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‘Popping the Clutch’: Novel Mechanisms Regulating Sexual Development in *Cryptococcus neoformans*

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Abstract

Sexual reproduction in fungal pathogens such as *Cryptococcus* provides natural selection and adaptation of the organisms to environmental conditions by allowing beneficial mutations to spread. However, successful mating in these fungi requires a time-critical induction of signaling pheromones when appropriate partners become available. Recently, it has been shown that the fungus uses the transcriptional equivalent of the racing technique: ‘popping the clutch’-- pushing in the clutch pedal, putting the car in gear, revving with the gas pedal and then dropping the clutch pedal to accelerate rapidly. In the same way, *Cryptococcus* during vegetative growth constitutively matches a high rate of pheromone synthesis with a high rate of degradation to produce repressed levels of transcript. Then, when mating is required, the fungus drops the degradative machinery, resulting in a rapid induction of the pheromone. Pairing with this novel regulatory cycle are a host of mitogen-activated protein (MAP) kinase cascades, cyclic AMP (cAMP) dependent, and calcium-calmodulin signaling pathways that maintain these high rates of pheromone synthesis and prime downstream pathways for an effective mating response. The intersection of a number of virulence-associated traits with sexual development such as the synthesis of an immune-disruptive laccase as well as a protective polysaccharide capsule makes these rapid regulatory strategies a formidable foe in the battle against human disease.

Keywords

Cryptococcus neoformans; mating pheromone; signaling pathway; sexual development

Introduction

Cryptococcus neoformans is a basidiomycete yeast with a defined sexual cycle [1–4] that causes life-threatening fungal meningoencephalitis in immunocompromised and in some cases immunocompetent hosts [5]. Infection occurs by inhalation of desiccated yeast cells which then spread hematogenously to the brain [6, 7], although spores produced by mating have been recently proposed as an infectious particle because of their superior infectivity in mice [8]. While the fungus is normally haploid during growth conditions including infection, under appropriate conditions and in response to mating pheromones, the two

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mating partners (MAT α and MATa) of this heterothallic organism produce conjugation tubes, and the cells fuse to produce a unique heterokaryon which rapidly develops into dikaryotic hyphae [2, 9–11]. A difficult paradox has become apparent in that, throughout evolution, *C. neoformans* has maintained an intact machinery of sexual development while at the same time mating in this yeast appears to be a rare event given the paucity of available ‘a’ mating type strains and the inefficiency of unisexual mating [12]. This stands in contrast to vector-borne pathogens for which sexual development is a mandatory event for infection [13]. However, for less sexually-dependent pathogens such as *Cryptococcus*, sexual development may contribute benefits to the available pool of infecting organisms by increasing genetic variation which, in turn, promotes adaptation to a variety of stressful conditions encountered in this globally-distributed opportunist pathogen [14]. Indeed, analysis of a cohort of isolates from Africa indicate that sexual recombination, though rare, is ongoing within niche populations containing both MAT α and MATa mating type strains [15, 16]. Thus, these rare events may serve to alter the overall population substructure of the pathogen, resulting in emerging virulence traits that could alter host-pathogen dynamics.

A principal genetic structure controlling and propagating sexual development is a large mating type locus of greater than 100 kb [17–19]. The MAT locus of *C. neoformans* is one of the largest among fungi and contains approximately 25 genes and includes pheromone and homeodomain genes as well as those involved in the pheromone-responsive MAPK cascade, meiosis and sporulation. The large size may reflect a fusion of the two mating type loci that are typical in each strain of basidiomycetes, producing one very large locus that conveys a bipolar mating system [17, 19, 20]. Bipolar systems allow mating between each of two possible mating types, and thus allows progeny to mate with half of the other members of that generation, whereas tetraploid mating strategies only allow mating between only 1 in 4 compatible first generation progeny. Such a biopolar mating system has thus been proposed to result in higher rates of inbreeding [21], because it theoretically results in greater propensity to mate with progeny from the same mating, rather than mating with unrelated strains. In the principal infectious species of *C. neoformans*, serotype A, the number of pheromone genes varies between the two stable mating types. The MATa alleles contain three unlinked 130-bp *MFa* pheromone genes embedded in 900–5,000 bp amplicons identical within an allele but not between species. In contrast, the α alleles contain four *MFa* pheromone genes embedded in approximately 500-bp conserved repeats [19, 22]. In addition to its well-known effects on mating, the *MFa* pheromone may have virulence-conditioning effects as well, as mutants with dysregulation of the pheromone also have attenuated virulence [23]. Also contained within the MAT locus are the Ste12 set of genes which was the first mating-type gene identified in *C. neoformans* [24]. In addition, a number of genes unrelated to mating include a myosin gene and a translation initiation factor gene, PRT [22]. The close physical proximity of more essential genes to many of the principal components of mating is unusual in yeast, but may have provided an additional evolutionary pressure to retain the machinery of sexual development in this fungus [21]. Here, we review findings on major signaling pathways for mating in *C. neoformans* with an emphasis on unique and unusual signaling approaches in the fungus.

Rapid Initiation of Sexual Development Relies on a Transcriptional Cycle to ‘Pop the Clutch’

To initiate sexual development, fungi produce signaling pheromones to indicate their presence and their mating compatibility. On natural environments, successful mating requires a close physical association that is easily disturbed for non-motile organisms such as yeast. This suggests that rapid induction might improve chances for successful reproduction. For *C. neoformans*, a particularly conducive environment for both pheromone production and mating is dried guano from pigeons, a principal global carrier of the fungus [16, 25]. Recent work showed that the fungus utilizes specifically-targeted mRNA degradative machinery to modulate a balance between high level pheromone transcript synthesis and high level degradation, akin to previously-described metabolic ‘futile’ cycles that utilize apparently excessive rates of metabolic synthesis and degradation. In the classic example from gluconeogenesis, inhibition of a degradative phosphatase results in a pulse of fructose 1,6-bisphosphonate required during the ‘fight or flight’ response [23, 26, 27]. In *C. neoformans* experiments, high level constitutive MF α transcript synthesis was balanced during vegetative growth by high level degradation by an mRNA decapping protein, Vad1, to yield repressed levels of transcript. Transfer to pigeon guano media resulted in inhibition of Vad1 degradation, resulting in a pulse of steady state levels of MF α transcript followed by sexual development. Genetic constructs that retained constitutive degradation by Vad1 were found to delay, but not prevent mating. These experiments showed that the fungus is much like a high speed drag racer who simultaneously revs the engine while pushing in the clutch pedal and holding the brake. When ready to launch, the driver ‘pops the clutch’, roaring off in a cloud of smoke to win the race. In the same way, by matching apparently wasteful high levels of MF α transcript synthesis and degradation during the vegetative phase when mating is repressed, the yeast retains the synthetic capacity to rapidly mobilize signals necessary for sexual development. In the case of *C. neoformans*, improving mating efficiency could be particularly important for an organism with such a severely skewed distribution of the two mating types [28, 29]. In addition, this property of rapid induction may be important for the establishment of infection since sexual spores resulting from mating have an approximate 100 fold increased infectious potential than the yeast form [8]. Unexplored aspects include whether the futile cycle involved in sexual development could also play a role in haploid fruiting or in uni-sexual mating. Such lack of apparent transcriptional efficiency in such cycles thus represents a paradigm shift in cell biology because it suggests that cells will waste large amounts of energy synthesizing transcripts only to break them down again during the vast periods of time when mating will not be activated. Since the paper by Park et al is only the first description of this phenomenon, it will require additional studies to explore its role in sexual development as well as other possible cellular pathways. Identification of additional transcription/degradation ‘futile’ cycles could thus allow new understanding of diverse eukaryotic regulatory systems including those where a rapid response is required.

Exploring the Mechanism of the Transcription/Degradation Cycle of *C. neoformans*

A principal component of the MF α degradative machinery includes a member of the RCK/p54 family of DEAD box proteins, Vad1, previously shown to be a global regulator of virulence in *C. neoformans* [30]. In both yeast and mammalian systems, numerous RNA-binding proteins (RBPs) have the ability to act as either enhancers or inhibitors of mRNA stability and translation efficiency [31]. RCK/p54 members such as Vad1 are key components of a multi-protein complex that, in combination with catalytic proteins such as the decapping protein, Dcp2, effect removal of the 5' cap of mRNA, followed by degradation [32–35]. Suggesting its role as an RNA transcriptional degradative factor (TDF), MF α mRNA accumulated in a *C. neoformans* *vad1* mutant and was markedly reduced after *VAD1* overexpression. Key to the demonstration of Vad1 as a TDF was the demonstration of a physiologic and dynamic regulation of MF α steady state levels and sexual development by alterations in Vad1-mediated MF α transcript degradation after transfer of cells from rich growth media to a physiologically relevant mating-induction media made from pigeon guano. Interestingly, both the 5' and 3' UTRs of the MF α target transcript were required to potentiate MF α 1 degradation in the presence of *VAD1* whereas typical genomic promoter regions played no role in the process. This allows an independent modulation of traditional transcription factor (TF)-mediated regulatory processes. The requirement for both 5' and 3' regions was likely due to binding of both regions to a Vad1-containing complex or restrictions in secondary structure requirements necessary for the formation of protein binding mRNA loops [36] (Fig. 1). Mechanisms for how signal transduction components mediate these environmental signals to TDFs such as the Vad1 complex is under active investigation and is expected to reveal novel mechanisms involved in signal transduction.

Mitogen-Activated Protein (MAP) Kinase Pathways: Downstream players in the response to mating pheromone

After expression of active mating pheromone, a dynamic series of cellular players interact to effect sexual development. Pheromone receptors transmit the pheromone response pathway through a Ste3 dependent signaling pathway. Unique to *C. neoformans*, a constitutively active Cpr2 pheromone-like receptor engages the same receptor pathway, competing for pathway activation [37]. MAPKs also play important roles in conveying varied environmental signals in eukaryotic cells. In *C. neoformans*, signal transduction pathways such as the pheromone-activated MAPK pathway are required for mating as well as for monokaryotic fruiting [28]. This pathway consists of Ste20, as well as MAPKKK Ste11, MAPKK Ste7 and MAPK Cpk1; these components are essential for dikaryotic filament formation as well as cell fusion [44]. Downstream of the Cpk1 MAPK pathway is the Mat2 allele which is also required for mating and monokaryotic fruiting [45] (Fig. 2). The budding yeast *S. cerevisiae* expresses five MAPKs including mating MAPK Fus3, which mediates the pheromone responses during sexual development [38–40]. Like MF α pheromones, Fus3 is largely inactive during vegetative growth, repressed by the HOG pathway which is mediated by the conserved high-osmolarity glycerol factor, HOG [41–43]. Disruption of

HOG1 downstream of the factors Ssk2 and Pbs2 in *C. neoformans* enhances capsule as well as laccase expression and mating efficiency [46]. Interestingly, *HOG1* also appears to have a proximal regulatory role in pheromone expression and appeared by epistatic experiments to be parallel to that of the *VAD1* global regulatory pathway [23, 46]. The *HOG1* signaling appears to act through a process of transcript synthesis, providing the ‘motor’ side of the *VAD1* futile cycle. Specifically, a model of *HOG1*-dependent activation is provided by precedents from the model yeast, *S. cerevisiae* where the factor is thought to act on *MF α* through stress response elements in the promoter region of mating pheromones [47]. This suggests that *HOG1* is involved in the opposing part of the transcriptional *MF α* cycle, regulating high level transcriptional synthesis of *MF α* similar to phosphorylation in the classic metabolic cycle. In our experiments, we did not find transcriptional induction upon a change from vegetative growth to mating media, suggesting the *HOG1* pathway is constitutively active during vegetative growth [48]. Indeed, paradigms from more conventional substrate cycles suggest that increases in induction and reductions in degradation are synchronized to effect large bursts of transcript steady states required for time-critical changes in cellular physiology.

Cyclic AMP Signaling Pathway

C. neoformans utilizes the highly conserved cyclic AMP (cAMP) signaling pathway to promote mating and virulence in response to environmental stressors [49, 50]. In the model yeast *S. cerevisiae*, phosphorylation of downstream targets by protein kinase A (PKA) induces cellular responses, including nutrient sensing and pseudohyphal growth [51]. Adenylyl cyclase (Cac1) is a membrane-bound protein activated via the Ras-GTPase Ras1 and Ras2, as well as the G-protein-coupled receptor system Gpr1-Gpa2 [52], and Cap/Srv2 [53]. Linking environmental stress response with virulence, the cAMP-mediated signaling pathway of *C. neoformans* is involved in expression of the virulence factors laccase as well as capsule production, in addition to its role in mating. Similarly, disruption of *CAC1* encoding adenylyl cyclase impairs laccase expression, capsule formation and mating [54]. Aca1, an ancillary adenylyl cyclase-associated protein, is another member required for mating and hyphal differentiation [55]. Upstream of the adenylyl cyclase-cAMP-PKA pathway is a G-protein-coupled receptor in *C. neoformans*. Disruption of *GPA1*, which encodes a G α subunit, again leads to loss of the same three functions--laccase, capsule and sexual development [49]. Gpb1, a G β subunit, has a more selective role in mating and monokaryotic fruiting [56]. *C. neoformans* encodes three regulators of G-protein signaling proteins, Crg1, Crg2 and Crg3, which act as GTPase-activating proteins that are negative regulators of G α subunits. Crg1 appears to be solely involved in pheromone-mediated mating [57] whereas Crg2 regulates the cAMP-mediated signaling pathway for pheromone-mediated mating as well as virulence [58, 59]. The functions of Crg3 are as yet not known. Nrg1, a transcription factor, was identified as a downstream target of the cAMP pathway, and disruption of *NRG1* results in delayed capsule formation and mating but not laccase expression, suggesting additional factors involved in Pka signaling of virulence in *C. neoformans* [60] (Fig. 2). In summary data regarding the Pka-dependent signaling pathway in *C. neoformans* suggest a tight interconnection between mating and virulence, unified by a response to environmental stress.

Calcium-Calcineurin Signaling Pathway

Transcriptional regulation via the calcium-calcineurin signaling pathway has been widely studied in fungi [61–63] and signaling elements are well conserved [64]. For example, in *S. cerevisiae*, calcineurin activity is required for recovery from a number of environmental stressors [65–69]. In *C. neoformans*, calcineurin is required for hyphal elongation during mating and monokaryotic fruiting in response to environmental stress [70]. Inhibition of calcineurin with the immunosuppressant drug FK506 or mutations of the genes encoding the calcineurin A or B subunits is a convenient method of probing the calcineurin pathway and has been found to prevent mating, monokaryotic fruiting and basidiospore production [70]. FK506 binding to the FK506-rapamycin target protein FKBP12 produces these effects and is a convenient method to produce calcineurin inhibition in yeast (Fig. 2). In the same way, calcium-dependent activation of calcineurin can be modeled by addition of high amounts of exogenous calcium, but sensitivity of *ena1 α* mutants to conditions of high carbon dioxide, alkaline pH and high concentrations of cations suggest that these environmental signals are the physiological conditions responsible for the activation of intracellular calcium stores. In addition, calcium signaling through calmodulin and the phosphatase calcineurin are required for mating and virulence of *C. neoformans* [71–73]. An increased level of calcium is sensed by calmodulin, which activates the conserved serine/threonine phosphatase calcineurin (Fig. 2). A conserved member of the calcipressin family, Cbp1/Rcn1, has also been shown to play a role in mating and members of this family interacting with calcineurin and acting as calcineurin effectors or feedback regulators in *C. neoformans* [72, 74]. The deleterious effects on the fungus by calcineurin inhibitors has been implicated in the anti-fungal effects of these immunosuppressants in treated patients [75], suggesting roles for these pathways in developing novel methods of disease control.

Conclusion

Cryptococcus neoformans has developed elaborate signaling mechanisms involved in sexual development. These mechanisms involve at least three pathways: mitogen-activated protein kinase (MAPK) cascades, cyclic AMP (cAMP), and calcium-calcineurin signaling pathways in concert with a transcriptional ‘futile’ cycle that rapidly activates the mating response. The challenge in future studies will be to identify the effectors and/or transcription factors and to define the cross-talk between these major pathways and others mediating the induction of sexual development in response to environmental signals.

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References

1. Alspaugh, JA.; Davidson, RC.; Heitman, J. Morphogenesis of *Cryptococcus neoformans*. In: Ernst, JF.; Schmidt, A., editors. Dimorphism in human pathogenic and apathogenic yeasts. Karger, Basel, Switzerland: 2000. p. 217-238.
2. Kwon-Chung KJ. A new genus, *filobasidiella*, the perfect state of *Cryptococcus neoformans*. *Mycologia*. 1975; 67(6):1197–1200. [PubMed: 765816]

3. Kwon-Chung KJ. A new species of *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. *Mycologia*. 1976; 68(4):943–946. [PubMed: 790173]
4. Sia RA, Lengeler KB, Heitman J. Diploid strains of the pathogenic basidiomycete *Cryptococcus neoformans* are thermally dimorphic. *Fungal Genet Biol*. 2000; 29(3):153–163. [PubMed: 10882532]
5. Casadevall, A.; Perfect, JR. *Cryptococcus neoformans*. Washington, D.C.: ASM Press; 1998.
6. Neilson JB, Fromtling RA, Bulmer GS. *Cryptococcus neoformans*: size range of infectious particles from aerosolized soil. *Infect. Immun*. 1977; 17(3):634–638. [PubMed: 332630]
7. Sukroongreung S, et al. Pathogenicity of basidiospores of *Filobasidiella neoformans* var. *neoformans*. *Med Mycol*. 1998; 36(6):419–424. [PubMed: 10206753]
8. Velagapudi R, et al. Spores as Infectious Propagules of *Cryptococcus neoformans*. *Infect. Immun*. 2009; 77(10):4345–4355. [PubMed: 19620339]
9. Kwon-Chung KJ. Morphogenesis of *Filobasidiella neoformans*, the sexual state of *Cryptococcus neoformans*. *Mycologia*. 1976; 68:821–833. [PubMed: 790172]
10. Davidson RC, et al. Characterization of the MFalpha pheromone of the human fungal pathogen *Cryptococcus neoformans*. *Molecular Microbiology*. 2000; 38(5):1017–1026. [PubMed: 11123675]
11. Shen W-C, et al. Pheromones Stimulate Mating and Differentiation via Paracrine and Autocrine Signaling in *Cryptococcus neoformans*. *Eukaryotic Cell*. 2002; 1(3):366–377. [PubMed: 12455985]
12. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. *Am J Epidemiol*. 1984; 120(1):123–130. [PubMed: 6377880]
13. Williamson KC. Pfs230: from malaria transmission-blocking vaccine candidate toward function. *Parasite Immunol*. 2003; 25(7):351–359. [PubMed: 14521577]
14. Goddard MR, Godfray HCJ, Burt A. Sex increases the efficacy of natural selection in experimental yeast populations. *Nature*. 2005; 434(7033):636–640. [PubMed: 15800622]
15. Litvinseva AP, et al. Evidence of Sexual Recombination among *Cryptococcus neoformans* Serotype A Isolates in Sub-Saharan Africa. *Eukaryotic Cell*. 2003; 2(6):1162–1168. [PubMed: 14665451]
16. Litvinseva AP, et al. Comparative Analysis of Environmental and Clinical Populations of *Cryptococcus neoformans*. *J. Clin. Microbiol*. 2005; 43(2):556–564. [PubMed: 15695645]
17. Fraser JA, et al. Convergent Evolution of Chromosomal Sex-Determining Regions in the Animal and Fungal Kingdoms. *PLoS Biol*. 2004; 2(12):e384. [PubMed: 15538538]
18. Morrow CA, Fraser JA. Sexual reproduction and dimorphism in the pathogenic basidiomycetes. *FEMS Yeast Research*. 2009; 9(2):161–177. [PubMed: 19220864]
19. Lengeler KB, et al. Mating-Type Locus of *Cryptococcus neoformans*: a Step in the Evolution of Sex Chromosomes. 704–718. 2002. *Eukaryotic Cell*. 2002; 1(5):704–718. [PubMed: 12455690]
20. Loftus BJ, et al. The Genome of the Basidiomycetous Yeast and Human Pathogen *Cryptococcus neoformans*. *Science*. 2005; 307(5713):1321–1324. [PubMed: 15653466]
21. Nielsen K, Heitman J. Sex and virulence of human pathogenic fungi. *Adv Genet*. 2007; 57:143–173. [PubMed: 17352904]
22. Nielsen, K.; Heitman, J.; Jay, CD. Sex and Virulence of Human Pathogenic Fungi, in *Advances in Genetics*. Academic Press; 2007. p. 143–173.
23. Park Y-D, et al. Mating Pheromone in *Cryptococcus neoformans* Is Regulated by a Transcriptional/Degradative "Futile Cycle". *Journal of Biological Chemistry*. 2010; 285(45): 34746–34756. [PubMed: 20801870]
24. Karos M, et al. Mapping of the *Cryptococcus neoformans* MATalpha locus: presence of mating type-specific mitogen-activated protein kinase cascade homologs. *J Bacteriol*. 2000; 182(21): 6222–6227. [PubMed: 11029445]
25. Nielsen K, De Obaldia AL, Heitman J. *Cryptococcus neoformans* Mates on Pigeon Guano: Implications for the Realized Ecological Niche and Globalization. *Eukaryotic Cell*. 2007; 6(6): 949–959. [PubMed: 17449657]

26. Shulman GI, et al. Substrate cycling between gluconeogenesis and glycolysis in euthyroid, hypothyroid, and hyperthyroid man. *The Journal of Clinical Investigation*. 1985; 76(2):757–764. [PubMed: 4031071]
27. Suh S, Paik I, Jacobs K. Regulation of blood glucose homeostasis during prolonged exercise. *Mol cells*. 2007; 23(3):272–279. [PubMed: 17646701]
28. Lin X, Hull CM, Heitman J. Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. *Nature*. 2005; 434(7036):1017–1021. [PubMed: 15846346]
29. Fraser J, et al. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. *Nature*. 2005; 437(7063):1360–1364. [PubMed: 16222245]
30. Panepinto J, et al. The DEAD-box RNA helicase Vad1 regulates multiple virulence-associated genes in *Cryptococcus neoformans*. *J. Clin. Invest*. 2005; 115(3):632–641. [PubMed: 15765146]
31. Wilusz CJ, Wormington M, Peltz SW. THE CAP-TO-TAIL GUIDE TO MRNA TURNOVER. *Nat Rev Mol Cell Biol*. 2001; 2(4):237–246. [PubMed: 11283721]
32. Beelman CA, Parker R. Degradation of mRNA in eukaryotes. *Cell*. 1995; 81(2):179–183. [PubMed: 7736570]
33. Mitchell P, Tollervey D. mRNA turnover. *Current Opinion in Cell Biology*. 2001; 13(3):320–325. [PubMed: 11343902]
34. van Hoof A, Parker R. The Exosome: A Proteasome for RNA? *Cell*. 1999; 99(4):347–350. [PubMed: 10571176]
35. Tucker M, Parker R. Mechanisms and control of mRNA decapping in *Saccharomyces cerevisiae*. *Annual Review of Biochemistry*. 2000; 69:571–595.
36. Mao H, White SA, Williamson JR. A novel loop-loop recognition motif in the yeast ribosomal protein L30 autoregulatory RNA complex. *Nat Struct Mol Biol*. 1999; 6(12):1139–1147.
37. Hsueh YP, Xue C, Heitman J. A constitutively active GPCR governs morphogenic transitions in *Cryptococcus neoformans*. *EMBO J*. 2009; 28(9):1220–1233. [PubMed: 19322200]
38. Seger, R.; Elion, EA.; Sahoo, R. *MAP Kinase Signaling Protocols*. Humana Press; 2010. Analysis of Mitogen-Activated Protein Kinase Activity in Yeast; p. 387–399.
39. Elion EA, Brill JA, Fink GR. FUS3 represses CLN1 and CLN2 and in concert with KSS1 promotes signal transduction. *PNAS*. 1991; 88(21):9392–9396. [PubMed: 1946350]
40. Ma D, Cook JG, Thorner J. Phosphorylation and localization of Kss1, a MAP kinase of the *Saccharomyces cerevisiae* pheromone response pathway. *Mol. Biol. Cell*. 1995; 6(7):889–909. [PubMed: 7579701]
41. Hall JP, et al. The osmoregulatory pathway represses mating pathway activity in *Saccharomyces cerevisiae*: isolation of a FUS3 mutant that is insensitive to the repression mechanism. *Mol. Cell. Biol*. 1996; 16(12):6715–6723. [PubMed: 8943326]
42. O'Rourke SM, Herskowitz I. A Third Osmosensing Branch in *Saccharomyces cerevisiae* Requires the Msb2 Protein and Functions in Parallel with the Sho1 Branch. *Mol. Cell. Biol*. 2002; 22(13):4739–4749. [PubMed: 12052881]
43. Hohmann, S.; Krantz, M.; Nordlander, B. *Osmosensing and Osmosignaling*. San Diego: Elsevier Academic Press Inc; 2007. Yeast osmoregulation. 29–+
44. Davidson RC, et al. A MAP kinase cascade composed of cell type specific and non-specific elements controls mating and differentiation of the fungal pathogen *Cryptococcus neoformans*. *Mol Microbiol*. 2003; 49(2):469–485. [PubMed: 12828643]
45. Lin X, et al. Transcription Factors Mat2 and Znf2 Operate Cellular Circuits Orchestrating Opposite- and Same-Sex Mating in *Cryptococcus neoformans*. *PLoS Genet*. 2010; 6(5):e1000953. [PubMed: 20485569]
46. Bahn Y-S, et al. Specialization of the HOG Pathway and Its Impact on Differentiation and Virulence of *Cryptococcus neoformans*. *Mol. Biol. Cell*. 2005; 16(5):2285–2300. [PubMed: 15728721]
47. Susan Wohler S, et al. Expression of the yeast glycogen phosphorylase gene is regulated by stress-response elements and by the HOG MAP kinase pathway. *Yeast*. 2001; 18(16):1505–1514. [PubMed: 11748727]

48. Alonso-Monge R, et al. The Hog1 Mitogen-Activated Protein Kinase Is Essential in the Oxidative Stress Response and Chlamydospore Formation in *Candida albicans*. *Eukaryotic Cell*. 2003; 2(2): 351–361. [PubMed: 12684384]
49. Alspaugh JA, Perfect JR, Heitman J. *Cryptococcus neoformans* mating and virulence are regulated by the G-protein α subunit GPA1 and cAMP. *Genes & Development*. 1997; 11(23):3206–3217. [PubMed: 9389652]
50. D'Souza CA, et al. Cyclic AMP-Dependent Protein Kinase Controls Virulence of the Fungal Pathogen *Cryptococcus neoformans*. *Mol. Cell. Biol.* 2001; 21(9):3179–3191. [PubMed: 11287622]
51. Lengeler KB, et al. Signal Transduction Cascades Regulating Fungal Development and Virulence. *Microbiol. Mol. Biol. Rev.* 2000; 64(4):746–785. [PubMed: 11104818]
52. Toda T, et al. In yeast, RAS proteins are controlling elements of adenylate cyclase. *Cell*. 1985; 40(1):27–36. [PubMed: 2981630]
53. Shima F, et al. Association of Yeast Adenylyl Cyclase with Cyclase-Associated Protein CAP Forms a Second Ras-Binding Site Which Mediates Its Ras-Dependent Activation. *Mol. Cell. Biol.* 2000; 20(1):26–33. [PubMed: 10594005]
54. Alspaugh JA, et al. Adenylyl Cyclase Functions Downstream of the G $\{\alpha\}$ Protein Gpa1 and Controls Mating and Pathogenicity of *Cryptococcus neoformans*. *Eukaryotic Cell*. 2002; 1(1):75–84. [PubMed: 12455973]
55. Bahn Y-S, et al. Adenylyl Cyclase-Associated Protein Aca1 Regulates Virulence and Differentiation of *Cryptococcus neoformans* via the Cyclic AMP-Protein Kinase A Cascade. *Eukaryotic Cell*. 2004; 3(6):1476–1491. [PubMed: 15590822]
56. Wang P, Perfect JR, Heitman J. The G-Protein beta Subunit GPB1 Is Required for Mating and Haploid Fruiting in *Cryptococcus neoformans*. *Mol. Cell. Biol.* 2000; 20(1):352–362. [PubMed: 10594037]
57. Wang P, et al. Mutation of the Regulator of G Protein Signaling Crg1 Increases Virulence in *Cryptococcus neoformans*. *Eukaryotic Cell*. 2004; 3(4):1028–1035. [PubMed: 15302835]
58. Shen G, et al. The RGS Protein Crg2 Regulates Pheromone and Cyclic AMP Signaling in *Cryptococcus neoformans*. *Eukaryotic Cell*. 2008; 7(9):1540–1548. [PubMed: 18658258]
59. Xue C, et al. The RGS protein Crg2 regulates both pheromone and cAMP signalling in *Cryptococcus neoformans*. *Molecular Microbiology*. 2008; 70(2):379–395. [PubMed: 18761692]
60. Cramer KL, et al. Transcription Factor Nrg1 Mediates Capsule Formation, Stress Response, and Pathogenesis in *Cryptococcus neoformans*. *Eukaryotic Cell*. 2006; 5(7):1147–1156. [PubMed: 16835458]
61. Hagiwara D, et al. Functional analysis of C α 2H α 2; zinc finger transcription factor CrzA involved in calcium signaling in *Aspergillus nidulans*. *Current Genetics*. 2008; 54(6):325–338. [PubMed: 19002465]
62. Soriani F, et al. Identification of possible targets of the *Aspergillus fumigatus* CRZ1 homologue, CrzA. *BMC Microbiology*. 2010; 10(1):12. [PubMed: 20078882]
63. Choi J, et al. MoCRZ1, a gene encoding a calcineurin-responsive transcription factor, regulates fungal growth and pathogenicity of *Magnaporthe oryzae*. *Fungal Genetics and Biology*. 2009; 46(3):243–254. [PubMed: 19111943]
64. Zelter A, et al. A comparative genomic analysis of the calcium signaling machinery in *Neurospora crassa*, *Magnaporthe grisea*, and *Saccharomyces cerevisiae*. *Fungal Genetics and Biology*. 2004; 41(9):827–841. [PubMed: 15288019]
65. Cyert MS, Thorner J. Regulatory subunit (CNB1 gene product) of yeast Ca $^{2+}$ /calmodulin-dependent phosphoprotein phosphatases is required for adaptation to pheromone. *Mol. Cell. Biol.* 1992; 12(8):3460–3469. [PubMed: 1321337]
66. Poor F, et al. Calcineurin mediates inhibition by FK506 and cyclosporin of recovery from [math>\alpha]-factor arrest in yeast. *Nature*. 1992; 360(6405):682–684. [PubMed: 1281518]
67. Moser MJ, Geiser JR, Davis TN. Ca $^{2+}$ -calmodulin promotes survival of pheromone-induced growth arrest by activation of calcineurin and Ca $^{2+}$ -calmodulin-dependent protein kinase. *Mol. Cell. Biol.* 1996; 16(9):4824–4831. [PubMed: 8756641]

68. Withee JL, et al. An essential role of the yeast pheromone-induced Ca²⁺ signal is to activate calcineurin. *Mol. Biol. Cell.* 1997; 8(2):263–277. [PubMed: 9190206]
69. Cyert MS. Calcineurin signaling in *Saccharomyces cerevisiae*: how yeast go crazy in response to stress. *Biochemical and Biophysical Research Communications.* 2003; 311(4):1143–1150. [PubMed: 14623300]
70. Cruz MC, Fox DS, Heitman J. Calcineurin is required for hyphal elongation during mating and haploid fruiting in *Cryptococcus neoformans*. *EMBO J.* 2001; 20(5):1020–1032. [PubMed: 11230126]
71. Kozubowski L, Lee SC, Heitman J. Signalling pathways in the pathogenesis of *Cryptococcus*. *Cellular Microbiology.* 2009; 11(3):370–380. [PubMed: 19170685]
72. Fox DS, Heitman J. Calcineurin-Binding Protein Cbp1 Directs the Specificity of Calcineurin-Dependent Hyphal Elongation during Mating in *Cryptococcus neoformans*. *Eukaryotic Cell.* 2005; 4(9):1526–1538. [PubMed: 16151246]
73. Kmetzsch L, et al. Calcium signaling components in the human pathogen *Cryptococcus neoformans*. *Commun Integr Biol.* 2011; 4(2):186–187. [PubMed: 21655435]
74. Gorlach J, et al. Identification and characterization of a highly conserved calcineurin binding protein, CBP1/calciressin, in *Cryptococcus neoformans*. *EMBO J.* 2000; 19(14):3618–3629. [PubMed: 10899116]
75. Steinbach WJ, et al. Harnessing calcineurin as a novel anti-infective agent against invasive fungal infections. *Nat Rev Microbiol.* 2007; 5(6):418–430. [PubMed: 17505522]

Fig. 1.
An mRNA transcription/degradation 'futile' cycle regulates *MFα* via *Vad1* in *Cryptococcus neoformans*.

Fig. 2.
Signaling pathways for mating in *C. neoformans*.