Transcription factor Znf2 coordinates with the chromatin remodeling SWI/SNF complex to regulate cryptococcal cellular differentiation

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Cellular differentiation is instructed by developmental regulators in coordination with chromatin remodeling complexes. Much information about their coordination comes from studies in the model ascomycetous yeasts. It is not clear, however, what kind of information that can be extrapolated to species of other phyla in Kingdom Fungi. In the basidiomycete Cryptococcus neoformans, the transcription factor Znf2 controls yeast-to-hypha differentiation. Through a forward genetic screen, we identified the basidiomycete-specific factor Brf1. We discovered Brf1 works together with Snf5 in the SWI/SNF chromatin remodeling complex in concert with existent Znf2 to execute cellular differentiation. We demonstrated that SWI/SNF assists Znf2 in opening the promoter regions of hyphal specific genes, including the ZNF2 gene itself. This complex also supports Znf2 to fully associate with its target regions. Importantly, our findings revealed key differences in composition and biological function of the SWI/SNF complex in the two major phyla of Kingdom Fungi.
Cellular differentiation allows genetically identical cells to exhibit distinct phenotypes. Although functional and morphological differences are most pronounced in higher eukaryotes, cellular differentiation is a universal phenomenon, often directed by differential gene expression. In eukaryotes, nucleosomes serve as a general barrier preventing transcription and specific regulatory mechanisms make certain genomic DNA regions accessible to transcription factors and RNA polymerase II. The ATP-dependent SWI/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling family mainly facilitates in cis sliding and/or in trans ejecting of nucleosomes on DNA, allowing transcription activation or repression. Through modulating gene expression, the SWI/SNF family of complexes are critical to a variety of cellular processes including stemness and differentiation.

The SWI/SNF complex is composed of 12 subunits in Saccharomyces cerevisiae and 11–15 subunits in humans. However, most human SWI/SNF subunits have several isoforms, permitting dozens of combinatorial assemblies and a spectrum of related complexes. It is, therefore, challenging to attribute observed phenotypes based on overexpression of a particular subunit to the function of a specific complex. Here, we use the term family when we discuss the SWI/SNF family of complexes. In S. cerevisiae and Schizosaccharomyces pombe, there are only two complexes in the SWI/SNF family: the SWI/SNF complex and the RSC (remodeling structure of chromatin) complex. These two complexes have their distinct catalytic and accessory subunits while sharing some common subunits. The relative simplicity in these model yeasts has facilitated investigation of the molecular functions of SWI/SNF complexes and individual subunits. It is unclear, however, what functions and features of the SWI/SNF complexes established in these two model ascomycete yeasts can be extrapolated to other eukaryotic species, including other distantly related fungal species.

In contrast to these model yeasts, Cryptococcus neoformans belongs to a different phylum in Kingdom Fungi: Basidiomycota. Basidiomycetes diverged from ascomycetes about one billion years ago. They share key features with higher eukaryotes that are absent from the model yeasts. For instance, >90% of cryptococcal protein-coding genes contain multiple introns. Epigenetic regulation, such as RNAi and DNA methylation, plays important roles in cryptococcobiology. C. neoformans can exist in multiple morphotypes and morphogenesis is associated with its pathogenicity. For instance, yeasts and spores are infectious and virulent; titan cells are proposed to be dormant and stress-resistant in hosts; pseudohyphae and hyphae are attenuated in hosts. In the environment, however, hyphae are an integral part of its life cycle and confer cryptic virulence in mammalian hosts. It is, therefore, challenging to attribute observed phenotypes based on overexpression of a particular subunit to the function of a specific complex between the two major phyla in the Kingdom Fungi.

Results

Nuclear Znf2 fails to induce hyphae in inserational mutants. We previously demonstrated the key function of ZNF2 in regulating yeast-to-hypha transition. Here, we employed a reporter strain to identify Znf2’s partners through a forward genetic screen. In this reporter strain, the native ZNF2 gene is deleted and an ectopic copy of mCherry-fused ZNF2 is expressed under the control of an inducible promoter of a copper transporter CTR4. This reporter strain switched from yeasts to hyphae when ZNF2 was induced in the presence of the copper chelator bathocuproinedisulfonic acid (BCS) (Fig. 1a), as expected based on our previous studies. The production of Znf2 can be monitored through the nuclear-localized mCherry signal (Fig. 1a). Here we used inserional mutagenesis through Agrobacterium-mediated transformation (AMT) and visually screened 88,000 T-DNA inserational mutants made in this reporter strain for smooth yeast colonies on filamentation-inducing V8 agar medium containing BCS (Fig. 1b). Eighty-four mutants defective in filamentation were isolated (strains AMT001-AMT084, Supplementary Data 1).

Fifty-eight mutants showed nuclear mCherry-Znf2 while 26 had altered subcellular distribution of the fluorescence signal. The mutants with mis-localized Znf2 signal might be defective in Znf2 trafficking, post-translational modification, and/or stability. Here, we set to identify factors that are required for nuclear-localized Znf2 protein to exert its function. Among these 58 mutants, 25 candidates showed only yeast cellular morphology and nuclear Znf2 while 26 mutants showed nuclear Znf2 with Znf2 protein to exert its function.

Two candidates were identified as filamentation activators. To identify the genes affected by the T-DNA insertions in the selected eight mutants, we used the genome sequencing approach developed by Dr. Alspaugh’s group. Eight insertion sites were identified from the genome sequences of the progeny of the eight inserational mutants where only the HYGR (T-DNA) is present (Supplementary Data 1).

In the environment, however, hyphae are an integral part of its life cycle and confer cryptic virulence in mammalian hosts. It is, therefore, challenging to attribute observed phenotypes based on overexpression of a particular subunit to the function of a specific complex.
Brf1 functions in the same biological process as Snf5. As mentioned earlier, deletion or T-DNA disruption of SNF5 or BRF1 abolished or nearly abolished self-filamentation (Fig. 1e). Unilateral crosses between the snf5Δ or the brf1Δ mutant with a non-self-filamentous reference strain on V8 medium produced fewer mating hyphae (Supplementary Fig. 2a), likely due to reduced cell fusion efficiency (Supplementary Fig. 2b). Bilateral crosses between the mutant partners (snf5Δ × snf5Δ or brf1Δ × brf1Δ) did not produce any aerial mating hyphae or sexual spores (Fig. 2a). As bisexual mating or self-filamentation on V8 medium is mostly driven by the pheromone signaling cascade, we decided to examine the impact of these mutations on filamentation on V8 medium containing 500 μM of copper where self-filamentation is independent of the non-self-recognition system. Neither brf1Δ nor snf5Δ filamented under this condition (Fig. 2a), confirming the similarly critical role of Brf1 and Snf5 in filamentation.

Brf1 and Snf5 also share similar functions in other assays. Both brf1Δ and snf5Δ showed slightly increased sensitivity to osmotic stress (Fig. 2b). Both mutants grew similarly to WT in other conditions tested. The increased sensitivity to osmotic stress in brf1Δ might be due to the decreased transcript level of genes involved in anion and ion transportation (Supplementary Data 2), which was not observed in the znf2Δ mutant. SNFS is known to be required for normal growth on media with sucrose or raffinose as the sole carbon source. We found that the brf1Δ mutant and the snf5Δ mutant showed slightly increased sensitivity to osmotic stress on V8 medium containing 500 μM of copper where self-filamentation is independent of the non-self-recognition system. Neither brf1Δ nor snf5Δ filamented under this condition (Fig. 2a), confirming the similarly critical role of Brf1 and Snf5 in filamentation.

Given the striking resemblance of the brf1Δ mutant and the snf5Δ mutant, we postulated that Brf1 functions in the same biological pathway as Snf5. Indeed, the brf1Δsnf5Δ double mutant behaved similarly as the brf1Δ or the snf5Δ single mutant in all
assays (Fig. 2b–d). Constitutive expression of tdTomato-tagged Brf1 in brf1Δ restored filamentation (Supplementary Fig. 4a), indicating functionality of the tagged Brf1. The tagged Brf1 localized to the nucleus (Supplementary Fig. 4a), as reported previously for Snf5 (ref. 33). Taken together, we propose that Brf1 functions together with Snf5.

As Snf5 is a core subunit of the SWI/SNF complex, we postulate that this novel protein Brf1 may also function in this chromatin remodeling complex. Every known SWI/SNF complex incorporates a subunit with an ARID domain34–36. C. neoformans carries three genes encoding ARID-containing proteins: RUM1α (CND05870), AVC1 (CNK00710), and BRF1. None of these three cryptococcal genes showed high sequence homology to either SWI1 or SOL1, the ARID-containing subunit in the SWI/SNF complex in S. cerevisiae or S. pombe, respectively. However, among the three proteins, Brf1 is more similar to ScSwi1 or SpSol1 in terms of domain architecture (Fig. 3a). Deletion of BRF1, but not AVC1 or RUM1α, caused defects in filamentation (Fig. 3b) and slowed growth on raffinose medium (Supplementary Fig. 3). The result is consistent with the idea that Brf1, but not Ave1 or Rum1α, works in the same complex as Snf5. We, therefore, hypothesize that Brf1 is the ARID subunit of the SWI/SNF complex in this basidiomycete.

Brf1 is a basidiomycete-specific subunit of SWI/SNF. If Brf1 and Snf5 work together in the SWI/SNF complex, we expect that Brf1 and Snf5 interact with each other, either directly or indirectly through other subunits of the SWI/SNF complex. To test the hypothesis, we performed co-immunoprecipitation assays coupled with mass spectrometry using the FLAG-tagged Brf1 as the bait. As BRF1 is a lowly expressed gene based on our and others’ transcriptome data37, we used the constitutively active TEF1 promoter to drive the expression of FLAG-tagged Brf1, which restored brf1Δ’s mating deficiency (Supplementary Fig. 4b). We then carried out Co-IP/MS in two independent isolates, including the WT H99 strain without any tag as the negative control. In addition to the bait protein Brf1, we identified many proteins homologous to the SWI/SNF subunits in S. cerevisiae and/or S. pombe, including Snf5, Snf2, Arp4, Arp9, Rsc6, and Rsc8 (Table 1). The interaction between Brf1 and Snf5 was further confirmed by a reciprocal Co-IP/western where the mNeonGreen-tagged Snf5, when used as the bait, pulled down the FLAG-tagged Brf1 (Supplementary Fig. 4c).

The SWI/SNF and the RSC complexes are assembled modularly, with some subunits/modules shared by both complexes4,38,39. In S. cerevisiae and S. pombe, homologs of Arp4, Arp9, Rsc6, and Rsc8 participate in both complexes. Since Brf1 pulled down subunits unique to the SWI/SNF complex and the ones shared by both complexes, we speculate that Brf1 is a SWI/SNF-specific in C. neoformans. In most fungal species examined, the SWI/SNF complex and the RSC complex each harbors a Snf5 domain-containing subunit, with Snf5 in the SWI/SNF complex and Sfh1 in the RSC complex (Fig. 3c). The mNeonGreen-tagged RSC-specific protein Sfh1 (CNC06140) in C. neoformans pulled down RSC-specific subunits including Rsc1, Rsc7, and Rsc9. Sfh1 also pulled down the shared subunits including Snf2, Arp4, Arp9, Rsc6, and Rsc8 (Supplementary Table 2). However, Sfh1 did not
pull down Brf1 or the SWI/SNF-specific subunit Snf5. Collectively, these lines of evidence support that Brf1 is a subunit specific to the SWI/SNF complex.

SWI/SNF but not RSC is critical for hyphal differentiation. Based on the aforementioned evidence, we inferred that Brf1 and Snf5 are subunits specific to the SWI/SNF complex while Sfh1, Rsc1, Rsc4, Rsc7, and Rsc9 are subunits specific to the RSC complex in *C. neoformans* (Table 2). Snf2, Arp4, Arp9, Rsc6, and Rsc8 are the shared subunits (Table 2, Supplementary Fig. 5a). Consistent with the idea that these two complexes have overlapping and distinct subunits, the transcript levels of the shared components are generally higher (purple) than the complex-specific subunits (yellow or blue) based on a cell-cycle-regulated transcriptome data. In both *S. cerevisiae* and *S. pombe*,
many subunits in the RSC complex are essential for growth, including Sfh1 and Rsc9 (Table 2)\textsuperscript{3,40,41}. By contrast, the RSC-specific SFH1 and RSC9 genes are not essential for growth in C. neoformans, although the sfh1Δ and the rsc9Δ mutants grew slower (Fig. 3f, g). Surprisingly, deletion of the SNF/SWI complex-specific BRF1 or SNF5 did not cause any growth defects under the same conditions (Fig. 3f, g).Remarkably, the snf5Δsfh1Δ double mutant, where both the SWI/SNF-specific subunit Snf5 and the RSC-specific subunit Sfh1 were disrupted, was still viable despite a much more pronounced growth defect (Fig. 3g). Thus, the SWI/SNF complex functionally differ from the RSC complex in C. neoformans. Neither the RSC complex nor both RSC and SWI/SNF complexes together are essential in this basidiomycetous fungus, in contrast to what is known in ascomycetes.

### Table 1 The list of proteins identified from Co-IP/MS by Brf1-CBP-2×FLAG as bait

| Coding locus (D) | Coding locus (A) | Protein name | JL401 (strain 1) | JL402 (strain 2) |
|------------------|------------------|--------------|-----------------|-----------------|
| CNE04020         | CNAG_02134       | Rsc8\textsuperscript{a} | 12              | 20              |
| CKN02030         | CNAG_01863       | Snf2\textsuperscript{a} | 10              | 23              |
| CN00898          | CNAG_04460       | Arp9\textsuperscript{a} | 8               | 9               |
| CNA07190         | CNAG_00740       | Sfh1\textsuperscript{a} | 4               | 5               |
| CND01230         | CNAG_00995       | Msc1         | 4               | 3               |
| CNG02950         | CNAG_03285       | Rsc6\textsuperscript{a} | 3               | 10              |
| CNB05320         | CNAG_04048       | Arp4\textsuperscript{a} | 3               | 4               |
| CNE02000         | CNAG_02350       | Rss1\textsuperscript{a} | 3               | 3               |
| CNA02240         | CNAG_00240       | Brf1\textsuperscript{b} | 2               | 6               |
| CNA00820         | CNAG_00091       | N/A          | 2               | 2               |
| CNK02620         | CNAG_01920       | Ubi4         | 2               | 2               |

\textsuperscript{a}The SWI/SNF and RSC complex shared subunits \textsuperscript{b}The SWI/SNF-specific subunits
Given that the two SWI/SNF complex-specific subunits Brf1 and Snf5, and none of the RSC complex-specific subunits were identified from our genetic screen, we predict that the SWI/SNF complex, but not the RSC complex, specifically regulates the yeast-hypha differentiation in *C. neoformans*. To test this hypothesis, we examined the impact of the disruption of the SWI/SNF complex (*brf1Δ* and *snf5Δ*) or the RSC complex (*sfh1Δ* and *rsc9Δ*) on filamentation. Indeed, the RSC complex mutants, *sfh1Δ* and *rsc9Δ*, were still filamentous on V8 medium, in contrast to the yeast growth of the SWI/SNF complex mutants *brf1Δ* and *snf5Δ* (Fig. 3e).

**BRF1 is required for the induction of the ZNF2 transcripts.** As Brf1 and Snf5 work together in the SWI/SNF complex, we decided to use Brf1 to further dissect the relationship between the SWI/SNF complex and Znf2 in controlling morphogenesis in *C. neoformans*. To study the genetic relationship between *BRF1* and *ZNF2*, we crossed a *brf1Δ/BRF1*oe strain with a *znf2Δ/ZNF2*oe strain. We micro-dissected meiotic progeny from the cross and confirmed the genotypes of the progeny by diagnostic PCRs (Fig. 4a). As expected, the progeny of the wild-type genotype were self-filamentous, while the progeny of the *znf2Δ* mutant or the *brf1Δ* genotype were non-filamentous. *ZNF2*oe/*znf2Δ* strains were filamentous, but not *ZN2*oe/*brf1Δ* (Fig. 4b). This result is consistent with our earlier observation that overexpression of Znf2 did not restore filamentation in the *brf1*Δ insertional mutant (Fig. 1c).

Likewise, *BRF1*oe/*brf1Δ* strains were filamentous, but not *BRF1*oe/*znf2Δ* (Fig. 4b). Thus, overexpression of either *BRF1* or *ZNF2* cannot override the absence of the other, and both are essential for yeast-hypha differentiation in *C. neoformans*.

We next analyzed the transcriptomes of WT, *brf1Δ*, *brf1Δ/BRF1*oe, and *ZNF2*oe strains cultured under filamentation-repressing YPD medium and filamentation-inducing V8 medium (Supplementary Data 2). More than 60% of the differentially expressed genes (up- or down-regulated) in *brf1Δ* were also differentially expressed in the *znf2Δ* mutant under filamentation-inducing condition (Fig. 4c). Remarkably, >95% (95/99) of the overlapping genes were down-regulated upon deletion of *BRF1* or *ZNF2*, suggesting that Brf1 and Znf2 activate these genes during hyphal differentiation. As Znf2 filamentation is primarily induced by the pheromone pathway when cells are cultured on V8 medium (see Fig. 3a, right panel), we predicted that deletion of either *BRF1* or *ZNF2* dampened the induction of the pheromone pathway genes: pheromone *MFA* (181× reduction), pheromone receptor *STE3* (14× reduction), pheromone exporter *STE6* (7× reduction), MAPK *CPK1* (5.1× reduction), and pheromone transcription factor *MAT2* (9× reduction) (Fig. 4c, right panel). The reduced activation of the pheromone pathway in the *brf1Δ* mutant lowered cell fusion which we observed earlier (Supplementary Fig. 2b). On the other hand, much fewer genes were differentially expressed in *brf1Δ* and *znf2Δ* under filamentation-suppression YPD condition (Supplementary Fig. 6, Supplementary Data 2).

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**Fig. 4** *BRF1* is required for ZNF2 transcription induction during hyphal differentiation. **a** The Venn diagram showing the number of progeny for each genotype from a cross between *znf2Δ/ZNF2*oe and *brf1Δ/BRF1*oe. *n* indicates the total meiotic spores analyzed. **b** WT XL280 and selected progeny of the following genotypes (*brf1Δ, brf1Δ + BRF1*oe, *brf1Δ + ZNF2*oe, *znf2Δ, znf2Δ + ZNF2*oe, and *znf2Δ + BRF1*oe) were cultured on V8 + BCS medium (to induce ZNF2) at 22 °C in dark for 5 days. **c** Volcano plots of fold changes in transcript level in the *znf2Δ* mutant and the *brf1Δ* mutant compared to WT cultured on V8 agar medium for 24 h. Each dot in the plots indicates a protein-coding gene. The vertical dash lines indicate the |log2FC| = 2 and the horizontal dash line shows the FDR = 0.05. The differentially up-regulated genes are indicated in red and the differentially down-regulated genes are indicated in blue. The shared DEGs between the *znf2Δ* and the *brf1Δ* mutants are colored in orange. **d** The relative transcript levels of ZNF2 in WT, *brf1Δ*, and *brf1Δ/BRF1*oe cultured on V8 agar medium and YPD medium. The ZNF2 transcript level in WT cultured on YPD medium was used for normalization and was set as 1
The transcript level of ZNF2 in WT increased 32-fold under filamentation-inducing condition compared to filamentation-suppressing conditions. In the absence of BRF1, however, the ZNF2 transcript level remained at the basal level as observed under filamentation-suppressing conditions (Fig. 4d). Ectopic overexpression of BRF1 in the brf1Δ strain restored the dramatic induction of the ZNF2 transcript level under filamentation-inducing conditions (Fig. 4d). By contrast, constitutive expression of ZNF2 (54.75× increase) had minimal impact on the transcript level of BRF1 (1.27× increase). The transcript of BRF1 remained at a low and steady level when cells were cultured either in YPD or on V8 medium. The steady level of BRF1 transcripts was also observed at different cell cycle stages (Fig. 3d). The results indicate that BRF1 is expressed at a low but constant level and is not influenced by Znf2. The induction of ZNF2, however, requires Brf1.

Brf1 is required for Znf2’s full association to its targets. Brf1 is required for ZNF2 induction; however, even when ZNF2 was ectopically overexpressed, Znf2 still failed to drive filamentation in the absence of Brf1 (Figs. 1c and 4b). Therefore, beyond transcription activation of ZNF2, Brf1 is critical for existent Znf2 protein to execute its function. We hypothesize that Znf2’s association with DNA may require Brf1.

To determine if the ability of Znf2 to bind to its downstream targets is affected by the SWI/SNF complex, we conducted chromatin immunoprecipitation (ChIP) experiments using 3xFLAG-tagged Znf2 as the bait in the WT or the brf1Δ mutant background. We first used qPCR to compare the relative abundance of the precipitated genetic locus of the filamentation marker gene CFO1, a proxy for Znf2 downstream targets. We found that Znf2 strongly binds to the CFO1 promoter (Supplementary Fig. 7a). We also found that Znf2 strongly binds to its own promoter (Supplementary Fig. 7b), suggesting that Znf2 likely autoregulates itself. In the absence of Brf1, the association of Znf2 to the promoter regions of CFO1 and ZNF2 decreased 2–3-fold (Fig. 5a, Supplementary Fig. 7). Thus, the SWI/SNF complex helps Znf2 associate with its downstream targets, including ZNF2 itself.

We further investigated the requirement of Brf1 for Znf2’s binding to its targets by analyzing the sequencing results of the same ChIP samples. A total of 361 potential Znf2-binding regions were identified (Fig. 5b, Supplementary Data 3). Consistent with our ChIP-qPCR results (Fig. 5a), Znf2’s binding to most of its downstream sites, including the ZNF2 and CFO1 promoter regions, was dampened upon the disruption of Brf1 (Fig. 5b, f). The finding is consistent with our earlier transcriptome data showing that BRF1 is required for ZNF2 transcription induction and that brf1Δ shares more than 60% of the differentially expressed genes with znf2Δ when cultured under filamentation-inducing conditions (Fig. 4d). Thus, Brf1, a subunit of the chromatin-remodeling SWI/SNF complex, is required for full association of the transcription factor Znf2 with its downstream targets.

Brf1 and Znf2 work in concert in cellular differentiation. The SWI/SNF complex is known to evict or slide nucleosomes to change chromatin structure. To test if Brf1 affects cell differentiation through chromatin remodeling of ZNF2 and Znf2 target regions, we employed the Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) and compared genetic regions with open chromatin structures in the WT strain, grown under filamentation-repressing or -inducing conditions, as well as in the brf1Δ, znf2Δ, and snf5Δ strains cultured under filamentation-inducing conditions. We included the brf1Δ/snf5Δ strain as an additional control. As expected, accessible chromatin fell in the promoter regions, with expressed genes having a higher level of accessibility based on the relative enrichment of ATAC-seq reads (Fig. 5c). Globally, chromatin accessibility was similar in all strains tested (Fig. 5d; Supplementary Data 4). When we examined the chromatin accessibility of the 361 regions that Znf2 potentially binds to based on the ChIP data (Fig. 5b; Supplementary Data 3), we noticed a significant reduction in chromatin accessibility in brf1Δ and snf5Δ across most of the regions, which now resembled the accessibility pattern for WT grown under the filamentation-suppression condition (Fig. 5e). K-means clustering further showed that Znf2-binding sites in clusters 1 and 2 (83 regions) did not strongly depend on Brf1, Snf5, or Znf2 to maintain open chromatin. Cluster 3 peaks (118 Znf2-binding regions) had low accessibility in YPD but high accessibility on V8. Accessibility of cluster 3 peaks was largely dependent on Brf1, Snf5, and Znf2. Cluster 5 (138 peaks) showed moderate accessibility on V8, which depended on Brf1, Snf5, and Znf2. Cluster 4 (22 peaks) was highly accessible on V8 and this depended on Brf1 and Snf5, but less dependent on Znf2. Fifteen out of the 19 genes potentially affected by the Brf1-dependent ATAC-seq peaks (cluster 4) overlapped with genes up-regulated under hyphal-promoting conditions based on the transcriptome data (Supplementary Data 3, colored in red). The genomic coordinates, cluster number, and nearest gene promoter for each Znf2-binding region are listed in Supplementary Data 3.

We next identified regions with differential ATAC enrichment in WT and brf1Δ cultured in filamentation-inducing conditions (Supplementary Data 5). Forty-one accessible regions were dependent on Brf1 (Supplementary Data 5, labeled in red), including regions immediately upstream of CFO1 and ZNF2. Surprisingly, 1727 regions were identified as hyper-accessible in brf1Δ compared to WT. A heatmap to visualize ATAC-reads across all differential peaks revealed that hyper-accessible regions identified in brf1Δ were associated with reduced accessibility at adjacent sites (Supplementary Fig. 8). Thus, these hyper-accessible regions may accumulate as a result of reduced nucleosome mobility at these sites in brf1Δ. Ninety-four percent of differential peaks in brf1Δ overlapped a differential peak identified in snf5Δ, consistent with Brf1 and Snf5 functioning together in the same complex (Fig. 5d, Supplementary Data 6).

RNA-seq, ChIP-seq, and ATAC-seq reads for three of these genes, along with a control gene CNA07690 that does not require Brf1 for its open chromatin are shown in Fig. 5f. Most noticeably, the promoter region of the filamentation marker gene CFO1 was the top differentially accessible region (10.02× change) (Fig. 5d, Supplementary Data 5). CFO1 is one of the highest induced genes controlled by Znf2 (ref. 21). Disruption of either Brf1, Snf5, or Znf2 abolished accessibility of the CFO1 promoter (ATAC-seq), where Znf2 binds to (ChIP-seq) (Fig. 5b, f). Accordingly, the CFO1 transcript level was almost undetectable low when BRF1 or ZNF2 was deleted. Overexpression of BRF1 in brf1Δ restored chromatin accessibility of the CFO1 promoter and also its transcript level (Fig. 5f). Similarly, we found that the ZNF2 promoter region became inaccessible in the brf1Δ and the znf2Δ mutants (5.88-fold change; Supplementary Data 5).

Collectively, the data support the interdependence between the transcription factor Znf2 and the SWI/SNF complex in opening up chromatin to facilitate transcription of filamentation genes.

Discussion

Chromatin remodeling plays critical roles in cellular differentiation in eukaryotes. The model ascomycetous yeasts S. cerevisiae and S. pombe have offered a relatively simple system for mechanistic
studies. Basidiomycetous fungi resemble more of the higher eukaryotes in terms of genome structures, epigenetic regulation, and transcriptome complexity, but research on chromatin remodeling in this major phylum of the fungal kingdom is scarce. To our knowledge, our study is the first to identify and characterize a basidiomycete-specific factor that serves as a critical subunit in the SWI/SNF complex. We demonstrated that this phylum-specific factor Brf1, together with the conserved known subunit Snf5 of the SWI/SNF complex, is essential for hyphal growth and sexual development in the basidiomycete 

C. neofor- manes.

This complex remodels the chromatin structure of the promoter regions of filamentation genes to make them accessible for transcription (Fig. 5g). Brf1 is vital for transcriptional induction of ZNF2 and also full association of Znf2 protein to the promoters of its downstream targets (e.g. CFL1 and ZNF2). Our findings are consistent with published literature in other organisms, in which the SWI/SNF complex contributes to the DNA binding of the transcription factor46,47. The SWI/SNF complex has been shown being targeted to specific genetic loci by sequence-specific transcription factors and acetylation of histone tails.46,48,49. In the ascomycetous fungus C. albicans, the transcription factor Efg1 recruits the histone acetyltransferase

Fig. 5 Znf2 and the SWI/SNF complex coordinate in transcription activation of filamentation genes. a The relative abundance of DNAs associated with Znf2 in designated strains grown in YPD + BCS media based on ChIP-qPCR. The points represent data from two technical replicates of each biological duplicate. b The heat map of the relative enrichment of Znf2-FLAG binding regions in WT and the brf1Δ mutant across all potential 361 Znf2-binding sites identified from ChIP-seq (lowest p value on top and the highest p value on bottom). c The heat map depicts relative enrichment of ATAC-seq reads from WT grown on V8 medium. The peaks are centered on the transcription start sites (TSS) for all genes arranged from the highest expression level (top) to the lowest expression level (bottom). d Global chromatin accessibility in the tested mutants. The relative enrichment of ATAC-seq reads for all ATAC-seq peaks from the WT strain grown on V8 medium is shown for each indicated strain. e Relative enrichment of the ATAC-seq peaks over the 361 Znf2-FLAG binding regions in WT, brf1Δ, brf1Δ + BRF1Δ, snf5Δ, and znf2Δ cells. f Genome browser images depict relative transcript levels based on RNA-seq (purple), ChIP-seq (blue), and ATAC-seq enrichment (red) for the four indicated genetic regions of the indicated strains. Images depicting representative ATAC-seq peaks at the three genetic loci that display reduced ATAC-seq enrichment in brf1Δ (CFL1, CND00490, and ZNF2) as well as one control genetic locus (CNA07690) that does not exhibit altered accessibility with or without Brf1. WT grown in YPD or on V8 medium is used as the negative or the positive control for filamentation. The down arrows indicate the location of differential accessible regions. The arrows at the bottom of the plot indicate direction of transcription.

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complex NuA4 to the promoters of hypha-associated genes, allowing, in turn, the recruitment of the SWI/SNF complex to activate their transcription. We reason that Znf2 may recruit the SWI/SNF complex to its target sites given that accessibility at the CFL1 promoter region is lost in the absence of ZNF2 (Fig. 5b–f). Although we could not establish direct physical interaction between Znf2 and the SWI/SNF complex via Co-IP experiment, the interaction may be too weak or transient to be captured by our assay. Alternatively, histone modifications or the modification enzymes may bridge the connection. Several SWI/SNF complex subunits possess domains that recognize histone modifications, such as bromodomains for histone acetylation.

How exactly the SWI/SNF complex works with Znf2 in C. neoformans to control differentiation warrants further investigation.

Our findings revealed striking differences in the two complexes (SWI/SNF and RSC) between Ascomycota and Basidiomycota. In Saccharomyces and Schizosaccharomyces species, most subunits in the RSC complex are essential for growth, including the catalytic ATPase subunit Snf2 (or SpSnf22), Sfh1, and Rsc9 (refs. 5, 40, 41) (Table 3). Snf2 is not essential in C. neoformans. Consistently, deletion of both SNF5 (SWI/SNF-specific) and SFH1 (RSC-specific) together slowed growth but did not cause lethality (Fig. 3g). Comparative functional analyses of the RSC complex among evolutionarily diverse species, therefore, provides a unique vantage point to understand the biological function of the RSC complex.

All SWI/SNF complexes have a subunit with a conserved Snf5 domain, which acts as a scaffold protein. The majority of fungi examined carry two proteins bearing a Snf5 domain (Pfam domain ID PF04855), presumably one acting as the Snf5 subunit in the SWI/SNF complex and one acting as the Sfh1 subunit in the RSC complex (Fig. 3c, Supplementary Table 3). Based on primary protein sequences, Snf5 proteins in Ascomycota separate into two clades, Snf5 and Sfh1 (Fig. 3c). C. neoformans and other basidiomycetes harbor two proteins with an Snf5 domain: SNF5 and SFH1 and they cluster closely with the Sfh1 clade in ascomycetes (Fig. 3c). The SWI/SNF complex assembles in an ordered modular fashion. In Saccharomyces, Snf5 and Sfh1 (the ARID-containing protein) likely belong to different modules given the phenotypical differences of the snf5 and swh1 mutants. However, Cryptococcus snf5 and bfr1 mutants have nearly identical phenotypes. Furthermore, Snf5 proteins in basidiomycetes (C. neoformans: 1784 aa, U. maydis: 2080 aa) are much larger in size compared to those in ascomycetes or in humans (S. cerevisiae: 905 aa, S. pombe: 632 aa, or humans: 385 aa) (Fig. 3c). Two internal repeats near the N-terminus of Snf5 proteins in basidiomycetes (Supplementary Fig. 5b) could potentially increase binding surface area and assist assembling the SWI/SNF complex.

The SWI/SNF family employs Snf2 as the ATPase catalytic subunit, which carries an HSA and a BROMO domain (the DEXDc and HELICc regions). A search for the HSA domain (PF07529) and the BROMO domain (PF00439) revealed that some fungi, including ascomycete S. cerevisiae and zygomycete Mucor circinelloides, have two distinct Snf2 family ATPases, presumably one for the SWI/SNF complex and one for the RSC complex. Some other fungi, including ascomycete Aspergillus nidulans and basidiomycete C. neoformans, however, have only one Snf2 ATPase complex, presumably shared by SWI/SNF and RSC (Table 2, Supplementary Table 4). In Drosophila melanogaster, the BAP (SNF/SWI) complex and the PBAP (RSC) complex share the same catalytic ATPase subunit (BRM). Human BAF (mSNF/SWI) complex and PBAP (mRSC) complex can share the same ATPase BRG-1 subunit or use different ATPases (hBRM and BRG-1). Thus, the ATPase could be shared by or be unique to the SWI/SNF and the RSC complexes. The copy number variation of SNF2 might have driven such divergence independent of the evolutionary distance of the species.

In conclusion, we discovered the basidiomycete-specific subunit of SWI/SNF chromatin remodeling complex and revealed the major differences in the composition and biology of this complex between ascomycetes and basidiomycetes. Given the universal importance of the SWI/SNF complex in cellular differentiation in eukaryotes, our findings provide an important platform for future comparative functional analyses of this complex in diverse eukaryotic lineages.
ZNF2 ORF was ligated into a vector where a 3xFLAG tag was downstream of the CTR4 promoter to allow tagging at the N-terminus. The functionality of the tagged proteins was confirmed by their ability to rescue/restore the phenotypes in the corresponding gene deletion strains. All constructed plasmids used in this study are listed in Supplementary Table 6. All the primers used for constructing or confirming gene deletion, gene overexpression, or protein tagging are listed in Supplementary Table 7.

### Phenotypic assays.

For phenotypic analyses, cells of the tested strains were cultured in YPD broth overnight at 30 °C with shaking at 230 r.p.m. Cells were collected by centrifugation, washed twice with sterile water, and then suspended with water to the same optical density at 600 nm (OD\textsubscript{600} = 3). Three microliters of cell suspension and 10× serial dilutions were spotted onto YPD agar medium or YPD with the supplement of Congo Red (0.3–0.5%) and were incubated at 30 or 37 °C for 1–2 days. To test carbon source utilization, glucose in the YNB medium was added to 950 μM in V8. To examine filamentation during bisexual mating, an equal number of cells of compatible mating types were mixed, spotted onto V8 agar medium (pH 5 for mating assay of serotype A strains and pH 7 for mating assay of serotype D strains) and incubated at 22 °C in dark for 2–10 days. To examine filamentation during bisexual mating, an equal number of cells of compatible mating types were mixed, spotted onto V8 agar medium (pH 5 for mating assay of serotype A strains and pH 7 for mating assay of serotype D strains) and incubated at 22 °C in dark for 1–7 days.

For strains that use the CTR4-2-inducible promoter to drive the expression of the examined genes, cells were maintained on YPD medium with 50 μM CuSO\textsubscript{4} to suppress the gene expression. To induce the gene expression, the copper chelator BCS was supplemented to the medium with the final concentration of 200 μM in YPD and 50 μM in V8.

### Cell growth assay.

Cryptococcal cells from overnight culture in YPD broth were centrifuged, washed twice with water, and resuspended in water. The cell concentration was normalized to the same optical density at 600 nm (OD\textsubscript{600} = 3). Three microliters of cell suspension and 10× serial dilutions were spotted onto YPD agar medium or YPD with the supplement of Congo Red (0.3–0.5%) and were incubated at 30 or 37 °C for 1–2 days. To test carbon source utilization, glucose in the YNB medium was added to 950 μM in V8. To examine filamentation during bisexual mating, an equal number of cells of compatible mating types were mixed, spotted onto V8 agar medium (pH 5 for mating assay of serotype A strains and pH 7 for mating assay of serotype D strains) and incubated at 22 °C in dark for 2–10 days. To examine filamentation during bisexual mating, an equal number of cells of compatible mating types were mixed, spotted onto V8 agar medium (pH 5 for mating assay of serotype A strains and pH 7 for mating assay of serotype D strains) and incubated at 22 °C in dark for 1–7 days.

For strains that use the CTR4-2-inducible promoter to drive the expression of the examined genes, cells were maintained on YPD medium with 50 μM CuSO\textsubscript{4} to suppress the gene expression. To induce the gene expression, the copper chelator BCS was supplemented to the medium with the final concentration of 200 μM in YPD and 50 μM in V8.

### RNA extraction and RNA-seq.

RNAs were extracted from cells cultured on V8 medium at 22 °C in dark for 24 h and prepared for sequencing as previously described\textsuperscript{19,20,60}. HiSeq Rapid 175bp pair-end RNA sequencing was performed at the Georgia genome sequencing facility. The low-quality bases of the raw reads were trimmed with a custom perl script as published before\textsuperscript{60}. TopHat2 was used to map the processed reads to the reference genome. The program HTSeq-count and DESeq2 were used to count the reads and identify the differentially expressed genes. The raw sequencing reads were deposited at NCBI with the BioProject accession number PRJNA534125 and SRA file numbers from SRR8947060 to SRR8947075.

### Mating, genetic crosses, and cell fusion assays.

Strains of α and a mating partners were crossed on V8 juice agar medium (pH 5 or 7) and incubated at 22 °C in dark for 2–3 weeks until adequate spores were produced. Spores were micro-manipulated using a dissecting microscope. The mating type of the germinated spores was determined by successful mating with either JEC20α or JEC21α reference strain. Genetic linkage between the presence of the drug marker and the observed mutant phenotype was established by analyzing the dissected spores as we described previously\textsuperscript{97}. If the insertional mutant harbors only one copy of the T-DNA, which carries the hygromycin selection marker HYG\textsuperscript{5}, we would expect a 1:1 ratio of hygromycin-sensitive (HYG\textsuperscript{5}) and hygromycin-resistant (HYG\textsuperscript{5}) progeny. If that single insertion caused the non- filamentous phenotype, then all HYG\textsuperscript{5} progeny would be non-filamentous while all HYG\textsuperscript{5} progeny would be filamentous under a Znf2-inducing condition. In comparison, when multiple T-DNAs inserted in the genome, the ratio between HYG\textsuperscript{5} and HYG\textsuperscript{5} meiotic progeny would be 1:3 (two unlinked insertions) or lower (>2 unlinked T-DNA insertions). For each genetic linkage assay, approximately 32 viable spores were analyzed, which gave a 97% confidence level of our analysis (confidence = 100% – (1/log\textsubscript{2} # of spores))

To determine the cell–cell fusion efficiency, mutants of the mating type α (znf2::NAT; mat2::NAT; brf1::NAT; and sfp5::NAT) and the control strain (pfi1::NAT) were collected and suspended in water to the same optical density (the calculated OD\textsubscript{600} = 0.3 × 10\textsuperscript{6}) Each mutant with NAT\textsuperscript{5} was mixed with the mating type α tester strain with NEO\textsuperscript{5} marker (strain YSB133) with equal volume\textsuperscript{58}. Three microliters of cell mixture were spotted onto V8 medium and incubated at 22 °C in dark for 24 h before they were collected, plated (in serial dilution) onto YPD + NEO + NAT agar medium, and incubated at 30 °C for 3–5 days to select for fusion products.

### Databases and online tools.

We used FungiDB to acquire the gene/protein sequences or the normalized FPKM data of C. neoformans H99 genes, S. cerevisiae genes and genes in other fungi. Clustal Omega was used for protein multiple sequence alignment and phylogenetic tree analysis. The gene tree of BRF1 (CNA02310) was retrieved from the Ensembl Fungi database. Simple Modular Architecture Research Tool (SMART) online server was used to analyze the domain layouts for proteins. IBS online illustrator tool was used to illustrate all the protein domain layouts\textsuperscript{59}.

Co-immunoprecipitation/mass spectrometry and co-IP/western. Strains with tagged proteins were cultured in 15 mL YPD media overnight, washed twice with cold water, flash frozen in liquid nitrogen, and lyophilized. Lyophilized cells were broken into fine powder with silica beads in a Bullet Blender Blue™ (Next Advance) without buffer for five cycles (60 s maximum blending followed by 90 s chilling on ice) and then with 1 mL of lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 5 mM MgCl\textsubscript{2}, 1 mM DTT, 10% glycerol, 0.5 mM PMSF, and protease inhibitors (#A32963, ThermoFisher)) for another five cycles. Cell lysates were centrifuged at 500 × g at 4 °C for 5 min. The supernatant was collected and centrifuged again at 10,000 × g at 4 °C for 20 min. The supernatant was then incubated with pre-washed anti-FLAG M2 agarose beads (#F2426, Sigma) at 4 °C on a rotator overnight. For immunoprecipitation with mCherry or mNeonGreen-tagged proteins, RFP-Trap®_MA or mNeonGreen-
Trap_MA beads from Chromotek (Germany) were used following the manufacturer’s instructions.

After washing, immunoprecipitated sample proteins were released from beads in the 2×SDS loading buffer (120mM Tris-HCl pH 6.8, 20% glycerol, 4% SDS, 0.04% bromophenol blue, and 10% β-mercaptoethanol) by boiling for 10 min.

For Co-IP/MS, protein samples were then loaded into pre-casted 4–12% SurePAGE™ Bis-Tris Gel (GeneScript), run for 5 min, and visualized by Coomasie Blue staining. The total pulled down protein samples were excised from the gel and sent to the proteomics and mass spectrometry facility (https://pams.uga.edu/) at the University of Georgia for identification. When analyzing the results, we applied the following criteria to exclude nonspecific proteins: proteins from the negative control, proteins not shared between samples of the two P\textsubscript{TELF}:BRF1-CBP-2×FLAG strains, and non-nuclear proteins as Brf1 localizes to the nucleus.

For Co-IP/western, IP proteins samples from mNeonGreen-trap along with the whole protein extracts (WCE) were run in an SDS-PAGE gel (30 μL of WCE and IP), transferred onto PVDF membrane, and western-blotted with anti-FLAG M2 antibodies (#F3165, Sigma).

Chromatin immunoprecipitation, ChIP-qPCR, and ChIP-seq.

FLAG-tagged Znf2 strains (strain JL653 and JL665) (initial OD\textsubscript{600} = 0.2) were incubated in 50mL YPD + BCS broth at 30 °C until the optical density reached OD\textsubscript{600} = 1. Cells were then fixed in the medium with formaldehyde at 1% final concentration at 22 °C for 15 min with occasional swirling. Glycine with the final concentration of 0.125 M was added to quench the crosslinking at 22 °C for 5 min. The cells were then washed twice with cold sterile water and lyophilized. Lyophilized cells were broken as described above for Co-IP experiment with a different lysis buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% NaDOC, 1 mM of PMSF, and proteinase inhibitor cocktail). The following steps were similar to what has been described previously\textsuperscript{43,61–63} and briefly, the cell lysates were centrifuged at 8000 r.p.m. at 4 °C for 10 min to enrich nuclei. The released nuclei were resuspended in 350 μL of lysis buffer. The nuclear suspensions were sheared by sonication in a Diagenode Bioruptor™ for 25 cycles at 4 °C (30 s on, 30 s off; cycle numbers varied). The protein–chromatin complex was recovered from the supernatant after centrifugation at 14,000 r.p.m. at 4 °C for 10 min and the volume was brought to 1 mL. Fifty microliters of each protein–chromatin suspension was saved as Input DNA. The rest of the sample was added to 30 μL anti-FLAG M2 monoclonal antibodies on magnetic beads (#M8823, Sigma) to precipitate the FLAG-tagged protein. After incubation with antibodies at 4 °C overnight, the beads were washed twice with 1 mL ChIP lysis buffer, once in 1 mL ChIP lysis buffer containing 0.5 M NaCl, once in 1 mL ChIP wash buffer (10 mM Tris-HCl, pH 8.0; 0.25 M LiCl; 0.5% NP-40; 0.5% NaDOC; 1 mM EDTA), and once in 1 mL TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA), all at 4 °C. The immunoprecipitated Znf2-3xFLAG was eluted twice by adding 200 μL of TES buffer (10 mM Tris-HCl, pH 8.0; 10 mM EDTA; 1% SDS) and incubated at 75 °C for 15 min before the supernatant was transferred to a new tube. Twenty microliters of 5 M NaCl was added to each sample to de-crosslink at 65 °C for 4 h. Three hundred and fifty microliters of TES was added to each Input DNA sample before adding 20 μL of 5 M NaCl to reverse formaldehyde crosslinks. One microliter of RNAse A (10 μg/μL) was added and the sample was incubated at 37 °C for 30 min. Four microliters of Proteinase K (20 μg/μL) was then added to each sample and incubated at 45 °C for at least 1 h to digest all the proteins. Eventually, 2 μL of glycogen (molecular level, 20 mg/mL) was added to chromatin DNA samples. Ethanol of 2.5 volumes was added to each sample to precipitate the glycogen tangled DNA. The precipitated DNA sample was then suspended in 80 μL of nuclease-free water.

For ChIP-qPCR, 3 μL of five times diluted DNA sample and 1 μL each from two primers (50 μM) were mixed with 5 μL of SYBR Green 2x qPCR premix reagents (Invitrogen). The qPCR reactions were carried out in a Realpex system (Eppendorf) with technical duplicates. The % Input is calculated as 2 (ΔC[T normal ChIP] - ΔC[T input] - log[ΔC(T input dilution factor)]) as described previously\textsuperscript{63}.

The same ChIP samples for qPCRs were sent for DNA sequencing at Georgia Genomics Facility (UGA) on an Illumina NextSeq500 platform. Input from ChIP-seq experiments in WT strains was used as a background control for all samples. Differential peaks called using the findPeaks tool, part of them were annotated using ChipSeeker\textsuperscript{64,65}. ChIP-seq have been submitted to the GEO database (accession # GSE137248).

ATAC-seq and data analysis.

\textit{C. neoformans} cells were cultured in 15 mL YPD liquid media at 30 °C overnight or on V8 medium (pH 7) at 22 °C in dark for 24 h. Cells were collected and washed two times with cold sterile water. Five hundred microliters of cold lysis buffer (15 mM Tris pH 7.5; 2 mM EDTA; 0.5 mM spermine; 80 mM KCl; 20 mM NaCl; 15 mM (or 0.1% w/v) β-me; 0.3% TritonX-100) and 200 μL (~1 PCR tube) of acid-washed glass beads (0.5mm, #9831 RPI) were added to the cell pellet of about 10\textsuperscript{8} cells in 1.7 mL Eppendorf tubes. Cells were broken in a cell disruptor (Scientific Industries, Inc., SI-D238) at 4 °C with maximum speed for 2 min and then spun down at 50 × g at 4 °C for 2 min to remove the glass beads. The supernatant was transferred into a new Eppendorf tube and spun at 200 × g for another 2 min to remove most of the broken cells and debris. The enrichment of nuclei in the supernatant was verified by microscopic observation and the nuclei were collected for making the ATAC-seq library.

ATAC-seq libraries were generated as follows. Briefly, about 0.2 million nuclei were incubated with Tn5 transposase preloaded with Illumina sequencing adapters at 37 °C for 30 min followed by purification of the DNA fragments by a reaction cleanup kit (Qiagen, # 28204). Libraries were PCR amplified for 10 cycles with Phusion polymerase (Thermofisher Scientific, # F3530L). Sequencing libraries were then cleaned with magnetic beads to remove free adapters and primer dimers (Beckman Coulter, #A63880). Libraries were mixed in equimolar ratios and pair-end sequenced by the Georgia Genomics Facility (UGA) on an Illumina NextSeq500 platform.

ATAC-seq reads were filtered for low-quality and short reads with Trim Galore (https://github.com/FelixKrueger/TrimGalore). Duplicate reads were removed using Picard MarkDuplicates tool (http://broadinstitute.github.io/picard/). Reads were aligned to the JEC21 reference genome (Refseq assembly GCF_000091045.1) with HISAT2 using non-spliced alignment and a maximum fragment length of 2000 bp. Peak calling was performed with MACS2 using a q-value of 0.01, extension size of 73, and shifting reads by 37 bp to center on the insertion site. MACS2 was also used to call differential peaks\textsuperscript{66}. Differential peak files were first sorted by fold change and then combined into a single file. Genes nearby Znf2 peaks were annotated using ChipSeeker\textsuperscript{64,65}. ATAC-seq enrichment was calculated across Znf2 peaks or differential peaks between WT and \textit{brf1Δ} using the annotatePeaks function, part of the HOMER (Hypergeometric Optimization of Motif EnRichment) suite of informatics tools, with parameters -size 3000 and -ghist\textsuperscript{67}. Heatmaps were generated in R using the heatmap package version 1.0.12. Differential peaks from ATAC-seq were compared to each other or Znf2 peaks or to genes identified as being up-regulated in WT V8 from RNA-seq analysis using
Monahan, B. J. et al. Fission yeast SWI/SNF and RSC complexes show shared mechanistic and functional differences from budding yeast. Chromatin remodeling complexes. Nat. Rev. Mol. Cell Biol. 18, 407–422 (2017).

Mohrmann, L. & Verrijzer, C. P. Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Biochim. Biophys. Acta 1681, 59–73 (2005).

Tang, L., Nogales, E. & Ciferri, C. Structure and function of SWI/SNF chromatin remodeling complexes and mechanistic implications for transcription. Prog. Biophys. Mol. Biol. 102, 122–128 (2010).

Mashtalir, N. et al. Modular organization and assembly of SWI/SNF family chromatin remodeling complexes. Cell 175, 1272–1288 e1220 (2018).

Monahan, B. J. et al. Fission yeast SWI/SNF and RSC complexes show compositional and functional differences from budding yeast. Nat. Struct. Mol. Biol. 15, 873–880 (2008).

Vignali, M., Hassan, A. H., Neely, K. E. & Workman, J. L. ATP-dependent chromatin-remodeling complexes. Mol. Cell. Biol. 20, 1899–1910 (2000).

Feretzi, M., Billmyre, B. R., Clancy, S. A., Wang, X. & Heitman, J. Gene network polymorphism illuminates loss and retention of novel RNAi silencing components in the Cryptococcus pathogenic species complex. PLoS Genet. 12, e1005688 (2016).

Loftus, B. J. et al. The genome of the basidiomycetous yeast and human fungal pathogen Cryptococcus neoformans. Science 307, 1321–1324 (2005).

Jambon, G. et al. Analysis of the genome and transcriptome of Cryptococcus neoformans var. grubii reveals complex RNA expression and microevolution leading to virulence attenuation. PLoS Genet. 10, e1004261 (2014).

Catania, S. et al. Evolutionary persistence of DNA methylation for millions of years after ancient loss of a de novo methyltransferase. Preprint at bioRxiv https://www.biorxiv.org/content/10.1101/149385v2 (2019).

Zhao, Y., Lin, J., Fan, Y. & Lin, X. Life Cycle of Cryptococcus neoformans. Annu. Rev. Microbiol. 73, 17–42 (2019).

Velagapudi, R., Hsoe, Y. P., Geunes-Boyer, S., Wright, J. R. & Heitman, J. Spores as infectious propagules of Cryptococcus neoformans. Infect. Immun. 77, 4345–4355 (2009).

Giles, S. S., Dagenais, T. R., Botts, M. R., Keller, N. P. & Hull, C. M. Elucidating the pathogenesis of spores from the human fungal pathogen Cryptococcus neoformans. Infect. Immun. 77, 3491–3500 (2009).

Ogakagi, I. H. & Nielsen, K. Titan cells confer protection from phagocytosis in Cryptococcus neoformans infections. Eukaryot. Cell 11, 826–826 (2012).

Zaragoza, O. & Nielsen, K. Titan cells in Cryptococcus neoformans: cells with a giant impact. Curr. Opin. Microbiol. 16, 409–413 (2013).

Lin, X., Jackson, J. C., Feretzi, M., Xue, C. & Heitman, J. Transcription factors Mat2 and Znf2 operate cellular circuits orchestrating opposite- and same mating in Cryptococcus neoformans. PLoS Genet. 6, e1000953 (2010).

Lin, J., Igdoura, A. & Lin, X. Morphology and its underlying genetic regulation impact the interaction between Cryptococcus neoformans and its hosts. Med. Mycol. 53, 493–504 (2015).

Lin, X. R., Hull, C. M. & Heitman, J. Sexual reproduction between partners of the same mating type in Cryptococcus neoformans. Nature 434, 1017–1021 (2005).

Gyawali, R. et al. Pheromone independent unisexual development in Cryptococcus neoformans. PLoS Genet. 13, e1006772 (2017).

Xu, X. et al. Glucosamine stimulates pheromone-independent dimorphic transition in Cryptococcus neoformans by promoting Ccz1 nuclear translocation. PLoS Genet. 13, e1006982 (2017).

Wang, L., Zhai, B. & Lin, X. The link between morphotype transition and virulence in Cryptococcus neoformans. PLoS Pathog. 8, e1002765 (2012).

Neiberg, L. & Carlson, M. Genes affecting the regulation of sexual development. Genetics 108, 865–884 (1984).

Clapier, C. R., Iwasa, J., Cairns, B. R. & Peterson, C. L. Mechanisms of action of chromatin-remodeling complexes. Nat. Rev. Mol. Cell Biol. 8, 57–68 (2007).

Bresch, P. D., Morschhäuser, J., Bücheler, E. & Schüller, C. The Czc1p Cytidine Kinase is a Virulence Factor of Cryptococcus neoformans. Cell Rep. 18, 2124–2134 (2017).

Sen, P. et al. Loss of Snf5 induces formation of an aberrant SWI/SNF complex. Cell Rep. 18, 2135–2147 (2017).

Cairns, B. R. et al. RSC, an essential, abundant chromatin-remodeling activity. FEBS Lett. 580, 2615–2622 (2006).

Wang, L. et al. Morphotype transition and sexual reproduction are genetically associated in a ubiquitous environmental pathogen. PLoS Pathog. 10, e1004185 (2014).

Tian, X. Y. et al. Cryptococcus neoformans sexual reproduction is controlled by a quorum sensing peptide. Nat. Microbiol. 3, 698–707 (2018).

BEDTools intersect to isolate these genes from the JEC21 annotation file for determining genes associated with differential peaks. ATAC-seq data have been submitted to the GEO database (accession # GSE137248).
44. Buenrostro, J. D., Bu, W., Chang, H. Y. & Greenleaf, W. J. ATAC-seq: A method for assaying chromatin accessibility genome-wide. *Carr. Protoc. Mol. Biol.* **109**, 21–29, https://doi.org/10.1007/0071142775.82139(109) (2015).

45. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat. Methods* **10**, 1213–1218 (2013).

46. Breyer, D. G. & Peterson, C. L. The yeast SWI-SNF complex facilitates binding of a transcriptional activator to nucleosomal sites in vivo. *Mol. Cell. Biol.* **17**, 4811–4819 (1997).

47. Wallberg, A. E. et al. Recruitment of the SWI-SNF chromatin remodeling complex as a mechanism of gene activation by the glucocorticoid receptor tau 1 activation domain. *Mol. Cell. Biol.* **20**, 2004–2013 (2000).

48. Kim, J. H., Saraf, A., Florens, L., Washburn, M. & Workman, J. L. Gcn5 regulates the dissociation of SWI/SNF from chromatin by acetylation of Swi5/Snf2. *Genes Dev.* **24**, 2766–2771 (2010).

49. Neely, K. E., Hassan, A. H., Brown, C. E., Howe, L. & Workman, J. L. Transcription activator interactions with multiple SWI/SNF subunits. *Mol. Cell. Biol.* **22**, 1615–1625 (2002).

50. Lu. Y. et al. Egr1-mediated recruitment of NuA4 to promoters is required for hypha-specific Swi/Snf binding and activation in *Candida albicans*. *Mol. Biol. Cell* **19**, 4260–4272 (2008).

51. Yun, M., Wu, J., Workman, J. L. & Li, B. Readers of histone modifications. *Cell Res.* **21**, 564–578 (2011).

52. Giaever, G. et al. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418**, 387–391 (2002).

53. Idnurm, A., Reedy, J. L., Nuussbaum, J. C. & Heitman, J. *Cryptococcus neoformans* virulence gene discovery through insertional mutagenesis. *Eukaryot. Cell* **3**, 420–429 (2004).

54. Mondon, P. et al. Heteroresistance to fluconazole and voriconazole in *Cryptococcus neoformans*. *Antimicrobial Agents Chemother.* **43**, 1856–1861 (1999).

55. Toffalatti, D. L., Rude, T. H., Johnston, S. A., Durack, D. T. & Perfect, J. R. Gene transfer in *Cryptococcus neoformans* by use of biolistic delivery of DNA. *J. Bacteriol.* **175**, 1405–1411 (1993).

56. Fan, Y. & Lin, X. Multiple applications of a Transient CRISPR-Cas9 Coupled with Electroporation (TRACE) system in the *Cryptococcus neoformans* species complex. *Genetics* **208**, 1357–1372 (2018).

57. Zhai, B. et al. Congenic strains of the filamentous form of *Cryptococcus neoformans* for studies of fungal morphogenesis and virulence. *Infect. Immun.* **81**, 2626–2637 (2013).

58. Bahn, Y. S., Cox, G. M., Perfect, J. R. & Heitman, J. Carbonic anhydrase and CO2 sensing during *Cryptococcus neoformans* growth, differentiation, and virulence. *Curr. Biol.* 15, 2013–2020 (2005).

59. Liu, W. et al. IBS: an illustrator for the presentation and visualization of biological sequences. *Bioinformatics* **31**, 3359–3361 (2015).

60. Zhao, Y., Upadhyay, S. & Lin, X. PAS domain protein PaX3 interacts with the Chromatin Modifier Bcr1 in regulating Cryptococcal morphogenesis. *MBio* 9, https://doi.org/10.1128/mBio.02135-18 (2018).

61. Dunescu, P. A. et al. Product binding enforces the genomic specificity of a yeast polycomb repressive complex. *Cell* **160**, 204–218 (2015).

62. Garcia-Santamarina, S. et al. Genome-wide analysis of the regulation of Cu metabolism in *Cryptococcus neoformans*. *Mol. Microbiol.* **108**, 473–494 (2018).

63. Kim, T. H. & Dekker, J. ChIP-quantitative polymerase chain reaction (ChIP-qPCR). *Cold Spring Harb. Protoc.* 2018, pdb prot082628, https://doi.org/10.1101/pdb.prot082628 (2018).

64. Yu, G., Wang, L. G. & He, Q. Y. ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. *Bioinformatics* **31**, 2382–2383 (2015).

65. Stark, R. & Brown, G. *DiffBind: Differential Binding Analysis of ChIP-Seq Peak Data*. R. package version 100, 4–3 https://bioconductor.org/packages/release/bioc/html/DiffBind.html (2011).

66. Zhang, Y. et al. Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* **9**, R137 (2008).

67. Heinz, S. et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell* **38**, 576–589 (2010).

68. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).

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Author contributions

J.L., Z.A.L., and X.L. designed the study. J.L., A.R.F., and Y.Z. conducted the experiments. Y.Z. and E.Y. analyzed the DNA sequencing data; Y.Z. and J.L. analyzed RNA sequencing data; A.R.F. and Z.A.L. analyzed the ATAC and ChIP-DNA sequencing data. J.L. and X.L. wrote the manuscript with contributions from A.R.F. and Z.A.L.

Competing interests

The authors declare no competing interests.

Additional information

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