Copper is an essential nutrient that drives a wide variety of essential biochemical processes through its function as an enzyme cofactor. Excess copper is toxic because it can lead to the generation of reactive oxygen species, via the Fenton reaction, which can, in turn, react with and damage nucleic acids, proteins, and lipids (1, 2). The biocidal properties of copper have been known for centuries and have long been exploited to control or prevent the growth of a wide variety of microbial organisms (3). Furthermore, an emerging theme in the field of host-pathogen interactions is the idea, for which there is considerable evidence, that macrophages kill phagocytized microbes by pumping copper into the phagolysosome to inflict copper poisoning upon them (4–6). The ability of microbes to survive in the presence of excess copper can be achieved by three key mechanisms—reducing copper influx, increasing copper efflux, and expressing metallothioneins, which function as copper storage proteins by chelating excess copper (7–12).

In *Candida albicans*, the major invasive fungal pathogen in humans, copper import is mediated by the high-affinity copper transporter, Ctr1p, and the action of two proteins with cupric reductase activity, Fre7p and Fre10p (13, 14). Under copper-limiting conditions, *CTR1* and *FRE7* are expressed at high levels, and this expression is dependent on the copper-sensing transcription factor encoded by *MAC1*, which binds to copper response elements in the promoters of *CTR1* and *FRE7*. Under copper-replete conditions, the expression of *CTR1* and *FRE7* is repressed (15). Although the mechanism of repression in the presence of copper has not been determined in *C. albicans*, it is likely to occur by the same molecular mechanism as used by *Saccharomyces cerevisiae*, whereby copper binds to and inhibits the ability of ScMac1p to bind DNA and subsequently prevents gene expression (7, 16, 17). (Herein, we use the prefix “Sc” to indicate *S. cerevisiae* gene products, and genes without a prefix are carried by *C. albicans*). As an added barrier to prevent excess copper accumulation, the presence of high copper levels induces the degradation of the copper transporter, ScCtr1p, at the plasma membrane in a manner that is dependent on ScMac1p (11, 18).

Essential for the ability of *C. albicans* to resist copper toxicity is the function of the proteins encoded by *CRP1*, *CUP1*, and *CRD2*. Homozygous deletion of any of these genes results in increased sensitivity to excess copper (19, 20). *CRP1* encodes a P1-type ATPase copper transporter that actively pumps excess copper out of the cell, a feature that appears to be absent in *S. cerevisiae* (19, 20). *CUP1* and *CRD2* encode copper metallothioneins (19, 20). The expression of *CRP1* and *CUP1* is stimulated by growth in excess copper, while *CRD2* expression is insensitive to copper levels (19, 20). The mechanism responsible for the copper-inducible expression of *CUP1* has not been analyzed in *C. albicans*, but it likely mimics the scenario in *S. cerevisiae*, in which copper binds to and activates a second copper-sensing transcription factor, ScCup2p, and subsequently induces the expression of *ScCUP1* (21–23). The *C. albicans* genome encodes a strong homologue of *ScCUP2*, and deletion of either homologue confers copper hypersensitivity to its respective organism (23, 24).

Relatively little is known about the molecular pathways that pathogenic fungi use to sense and respond to excess copper apart from the function of the two copper-sensing transcription factors. Here we demonstrate that deletion of *GPA2*, which encodes a G-protein α subunit involved in filamentous growth, confers increased resistance to normally toxic levels of copper. We show that Gpa2p governs the expression of genes involved in copper uptake and chelation and provide the first evidence for the involvement of the protein kinase A (PKA) pathway in copper homeostasis.
**TABLE 1 Strains used in this study**

| Strain          | Relevant genotype | Reference |
|-----------------|-------------------|-----------|
| C. albicans     |                   |           |
| RBY1179         | GPA2/GPA2         | 26        |
| RBY1166         | gpa2::HIS1/gpa2::LEU2 | 26   |
| RBY1205         | gpa2::HIS1/gpa2::LEU2/GPA2::SAT1-FLIP | (addback) |
| CAI4-URA        | EFG1/EFG1         | 43        |
| DSC10          | efg1::hisGefg1::hisG | 43       |
| DSC11          | efg1::hisGefg1::EFG1-dpl200 | 43    |
| SN250          | MAC1/MAC1 CUP2/CUP2 | 24       |
| TF065-1        | mac1::HIS1/mac1::LEU2 | 24    |
| TF065-2        | cup2::HIS1/cup2::LEU2 | 24    |
| TF039-3        | cup2::HIS1/cup2::LEU2 | 24    |
| S. cerevisiae  |                   |           |
| BY4741          | GPA2              |           |
| gpa2Δ          |                   | 44        |
| C. neoformans   |                   |           |
| M049b          | GPA1              | 37        |
| AAC1           | gpa1Δ::ADE2       | 37        |
| AAC2           | gpa1Δ::ADE2 + pGPA1 on Cn Tel-Hyg | 37 |

**TABLE 2 qRT-PCR oligonucleotides used in this study**

| Oligonucleotide | Sequence (5’-3’) |
|----------------|------------------|
| CTR1-qR        | TCTTTGTGTCCTTCCCTTGCT |
| CTR1-qF        | GTTCCAGGACCGTTTGGTGT |
| CTR2-qF        | TTGTACAAATATGGCTTAGA |
| CTR2-qR        | ACCAAATGGCAATTCAT |
| CRI1-qF        | GCCGAGAAGTATTTGATAGT |
| CRI1-qR        | CCAGCAAGGACCATGCAACAC |
| CRD2-qF        | CTGCTCAAAGTTGTGGTGGT |
| CRD2-qR        | CACAAATTAGATTGACACCAAC |
| CUP-qF         | AAAAACTATTGCAAGGAAAC |
| CUP-qR         | TACACCTACACAGGACACCAAC |
| CUP9-qF        | ATGGTCATGTGCTTCATCC |
| CUP9-qR        | TGGTGTGGTTGTTGTTGGT |
| MAC1-qF        | ACGACCCCTTGAACAAACAG |
| MAC1-qR        | AGAGAATGCTGCTGGAGAAG |
| FRE7-qF        | TCCAGGTAAGGTAGGTGTC |
| FRE7-qR        | ACCGGGAAATAGACCAACAG |
| CUP1-qF        | TTAATCTAGGATCTGGCTGG |
| CUP1-qR        | TTGGCTATTGCTTGGAGAAC |

**MATERIALS AND METHODS**

**Strains and media.** *Candida albicans*, *Saccharomyces cerevisiae*, and *Cryptococcus neoformans* strains were routinely passaged in YPD (2% dextrose, 2% Bacto peptone, 1% yeast extract) at 30°C. All strains are listed in Table 1.

**Growth assays.** Single colonies were inoculated into 3 ml of YPD and grown overnight in an orbital shaker at 30°C. For liquid growth assays, the cultures were split into two 50-ml aliquots. CuSO₄ was added to one of the aliquots to a final concentration of 12 mM. The other aliquot was left untreated. Growth was measured by optical density at 600 nm (OD₆₀₀) of 0.01 in a microtiter plate.

**Gene expression analysis.** Single colonies were inoculated into 3 ml of YPD and grown overnight at 30°C. These overnight cultures were used to inoculate 100-ml cultures of YPD, which were incubated at 30°C with orbital shaking to mid-log phase (OD₆₀₀ ~1) at which point the cultures were split into two 40-ml aliquots. CuSO₄ was added to one of the aliquots to a final concentration of 12 mM. The other aliquot was left untreated. Cultures were allowed to grow at 30°C for 1 h and then harvested by centrifugation. Pellets were washed 3 times with 40 ml of deionized water before being stored at −80°C until induction for protein expression. Frozen pellets were resuspended in 20% trichloroacetic acid (TCA) and stored at −80°C for 1 h. Samples were then transferred to 50-ml conical tubes, and the Eppendorf tubes were rinsed with 2% trichloroacetic acid (TCA) to ensure complete transfer of metal ions. An internal standard was incorporated into each sample to verify the concentration of the standard. Samples were then centrifuged, and the supernatant was collected for ICP-MS analysis. The supernatant was then analyzed by ICP-MS to determine the concentration of copper in each sample as measured by OD₆₀₀.

**RESULTS AND DISCUSSION**

**Deletion of C. albicans GPA2 confers resistance to copper.** During our studies to define the functions of novel unannotated transcripts in the *C. albicans* genome, we observed that a strain harboring a deletion of the transcript listed as NOVEL-Ca21chr3-018 (25) displayed increased growth relative to a wild-type (WT) strain on rich media supplemented with excess copper sulfate (CuSO₄) (data not shown). Since this transcript is in close proximity (350 bp) to the start codon of GPA2 on chromosome 3 and is transcribed on the same strand, we reasoned that deletion of this transcript might confer increased copper tolerance by reducing the expression of GPA2. To directly address this, we tested the ability of a strain carrying a homologous deletion of GPA2 (26) to grow in the presence of excess copper. The gpa2Δ/Δ strain exhibited significant growth in liquid medium supplemented with CuSO₄, while the growth of the WT strain was significantly inhib-
A gpdAΔ mutant is resistant to copper. (A) C. albicans WT (squares; RBY1179), gpdAΔ (circles; RBY1166), and gpdAΔ GPA2 (triangles; RBY1205) strains were inoculated into YPD (closed symbols) or YPD plus 12 mM CuSO₄ (open symbols) at an OD₆₀₀ of 0.01. The ODs were measured hourly over the course of 30 h. Shown are the averages of 3 biological replicates. (B) C. albicans WT (RBY1179), gpdAΔΔ (RBY1166), and gpdAΔΔ GPA2 (RBY1205) strains were grown overnight in YPD, serially diluted, and spotted onto YPD or YPD plus 10 mM CuSO₄ and photographed after 1 or 2 days, respectively.

We observed that the copper resistance of the gpdAΔ strain was more severe than that of the strain harboring the deletion of NOVEL-Ca21chr3-018 (data not shown), suggesting that deletion of NOVEL-Ca21chr3-018 partially reduced the expression of GPA2, resulting in a less severe phenotype. Taken together, these results indicate that GPA2, and not NOVEL-Ca21chr3-018, functions to govern copper homeostasis in C. albicans.

We considered whether or not the copper resistance phenotype of the gpdAΔ strain was indicative of a general defect in metal homeostasis by testing growth of the mutant in media depleted of or supplemented with other metals. We observed no difference between the WT and the gpdAΔ mutant in growth assays under iron-limiting conditions (500 μM ferrozine or 100 μM bathophenanthrolinedisulfonic acid) or copper-limiting conditions (100 μM bathocuproine disulfonate) or in media containing excess cadmium, silver, iron (Fig. 2), manganese, nickel, or cobalt (data not shown).

A gpdAΔΔ mutant displays altered regulation of copper-related genes. To determine whether GPA2 may influence copper resistance through altered regulation of one or more copper-related genes, we assayed the steady-state and copper-inducible expression of several copper-related genes by qRT-PCR in the WT, gpdAΔΔ mutant, and complemented strains.

The expression of the copper uptake genes, CTR1 and FRE7, was repressed after exposure to excess copper in both the WT and complemented strains (Fig. 3A and B). When only the untreated samples are considered, the gpdAΔΔ mutant had severely reduced expression of CTR1 and FRE7 (11.5-fold and 8.2-fold, respectively) relative to the WT strain, and this reduction was not apparent in the complemented strain. As expected, among the samples that were exposed to excess copper, both genes were expressed at comparably low levels in all 3 strains tested. Therefore, in the absence of excess copper, deletion of GPA2 confers a decrease in the expression of the copper uptake genes, CTR1 and FRE7.

The expression of the copper-efflux pump, CRP1, was induced upon exposure to excess copper in both the WT and complemented strains (Fig. 3C). When only the copper-treated samples are considered, the gpdAΔΔ strain displayed a 5.2-fold reduction in CRP1 expression relative to the WT strain that was not evident in the complemented strain (Fig. 3C). CRP1 was expressed at equivalently low levels in each of the 3 strains when they were left untreated (Fig. 3C). Therefore, deletion of GPA2 results in the

FIG 1 A gpdAΔ mutant is resistant to copper. (A) C. albicans WT (squares; RBY1179), gpdAΔ (circles; RBY1166), and gpdAΔ GPA2 (triangles; RBY1205) strains were inoculated into YPD (closed symbols) or YPD plus 12 mM CuSO₄ (open symbols) at an OD₆₀₀ of 0.01. The ODs were measured hourly over the course of 30 h. Shown are the averages of 3 biological replicates. (B) C. albicans WT (RBY1179), gpdAΔΔ (RBY1166), and gpdAΔΔ GPA2 (RBY1205) strains were grown overnight in YPD, serially diluted, and spotted onto YPD or YPD plus 10 mM CuSO₄ and photographed after 1 or 2 days, respectively.

FIG 2 A gpdAΔΔ mutant is not resistant to excess silver, cadmium, or iron. (A) C. albicans WT (RBY1179), gpdAΔΔ (RBY1166), and gpdAΔΔ GPA2 (RBY1205) strains were inoculated into YPD (gray bars), YPD plus 75 μM Ag₂SO₄ (black bars), or YPD plus 100 μM CdSO₄ (striped bars) at an OD₆₀₀ of 0.01. ODs were measured at 24 h. Shown are the averages of 3 biological replicates, with standard deviations. (B) C. albicans WT (RBY1179) and gpdAΔΔ (RBY1166) strains were inoculated into YPD or YPD plus 12 mM FeSO₄ at an OD₆₀₀ of 0.01. ODs were measured at 24 h. Shown are the averages of 3 biological replicates.
creased in expression of the copper metallothionein gene, complemented strain. Therefore, deletion of the WT strain, and this increased expression was not evident in the untreated sample, and expression was normalized to TEF1. logical replicates, with standard errors. *, grown under the same condition. #, grown under the same condition.

FIG 3 Altered expression of copper-related genes in a gpa2Δ/Δ mutant. WT (RBY1179), gpa2Δ/Δ (RBY1166), and gpa2Δ/Δ GPA2 (RBY1205) strains grown in YPD (gray bars) or YPD plus 12 mM CuSO4 for 30 min (black bars) were subjected to qRT-PCR expression analysis of the indicated copper-related genes: CTR1 (A), FRE7 (B), CRP1 (C), CRD2 (D), and MAC1 (E). RNA expression was normalized to TEF1 expression, and fold changes between strains were normalized to the WT strain (RBY1179) grown in the absence of copper, which was adjusted to a value of 1. Shown are the averages of 4 biological replicates, with standard errors. *, P < 0.01 compared to the WT strain grown under the same condition. #, P < 0.05 compared to the WT strain grown under the same condition.

inability of the CRP1 gene to be induced by exposure to excess copper.

The expression of the metallothionein gene, CRD2, did not respond to the addition of excess copper in any of the strains tested (Fig. 3D). Notably, under both conditions, the gpa2Δ/Δ strain exhibited increased expression (6.5-fold and 17.8-fold) relative to the WT strain, and this increased expression was not evident in the complemented strain. Therefore, deletion of GPA2 results in increased expression of the copper metallothionein gene, CRD2.

The expression of the gene encoding the copper-sensing tran-

scription factor, MAC1, was repressed after exposure to excess copper in both the WT and complemented strains (Fig. 3E). Among the copper treated samples, MAC1 was expressed at comparable levels in all three strains (Fig. 3E). However, MAC1 expression was reduced 2-fold in the untreated gpa2Δ/Δ sample relative to the untreated WT sample, and this reduction was not evident in the complemented strain (Fig. 3E). Therefore, in the absence of excess copper, deletion of GPA2 results in a decrease in MAC1 expression.

We were unable to detect a reproducible, statistically significant difference in the expression of CUP1, CTR2, or CUP2 between the WT and gpa2Δ/Δ strains (data not shown). Taken together, the results of our gene expression analyses suggest that the increased copper resistance observed in the gpa2Δ/Δ mutant results from a combination of decreased copper uptake and increased synthesis of metallothioneins. Although our results suggest that increased copper efflux is not a factor contributing to the copper resistance phenotype, the possibility exists that a previously undescribed posttranscriptional mechanism could be increasing the amount of efflux pumps without altering the amount of RNA message.

The decreased expression of CTR1 (Fig. 3A), FRE7 (Fig. 3B), and MAC1 (Fig. 3E) in the gpa2Δ/Δ mutant is likely to reflect a decrease in expression of the copper-responsive transcription factor, Mac1p, which activates transcription in the absence of excess copper by binding to copper responsive elements (CuREs) in the promoters of its target genes (15). We examined the expression of CTR1 in independently generated strains that each harbor a homozygous deletion of MAC1 (24). As expected, the mac1Δ/Δ strains exhibited significantly reduced expression of CTR1 (Fig. 4A) compared to the wild-type strain.

A gpa2Δ/Δ mutant accumulates less copper. To extend upon the changes we observed in the expression of the copper uptake genes, CTR1 and FRE7, we tested the strains’ sensitivity to cisplatin, a chemotherapeutic drug that is toxic to yeast cells. In S. cerevisiae and mammals, cisplatin enters cells through the copper transporter encoded by ScCTR1 (30). A S. cerevisiae deletion of ScCTR1 displays increased resistance to cisplatin (30). Although the mechanism by which cisplatin is taken up into S. cerevisiae displays increased resistance to cisplatin (30). Among the copper treated samples, the expression of CTR1 (Fig. 3A) in the untreated gpa2Δ/Δ mutant displayed increased resistance to cisplatin (30). Although the mechanism by which cisplatin is taken up into C. albicans cells has not been elucidated, we reasoned that it is likely to be mediated by the C. albicans CTR1 gene, given its sequence and functional homology to the ScCTR1 gene of S. cerevisiae (31). Thus, an increased resistance to cisplatin would imply a decrease in expression or function of the C. albicans CTR1 gene. Consistent with the gene expression data, the gpa2Δ/Δ strain displayed increased growth in the presence of cisplatin compared to WT and complemented strains (Fig. 5). Therefore, deletion of GPA2 results in an increase in resistance to cisplatin. This result is consistent with the notion that a decrease in copper uptake is a contributing factor in the copper resistance of the gpa2Δ/Δ strain.

The cisplatin (Fig. 5) and copper (Fig. 1) resistance phenotypes, as well as the decrease in CTR1 gene expression (Fig. 3A) in the gpa2Δ/Δ strain, led us to examine the cell-associated copper content of these strains. To accomplish this, we performed inductively coupled plasma mass spectrometry (ICP-MS) on the WT, gpa2Δ/Δ, and complemented strains grown in YPD alone or supplemented with 12 mM CuSO4. All three of the strains had equivalent, low levels of cell-associated copper when grown in YPD. As expected, treatment of WT cells with CuSO4 for 1 h resulted in a significant increase (175-fold) in the cellular copper content.
However, following exposure to 12 mM CuSO₄ for an hour, the gpa2/H9004/H9004 mutant had reduced (2.1-fold) copper content compared to those of the WT and complemented strains (Fig. 6A). Therefore, deletion of GPA2 results in a decrease in cell-associated copper content upon exposure to copper. This result is consistent with the increased cisplatin resistance and the decreased CTR1 expression that we have observed for the gpa2/H9004/H9004 mutant. Together, these observations favor the model that reduced copper uptake contributes to the copper resistance phenotype of the gpa2ΔΔ mutant.

We noticed a difference in CTR1 and FRE7 expression between the WT and gpa2ΔΔ strains under conditions where no extra copper was added to the medium but not under conditions of excess copper (Fig. 3A and B), where we observed the difference in copper accumulation (Fig. 6). This difference can be explained by copper-inducible protein degradation. S. cerevisiae Ctr1p is degraded at the plasma membrane when cells are exposed to high levels of copper (11, 18). While this aspect of regulating Ctr1p function in C. albicans has not been authenticated, it is reasonable to predict a similar role. Based on the gene expression data, one likely scenario is that the gpa2ΔΔ strain has severely reduced steady-state levels of Ctr1p. Thus, the amount of Ctr1p that needs to be degraded to adequately shut down copper import is much lower, allowing the gpa2ΔΔ strain to turn off copper import more rapidly and subsequently achieve lower copper levels than the WT strain. Further experiments are required to conclusively determine this mechanism.

The decreased accumulation of cell-associated copper in the gpa2ΔΔ mutant is most likely the result of decreased expression of MAC1 in the gpa2ΔΔ mutant, which, as demonstrated above, confers decreased expression of the copper transporter, CTR1 (Fig. 4A). We measured the amount of cell-associated copper in the mac1ΔΔ strains grown in YPD alone or YPD supplemented with 12 mM CuSO₄. Deletion of MAC1 confers a defect in cell-
associated copper accumulation (~2-fold decrease) very similar to that conferred by deletion of GPA2 (Fig. 6). These results are consistent with the model in which deletion of GPA2 leads to reduced copper accumulation by decreasing the expression of the copper-sensing transcription factor MAC1.

The expression of CRP1 is induced when excess copper is added to the medium (19, 20). The decreased accumulation of copper in the gpa2Δ/Δ strain (Fig. 6A) might explain the lack of induction of the copper efflux pump, CRP1 (Fig. 3C). In S. cerevisiae, copper-inducible gene expression is mediated by the function of ScCuP2. Under conditions where copper is in excess, ScCuP2 binds to copper and activates the expression of ScCuP1, which specifies a metallothionein (21–23, 32, 33). The C. albicans CUP2 homologue is required to resist high copper levels because deletion of this gene confers hypersensitivity to excess copper (24), making it a likely candidate for the transcription factor that mediates the copper-inducible expression of CRP1. To test this in C. albicans, we examined the copper-inducible gene expression of CRP1 in two independently generated strains that each harbor a homozygous deletion of the CUP2 gene (24). As expected, the cup2Δ/Δ strains exhibited significantly reduced copper-induced expression of CRP1 (Fig. 4B) compared to the wild-type strain. To our knowledge, this is the first report that connects CRP1 expression to the activity of CUP2 in C. albicans.

**Copper resistance can be reversed by cyclic AMP.** The C. albicans GPA2 gene encodes a G-protein α subunit that functions with GPR1, a G-protein coupled receptor, as a nutrient sensor that regulates filamentous growth (34–36). GPA2 in hyphal morphogenesis is known to act through the cAMP-dependent PKA pathway, as deletion of GPA2 results in a morphogenesis defect that can be reversed by the addition of exogenous cAMP to the medium (34). To determine if GPA2 functions through the same pathway to govern copper homeostasis, we tested whether the addition of 10 mM N\textsuperscript{6},\textsuperscript{2’-O-dibutyryl-AMP (dbcAMP) would reverse the copper resistance of the gpa2Δ/Δ strain. DbcAMP is a cell-permeative, nonmetabolizable derivative of cAMP that has previously been used to mimic high cAMP levels in C. albicans (35). Addition of dbcAMP did not affect the growth of the WT or gpa2Δ/Δ strain in the absence of copper (Fig. 7). While the gpa2Δ/Δ mutant achieved significant growth in YPD supplemented with CuSO\textsubscript{4} (Fig. 1 and 7A), its growth was significantly inhibited in same medium to which dbcAMP was added (Fig. 7A). Therefore, addition of exogenous cAMP can bypass the effect on copper tolerance of the GPA2 deletion and render the gpa2Δ/Δ strain sensitive to copper. EFG1 encodes a transcription factor that functions at the end of the cAMP-PKA pathway to govern hyphal morphogenesis. In order to further investigate the role of the PKA pathway in copper toxicity, we tested whether mutations in the EFG1 branch of the C. albicans PKA pathway would confer increased resistance to excess copper. We observed that an efg1Δ/Δ mutant was significantly more resistant to excess copper than both the WT and the EFG1 complemented strain (Fig. 7B). This result is in agreement with the findings of Homann et al. (24).

Taken together, these results suggest that GPA2 functions through the PKA pathway to govern copper homeostasis.

**Conservation of GPA2 function among pathogenic yeasts.** We next considered whether this new role for GPA2 in copper homeostasis is conserved in other fungi. We decided to address this by analyzing deletion mutants in the nonpathogenic S. cerevisiae and the primary pathogen Cryptococcus neoformans. The GPA2 homologues in S. cerevisiae and C. neoformans are ScGPA2 and CnGPA1, respectively. Both genes have been shown to function upstream of the cAMP-PKA pathway in their respective organisms (37–41). We could not detect a difference between the WT S. cerevisiae strain and the gpa2Δ/Δ mutant in the presence of excess copper when grown in liquid or on solid medium (Fig. 8A and 8B, respectively). Therefore, deletion of ScGPA2 does not appear to confer copper resistance, suggesting that GPA2 does not govern copper homeostasis in S. cerevisiae. However, we cannot exclude the possibility that another gene provides redundant function in the Scgpa2Δ mutant.

The C. neoformans gpa1Δ strain exhibited an ~5-fold increase in growth in the presence of excess copper relative to the WT strain, and the resistance was reversed in the complemented strain carrying a WT copy of GPA1 (Fig. 8C). Therefore, deletion of GPA1 confers copper resistance to a similar extent as deletion of C. albicans GPA2.

There are several observations in the literature that are consistent with deletion of CnGPA1 resulting in decreased copper import. AlsOUGH et al. demonstrated that CnGPA1 regulates capsule production and melanization by showing that a strain harboring a deletion in CnGPA1 displays defects in these processes (37). In a study performed 14 years later, Silva et al. observed that treatment of C. neoformans cells with microplussin, a copper-chelating antimicrobial peptide, inhibited both capsule production and melanization (42). One possible
explanation for the similarities in phenotypes shared by deletion of CnGPA1 and depletion of copper by chelation is that a CnpalΔ mutant has a defect in copper import, similar to what we observed in C. albicans. More experiments are required to reach this conclusion definitively.

Relationship between GPA2 and copper homeostasis. When exposed to toxic concentrations of copper in the environment, C. albicans employs three key microbial mechanisms to ensure survival. It responds by reducing copper influx, increasing copper efflux, and expressing metallothioneins that function to chelate free copper ions (19, 20). Our understanding of the signaling pathways that mediate these responses is incomplete, with most of the knowledge being extrapolated from observations made in S. cerevisiae. In this report, we describe the novel role of GPA2 and the PKA pathway in copper homeostasis. We demonstrate that a strain harboring a deletion in GPA2 results in a copper resistance phenotype (Fig. 1) that stems from 2 of the 3 key copper survival mechanisms—decreased copper import (Fig. 3, 5, and 6) and increased metallothionein expression (Fig. 3D). The GPA2 gene encodes a G protein α subunit that functions through the CAMP-PKA pathway to govern hyphal morphogenesis (34, 35). Our observation that the copper resistance can be reversed by addition of dbcAMP suggests that GPA2 also functions through the CAMP-PKA pathway to govern copper homeostasis, presumably by controlling the expression of the copper import genes, CTR1 and FRE7, as well as the metallothionein gene, CRD2. Further experiments are required to determine the exact pathways and mechanisms that GPA2 uses to control the expression of these genes.

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