX and Mn-PPIX in wild-type but not cig1Δ or rim101Δ strains supports a role for Cig1 and Rim101 in heme uptake. This phenotype was only apparent when cells were forced to use heme as the sole iron source. Interestingly, cig1Δ was able to grow on heme in the presence of Ga-PPIX, perhaps because of residual iron reserves (ie, the cells were not prestarved for iron). Moreover, there may be a small amount of free iron contaminating the heme and/or the agarose in the medium to support some growth. The growth defect of cig1Δ cof1Δ on heme partially supports this explanation, although an additional system may allow heme iron acquisition in the absence of uptake (eg, by surface reduction and/or extraction of iron for uptake via the high-affinity system) and therefore not promote noniron MP susceptibility. The growth defect of cig1Δ cof1Δ on heme supports this hypothesis.

The contribution of Cig1 to virulence was evident only in a strain that lacked the high-affinity uptake system, thus supporting the idea that C. neoformans has multiple mechanisms for iron acquisition in the host. A contribution of Cig1 to fungal burden was not found, although it may have been masked because loss of the high-affinity system is known to block accumulation in the brain [11]. A more detailed analysis of the disease process will be needed to fully understand the contribution of Cig1.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary materials are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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