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F-box Protein of *Cryptococcus neoformans* and its Importance in Fungal Sexual Reproduction and Virulence

Ijaz Usman Ali[1], Muhammad Mumtaz Tahir[1], Muhammad Afzaal[1], Ahmad Waheed[2], Hina Naz[2]

[1] Department of Microbiology and Molecular Genetics, Faculty of Life Sciences, University of Okara, Okara 56130 Pakistan

[2] Department of Zoology, Faculty of Life Sciences, University of Okara, Okara 56130 Pakistan

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Address of Correspondence

Ahmad Waheed

wahmad09@yahoo.com

A B S T R A C T

Cryptococcus neoformans is a pathogenic yeast that poses significant health risks, particularly to immunocompromised individuals, leading to severe infections and high mortality rates. This review delves into the critical roles of the F-box protein in *C. neoformans*, emphasizing its involvement in sexual reproduction and virulence. The yeast's ability to form melanin and polysaccharide capsules and its growth at mammalian body temperature are critical factors in its pathogenicity. The F-box protein (Fbp1) is specifically linked to pathogenicity and immune evasion, playing a significant role in the synthesis of sexual spores and the regulation of meiosis and nuclear division. Mutants lacking Fbp1 exhibit defects in these processes, underscoring its essential role. Various experimental approaches, including in vivo pathogenicity assays, bioinformatics tools, transcriptomics and proteomics analysis, protein-protein interactions, and genetic knockouts, are suggested for evaluating the role of F-box proteins. Comparative genomics highlights the presence of 1796 F-box genes in the wheat genome, emphasizing their roles in cellular processes and amino acid functions. Studies on other fungi, such as *Schizosaccharomyces pombe* and *Candida albicans*, provide insights into the conserved and unique functions of F-box proteins. Understanding the molecular mechanisms of F-box proteins can provide valuable insights into fungal pathogenesis and potential therapeutic targets. Further research is essential to develop more effective treatments for infections caused by *C. neoformans*, particularly in light of emerging drug resistance. This review underscores the importance of F-box proteins in the biology and pathogenicity of *C. neoformans*, highlighting their potential as targets for therapeutic intervention.

Keywords: F-box protein, Cryptococcus neoformans, sexual reproduction, virulence

Introduction

The human central nervous system (CNS) is infected by *Cryptococcus neoformans,* which is a disease-causing basidiomycetous yeast. It can also induce meningoencephalitis, mainly in immunodeficient human beings.[1] This pathogen has appeared as one of the most prominent causes of meningitis due to fungi, with about a million cases accounting for 0.6 million deaths every year.[2] *C. neoformans was* used as an experimental organism to examine the genome as well as the pathogenesis of fungi. It is a haploid yeast that can grow at a very fast rate with a definite sexual cycle. Potent organism models are accessible in which evaluation of its virulence can be done. [1,3] Studies on large scales have been conducted to examine *C. neoformans* because of its significance and genetic tractability. Many factors of virulence, like the synthesis of capsule buildups of polysaccharide and melanin, as well as the capability of developing in the bodies of mammals at 37 °C, have been well distinguished. Sexual reproduction of fungi and virulence of fungi-related significant pathways that are used in signaling have also been recognized and studied at large scales. [4-7]

$CD4^+$ and $CDC8^+$ T cells had a prominent role in adaptive immunity while utilizing mouse models for studying cryptococcal infection .[8-12] In contrast, spontaneous effects of type 2 helper T cells, like the synthesis of helixin-4 (also called IL-4), as well as interleukin-13, were found to have adverse effects during cryptococcosis. [13-16] A remarkable number of virulence factors were shown by *C. neoformans* that are helpful for the cells of fungi to escape from the immunity of the host. [17-21] Regardless

of the high morbidity and death rate due to cryptococcosis, options of therapy for cryptococcosis are limited, and only 3 main groups of drugs are recently authorized for analytical purposes: polyenes, azoles, and the pyrimidine analogue flucytosine (5-FC). [13,22]

Schizosaccharomyces pombe is another yeast (containing numerous F-box proteins) that has been studied extensively. [23] As concerns about drug resistance as well as the progression of new virulent stains increase[24-27], there is a dire need to recognize the molecular analysis of cryptococcal sepsis conducive to identifying and establishing a secure and longer potent for fungal medicine.[28] Cdc4+ is another protein containing F-box that has been examined in *S. cerevisiae* [29-31] and *C. albicans*.[32-34] Importantly, an appealing query has currently been shown by researchers in which crucial cell cycle genes like GRR1 and CDC4, which are preserved all through growth, show no crucial part in the cycle of the cell but influence development in *C. albicans.* [32,35-37] *Candida albicans* is one of the main disease-causing fungi in humans, which can cause congenital diseases in immunosuppressed individuals and a significant number of external infections. [38-40]

Despite significant advancement regarding the understanding of *Cryptococcus neoformans* from a biological point of view, there is still a substantial gap in the literature regarding the role and function of F-box proteins in this organism. F-box proteins have been well-characterized in model organisms such as *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, revealing their roles in stress responses, nutrient sensing, and cell cycle progression [41]. Still, F-box proteins' characterization in *C. neoformans* remains largely unexplored. Cao and Xue [42] have suggested ubiquitin-mediated proteolysis's potential role in stress responses and virulence of *C. neoformans*. However, there is no systematic evaluation of the specific role of F-box protein in these processors.

So, this review article explores F-box proteins in *Cryptococcus neoformans*, with a particular focus on their involvement in sexual reproduction and virulence. Moreover, this review also seeks to bridge this knowledge gap by examining the molecular mechanisms through which F-box proteins influence the pathogenicity and immunity evasion strategies of *C. neoformans*.

F-box Proteins' Functional Role in the SCF Ubiquitin Ligase Complex

A critical component of the SCF (Skp1-Cullin-F-box) ubiquitin ligase complex is the F-box proteins [43]. A crucial role is played by the SCF complex in regulating degradation of proteins through the ubiquitin-proteasome system [44]. This complex also functions as an E3 ubiquitin ligase that transfers the ubiquitin molecules to specific protein substrates and marks them for degradation by the proteasome. [45]

Composition of the SCF Complex

There are four main components of the SCF complex: Skp1, Cullin, an F-box protein, and Rbx1 (RING-box protein). SKp1 is a scaffold protein linking the F-box protein to Cullin. Cullin is also a scaffold protein involved in the SCF complex organization and interaction with the Rbx1. Rbx1 is involved in the recruiting of an E2 ubiquitin-conjugating enzyme. The F-box protein is a key specificity factor within this complex that recognizes and binds to the target substrates [45,46].

Role of F-box Proteins in the SCF Complex

A vital role is played by F-box proteins to determine the substrate specificity of the SCF complex. [47] F-box proteins have two functional domains: the F-box motif and a substrate-binding domain. [48] The F-box motif (which is a structural domain of 40–50 amino acids) is responsible for the interaction between the F-box protein and Skp1, thereby anchoring the F-box protein to the SCF complex.[47] The substrate-binding domain can vary in different F-box proteins [48] and it mostly includes WD40 repeats, leucine-rich repeats (LRRs), or other protein-protein interaction motifs, that helps the F-box protein in recognizing and binding to other specific substrates. [49]

Phosphorylation-dependent mechanisms are used by F-box proteins in the process of recognition of specific substrates [50]. The SCF complex recognizes many substrates only after their phosphorylation at specific residues, which eventually causes the tight binding of substrates with the F-box protein. [51] Once attached, the SCF complex tags the substrate for later destruction by the 26S proteasome by facilitating the transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to the substrate.[44] So, F-box proteins are involved in the regulation of a variety of cellular processes, i.e., transcription, signal transduction, and cell cycle progression. Pathological conditions like neurodegenerative diseases and cancer can occur if there is dysregulation in the functioning of F-box proteins or associated SCF complex.

Potential experimental approaches for evaluating the role of F-box proteins

There are a variety of experimental approaches that can be used to predict the function of F-box proteins in *C. neoformans*, which are described below.

1. In vivo Pathogenicity Assays

In vivo pathogenicity assays can be used to find the potential role of F-box protein in the pathogenicity of *C. neoformans.* Previously, specific F-box proteins have been linked to immune evasion strategies during malaria infection in *Plasmodium* species. [52]

2. Bioinformatics Tools

Bioinformatics tools that are based on phylogenetic analysis, domain architecture, and sequence homology can be used to predict F-box proteins' functions. Szklarczyk et al. [53] have used bioinformatics tools like STRING and BLAST for predicting different proteins' functions and interaction networks in various pathogens.

3. Transcriptomics and Proteomics Analysis

Transcriptomics and Proteomics Analysis are used to evaluate the global impact of F-box protein knockouts on gene expression and protein abundance. For example, Gonzalez et al. [54] employed a transcriptomic analysis to find the impact of F-box protein knockouts on stress response pathways in pathogenic bacteria.

4. Protein-Protein Interactions

Protein-protein interactions (PPI) can be used to find the interaction partners of F-box proteins that could eventually help in finding the potential role of F-box proteins in the pathogen. PPI studies have been vital in identifying substrates of F-box proteins in model organisms like *Saccharomyces cerevisiae.* [55]

5. Genetic Knockouts

A Genetic knockout approach is used to identify the role of specific F-box proteins in the pathogen's response to environmental stresses, life cycle, or virulence. This technique was previously utilized by Chow et al. [56] for evaluating the role of F-box proteins in *Candida albicans* and found that knockouts led to defects in virulence and morphogenesis.

Genes of F-box protein of Wheat genome

The genome of wheat contained 1796 genes of F-box protein, which depend on the practical domain at C-end and are distributed into various sub-categories [57]. F-box proteins persist functionally in human beings due to amino acids like proline and leucine. These amino acids are important components of the F-box protein [23]. The overall 4363 probable genes of F-box protein were recognized by a hidden Markov model profiling F-box proteins against the database of regional proteins in the genome of wheat, utilizing biosequence analysis. About 1032 F-box genes out of 1796 genes of F-box protein were categorized into the main 3 gene ontology terms like biological activities, activities of molecules, and parts of a cell. Genes of F-box were found to be spread extensively all-round the complete genome of wheat, and localization was not associated with the elongation of chromosomes. [57]

F-box protein Fbp1 in C. neoformans

To assess the involvement of Cdc4 in fungi breeding, Wu et al. [58] synthesized the cdc4Δ mutants in KN99a as well as H99 strain environments. The air passage and lung-draining mediastinal lymph nodes of mice shows the cytokine profile of CD4+ T cells when mice are infected due to the fbp1 mutant compared with H99. Earlier studies shows that all the previous established methods have also been used to study CD4+ T cellular signals [59-61]. In mediastinal lymph nodes, a remarkable maturation of cytokines synthesizing CD4+ T cells was examined by Olson et al. [62] between 7 and 14 days after infection. Th2 responses were detrimental to *C. neoformans,* while enhanced responses of T helper cell type 1 were secured, according to previous studies [63-74].

Functional variation of F-box gene duplexes

During evolution, distinctive secular or spatial expression patterns are possible mechanisms for the functional variation of duplexed genes. The pattern of expression of genes of F-box protein was correlated among 7 growth phases in *C. elegans,* and mostly genes of F-box protein reveal a phase-particular pattern of expression. At the embryonic stage, few members have an extremely supreme expression, but in the larval phase, others members have a specifically high expression. [75,76] Because of their vast and diverse expression patterns, F-box proteins are thought to have a variety of roles throughout plant development, growth, and response to cellular or extracellular nodes. A large number of the F-box proteins are utilized as elements of Skp1-Cullin1-F-box (SCF)-type ubiquitin E3 ligases, in which F-box proteins work as the substrate-conclusive element. [77]

F-box protein in degradation

The significance of F-box proteins is being governed in many ways. Firstly, the substratum particularity complexes of Skp1-Cullin1-F-box depend upon F-box protein (Figure 1). The F-box proteins b-Trcp and Skp2 target substrates that command the addition of different cells in mammals. The degradation of p27 depends upon Skp2, a significant controller for S stage entrance and the remaining stages in the cycle of a cell [78-80]. F-box proteins are evolutionarily preserved in eukaryotes like *D. melanogaster* and *S. cerevisiae*. With the

strongest hereditary methods and absolute advancing devices, Drosophila is seen as a good model organism for observing the F-box proteins. Recently, the genome coded about 33 F-box proteins [81]. The variation in substrates implies an extensive range of biological activities that F-box proteins could plausibly regulate [82]

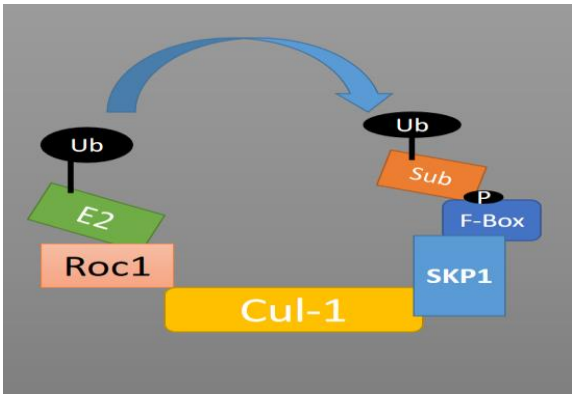


Figure 1. Schematic Diagram of SCF (Skp1–Cullin–F-box) complex with a RING-domain E3 ligases.

Fbp1 is significant for the synthesis of sexual spores

F-box protein 1 elimination mutants were developed by Liu et al. [83] in *C. neoformans* KN99a as well as H99 strain backgrounds. Liu et al. [83] also studied Dikaryotic hyphae as well as basidiospore development in F-box protein one-sided coupling (fbp1×violent type) and mutual coupling (fbp1 × fbp1). No apparent phenotypic variations were noticed by Liu et al. [83] in the fbp1 unilateral coupling analysis. So, the domain of F-box is a crucial part in the functioning of the protein. Liu et al. [83] examined the importance of the F-box domain in fbp1 activity by developing strains showing F-box protein 1 without the F-box domain (Fbp1ΔFB).

Importance of Fbp1 in meiosis and nuclear division

A yeast cell with a single nucleus was examined in the violent type by Liu et al. [83], and they found that cultivations of F-box protein 1 and 2 different nuclei can be recognized in the hypha of each dikaryotic cell developed from a bilateral coupling combination after plasmogamy. Although nuclei in the mutual coupling of fbp1 mutants flopped to encounter meiosis after amalgam, one nucleus was examined in each adult basidium after incubating fortnightly, while all basidia from violent-type coupling synthesized 4 nuclei. Because fbp1 mutants have a usual rate of development as well as contain regular nuclear segmentation when developed in filthy medium, F-box protein 1 acts only in regulating meiosis but does not take part in the cellular cycle in mitotic division [83].

CDC4 gene in C. neoformans

Stempinski et al. [84] indicated that a deeper understanding of the genetic and molecular processes that cause morphological changes in cryptococcal cells may improve our understanding of the role of cellular morphology in disease. The Cdc4 gene is used to regulate cell membrane integrity and is also known for repairing DNA damage [85]. Though Cdc4 is not associated with the growth of many well-defined factors of virulence, May et al. [13] studied the development of a *Cryptococcus* strain under pressure that parasitizes the environment of a malicious host. The Cdc4 gene is needed for the infection of fungi [85].

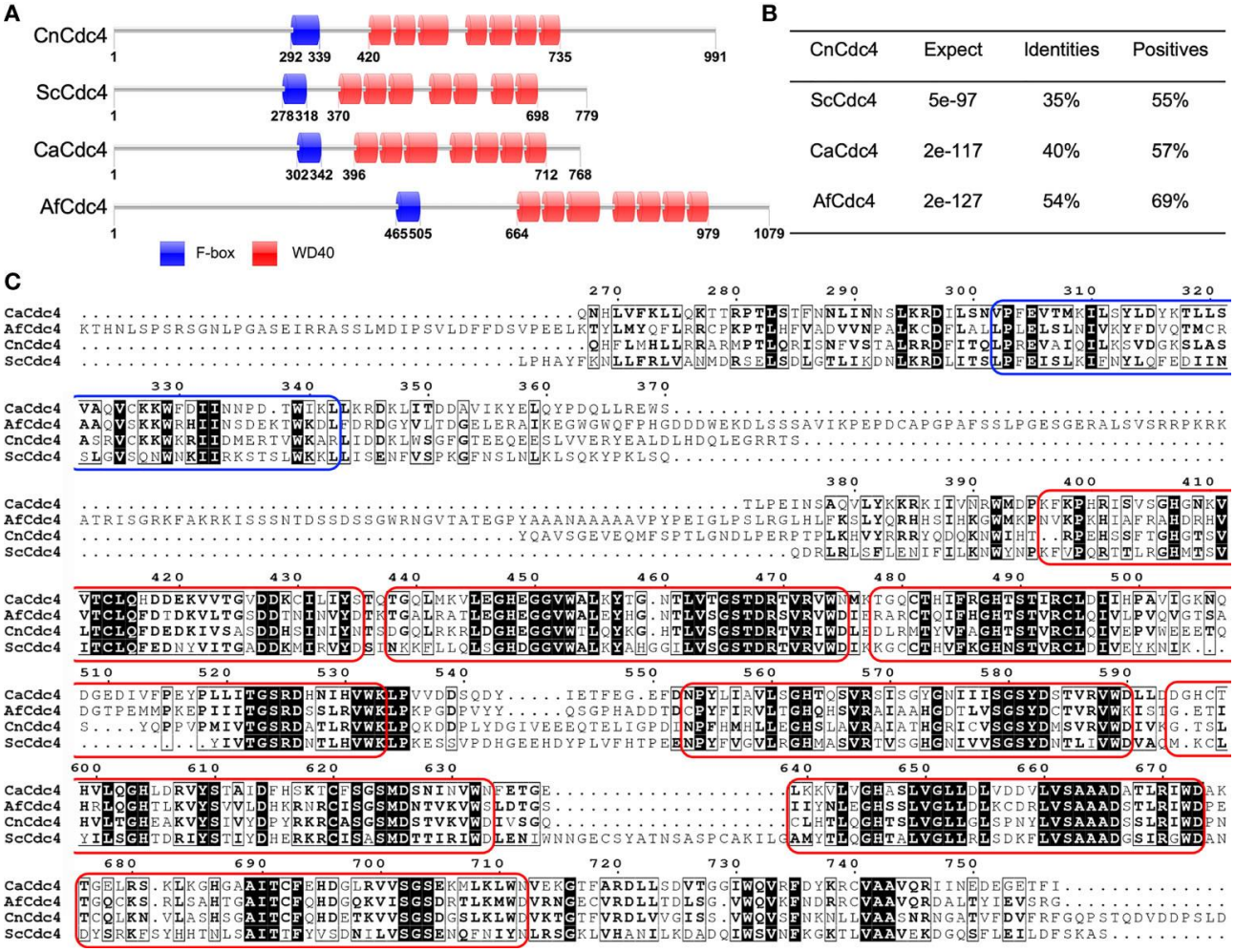
Cdc4 is not associated with the production factors that are involved in the virulence of pathogens [85]. It is also studied that Cdc4 is very essential for the proliferation inside the macrophages as well as in the endurance of the complement cascade of the host [58]. Diagrammatic demonstration of Cdc4 proteins in *C. albicans* (CaCdc4), *S. cerevisiae* (ScCdc4), and *C. neoformans* (CnCdc4) is shown in Figure 2 while the number of genes which code F-box protein recognized in nematode's 6 species are given in table I.

CDC4 in Sexual Reproduction

To assess the function of Cdc4 in the coupling of fungi, we produced the cdc4 mutants in H99 as well as KN99a strain backgrounds [86]. In the process of mating, the wild type and the cdc4 mutants had one nucleus that was fused in the immature basidium, revealing that both strains experience conventional fusion of a nucleus to form basidia, which is constant with earlier discoveries [83,87,88]. Cdc4 is needed for the surveillance of meiosis in the breading process, which is suggested by Wu et al. [58]

Table I: The no. of genes which code F-box protein recognized in nematode's 6 species.

Species	*Hmmer*	*Psscan*	*Hmmer & Psscan*	*Psiblast*	*F-box genes*	*Genome genes*	*Percent*
C. brenneri	559	471	437	1	594	30667	1.93%
C. briggsae	181	150	139	0	192	21936	0.88%
C. elegans	356	302	281	0	377	20532	1.84%
C. japonica	35	23	19	0	39	29964	0.13%
C. remanei	1377	1252	1203	1	1426	31444	4.54%
C. pacificus	95	79	77	0	97	29644	0.33%



CnCdc4	Expect	Identities	Positives
ScCdc4	5e-97	35%	55%
CaCdc4	2e-117	40%	57%
AfCdc4	2e-127	54%	69%

Figure 2. Diagrammatic demonstration of Cdc4 proteins in C. albicans (CaCdc4), S. cerevisiae (ScCdc4), and C. neoformans (CnCdc4). Source = Wu et al.[58]

Functions of F-box protein genes

The genes for the F-box protein contain a key role in the production of plants as well as protection from stress [77,89]. Particularly, homologous F-box genes, which are homologous in nature, could have the same applications. For instance, SmFBX353 and SmFBX241 in cluster 8 are similar [90]. It is also identified that SmFBX193 (PP2), SmFBX13 (N/A), SmFBX49 (GRAS), SmFBX13 (N/A), SmFBX172 (N/A), and SmFBX11 reacted conclusively to stress due to heat in eggplant [90].

Future Directions

Currently, *C. neoformans has* developed resistance to fluconazole (FLU), which generates a number of difficulties associated with the treatment of patients with infections due to *C. neoformans* [13,91]. To solve this problem, researchers are working to search out new agents or sensitizers against fungi (antifungal agents). It is also revealed that a number of antimicrobials and related analogs can increase the potency of antifungals in the treatment of *C. neoformans* [92,93]. Research has revealed that minocycline (MINO) has an effect of inhibition on *Acinetobacter baumannii*, *C. albicans,* and *Staphylococcus aureus* [94,95].

The key property of cryptococcal cells is their capacity to change the size of the encapsulated cells dramatically. These enlarged cells are “titan cells,” which are from 10 µm up to 100 µm in diameter [96-98]. It is also revealed that *C. neoformans* has a pseudohyphal physical composition. In *Cryptococcus*, pseudohyphae are in the form of chains that are partially completed and distinguished cells of yeast that look like real hyphae but are distinguished by contractions among cells as a substitute of septa [99]. In current years, a lot of discoveries and more research on pseudohyphal development must be required to assess the morphological mechanisms in *Cryptococcus*.

Conclusion

In conclusion, the F-box proteins are very important in reproducing sexually and in the virulence of *Cryptococcus*

neoformans. *C. neoformans* is a disease-causing yeast that is very lethal for humans of different ages and genders. So, it is required to study this yeast to better identify the genetics and their role in the pathogenesis of yeast. The genes of the F-box proteins are very vital in various roles of *Cryptococcus neoformans,* as well as their locations in the genome of *C. neoformans*. Different genes of *C. neoformans* are tailored for various functions and are very specific in their functions. Cdc4 gene is very crucial in the virulency and reproduction sexually in *C. neoformans*. It is unclear until now whether Cryptococcus has the same substrates, and further investigation is needed to explain this. Given the critical role that protein degradation plays in cellular homeostasis and the adaptation to environmental stress, further research is needed to identify and characterize the F-box proteins in *C. neoformans*. Such studies could elucidate their roles in pathogenicity, stress response, and survival within the host. Moreover, understanding the functions of these proteins could reveal new targets for antifungal therapy, addressing a significant unmet need in the treatment of cryptococcosis.

References

1. Casadevall A, Perfect JR. Cryptococcus neoformans. In: Book Name. 1998;595. Citeseer. https://doi.org/10.1128/9781555818241
2. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23(4):525-30. https://doi.org/10.1097/QAD.0b013e328322ffac
3. Hull CM, Heitman J. Genetics of Cryptococcus neoformans. Annu Rev Genet. 2002;36(1):557-615. https://doi.org/10.1146/annurev.genet.36.052402.152652
4. Alspaugh JA, Perfect JR, Heitman J. Cryptococcus neoformans mating and virulence are regulated by the G-protein α subunit GPA1 and cAMP. Genes Dev. 1997;11(23):3206-17. https://doi.org/10.1101/gad.11.23.3206
5. Idnurm A, Bahn Y-S, Nielsen K, Lin X, Fraser JA, et al. Deciphering the model pathogenic fungus Cryptococcus neoformans. Nat Rev Microbiol. 2005;3(10):753-64. https://doi.org/10.1038/nrmicro1245
6. Lengeler KB, Davidson RC, D'souza C, Harashima T, Shen W-C, et al. Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev. 2000;64(4):746-85. https://doi.org/10.1128/MMBR.64.4.746-785.2000
7. Wang P, Heitman J. Signal transduction cascades regulating mating, filamentation, and virulence in Cryptococcus neoformans. Curr Opin Microbiol. 1999;2(4):358-62. https://doi.org/10.1016/S1369-5274(99)80063-0
8. Huffnagle GB, Lipscomb MF, Lovchik JA, Hoag KA, Street NE. The role of CD4+ and CD8+ T cells in the protective inflammatory response to a pulmonary cryptococcal infection. J Leukoc Biol. 1994;55(1):35-42. https://doi.org/10.1002/jlb.55.1.35
9. Buchanan KL, Doyle HA. Requirement for CD4+ T lymphocytes in host resistance against Cryptococcus neoformans in the central nervous system of immunized mice. Infect Immun. 2000;68(2):456-62. https://doi.org/10.1128/IAI.68.2.456-462.2000
10. Hoag KA, Street NE, Huffnagle GB, Lipscomb MF. Early cytokine production in pulmonary Cryptococcus neoformans infections distinguishes susceptible and resistant mice. Am J Respir Cell Mol Biol. 1995;13(4):487-95. https://doi.org/10.1165/ajrcmb.13.4.7546779
11. Jarvis JN, Casazza JP, Stone HH, Meintjes G, Lawn SD, et al. The phenotype of the Cryptococcus-specific CD4+ memory T-cell response is associated with disease severity and outcome in HIV-associated cryptococcal meningitis. J Infect Dis. 2013;207(12):1817-28. https://doi.org/10.1093/infdis/jit099
12. Song SJ, Sanders JG, Delsuc F, Metcalf J, Amato K, et al. Comparative analyses of vertebrate gut microbiomes reveal convergence between birds and bats. MBio. 2020;11(1):e02901-19. https://doi.org/10.1128/mBio.02901-19
13. May RC, Stone NRH, Wiesner DL, Bicanic T, Nielsen K. Cryptococcus: from environmental saprophyte to global pathogen. Nat Rev Microbiol. 2016;14(2):106-17. https://doi.org/10.1038/nrmicro.2015.6
14. Voelz K, May RC. Cryptococcal interactions with the host immune system. Eukaryot Cell. 2010;9(6):835-46. https://doi.org/10.1128/EC.00039-10
15. Stenzel W, Müller U, Köhler G, Heppner FL, Blessing M, et al. IL-4/IL-13-dependent alternative activation of macrophages but not microglial cells is associated with uncontrolled cerebral cryptococcosis. Am J Pathol. 2009;174(2):486-96. https://doi.org/10.2353/ajpath.2009.080598
16. Wiesner DL, Specht CA, Lee CK, Smith KD, Mukaremera L, et al. Chitin recognition via chitotriosidase promotes pathologic type-2 helper T cell responses to cryptococcal infection. PLoS Pathog. 2015;11(3):e1004701. https://doi.org/10.1371/journal.ppat.1004701
17. Kronstad JW, Attarian R, Cadieux B, Choi J, D'souza CA, et al. Expanding fungal pathogenesis: Cryptococcus breaks out of the opportunistic box. Nat Rev Microbiol. 2011;9(3):193–203. https://doi.org/10.1038/nrmicro2522
18. Kronstad J, Jung WH, Hu G. Beyond the big three: systematic analysis of virulence factors in Cryptococcus neoformans. Cell Host Microbe. 2008;4(4):308–10. https://doi.org/10.1016/j.chom.2008.09.003
19. Zaragoza O, Rodrigues ML, De Jesus M, Frases S, Dadachova E, et al. The capsule of the fungal pathogen Cryptococcus neoformans. Adv Appl Microbiol. 2009;68:133–216. https://doi.org/10.1016/S0065-2164(09)01204-0
20. Coelho C, Bocca AL, Casadevall A. The intracellular life of Cryptococcus neoformans. Annu Rev Pathol. 2014;9:219–38. https://doi.org/10.1146/annurev-pathol-012513-104653
21. Almeida F, Wolf JM, Casadevall A. Virulence-associated enzymes of Cryptococcus neoformans. Eukaryot Cell. 2015;14(12):1173–85. https://doi.org/10.1128/EC.00103-15
22. Day JN, Chau TT, Wolbers M, Mai PP, Dung NT, et al. Combination antifungal therapy for cryptococcal meningitis. N Engl J Med. 2013;368(14):1291–302. https://doi.org/10.1056/NEJMoa1110404
23. Jonkers W, Rep M. Lessons from fungal F-box proteins. Eukaryot Cell. 2009;8(5):677–95. https://doi.org/10.1128/EC.00386-08
24. Bartlett KH, Kidd SE, Kronstad JW. The emergence of Cryptococcus gattii in British Columbia and the Pacific Northwest. Curr Infect Dis Rep. 2008;10(1):58–65. https://doi.org/10.1007/s11908-008-0011-1
25. Byrnes EJ III, Li W, Lewit Y, Ma H, Voelz K, et al. Emergence and pathogenicity of highly virulent Cryptococcus gattii genotypes in the northwest United States. PLoS Pathog.

2010;6(4):e1000850. https://doi.org/10.1371/journal.ppat.1000850
26. Fraser JA, Giles SS, Wenink EC, Geunes-Boyer SG, Wright JR, et al. Same-sex mating and the origin of the Vancouver Island Cryptococcus gattii outbreak. Nature. 2005;437(7063):1360–4. https://doi.org/10.1038/nature04220
27. Kidd SE, Hagen F, Tscharke R, Huynh M, Bartlett KH, et al. A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc Natl Acad Sci U S A. 2004;101(49):17258–63. https://doi.org/10.1073/pnas.0402981101
28. Liu TB, Xue C. Fbp1-mediated ubiquitin-proteasome pathway controls Cryptococcus neoformans virulence by regulating fungal intracellular growth in macrophages. Infect Immun. 2014;82(2):557–68. https://doi.org/10.1128/IAI.00994-13
29. Feldman RR, Correll CC, Kaplan KB, Deshaies RJ. A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. Cell. 1997;91(2):221-30. doi:10.1016/S0092-8674(00)80404-3.
30. Orlicky S, Tang X, Willems A, Tyers M, Sicheri F. Structural basis for phosphodependent substrate selection and orientation by the SCFCdc4 ubiquitin ligase. Cell. 2003;112(2):243-56. doi:10.1016/S0092-8674(03)00034-5.
31. Liu Q, Larsen B, Ricicova M, Orlicky S, Tekotte H, et al. SCFCdc4 enables mating type switching in yeast by cyclin-dependent kinase-mediated elimination of the Ash1 transcriptional repressor. Mol Cell Biol. 2011;31(3):584-98. doi:10.1128/MCB.00845-10.
32. Atir-Lande A, Gildor T, Kornitzer D. Role for the SCFCDC4 ubiquitin ligase in Candida albicans morphogenesis. Mol Biol Cell. 2005;16(6):2772-85. doi:10.1091/mbc.e05-01-0079.
33. Shieh J-C, White A, Cheng Y-C, Rosamond J. Identification and functional characterization of Candida albicans CDC4. J Biomed Sci. 2005;12:913-24. doi:10.1007/s11373-005-9027-9.
34. Tseng T-L, Lai W-C, Jian T, Li C, Sun H-FS, et al. Affinity purification of Candida albicans CaCdc4-associated proteins reveals the presence of novel proteins involved in morphogenesis. Biochem Biophys Res Commun. 2010;395(1):152-7. doi:10.1016/j.bbrc.2010.03.162.
35. Biswas S, Van Dijck P, Datta A. Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of Candida albicans. Microbiol Mol Biol Rev. 2007;71(2):348-76. doi:10.1128/MMBR.00009-06.
36. Whiteway M, Bachewich C. Morphogenesis in Candida albicans. Annu Rev Microbiol. 2007;61:529-53. doi:10.1146/annurev.micro.61.080706.093341.
37. Li WJ, Wang YM, Zheng XD, Shi QM, Zhang TT, et al. The F-box protein Grr1 regulates the stability of Ccn1, Cln3 and Hof1 and cell morphogenesis in Candida albicans. Mol Microbiol. 2006;62(1):212-26. doi:10.1111/j.1365-2958.2006.05361.x.
38. Calderone RA, Fonzi WA. Virulence factors of Candida albicans. Trends Microbiol. 2001;9(7):327-35. doi:10.1016/S0966-842X(01)02094-7.
39. Holdsworth C, Morgan D. Transitions in context: Leaving home, independence and adulthood. In: Book Name. McGraw-Hill Education (UK); 2005.
40. Richardson JT. Instruments for obtaining student feedback: A review of the literature. Assess Eval High Educ. 2005;30(4):387-415. doi:10.1080/02602930500099193.
41. Rutherford JC, Bahn Y-S, van den Berg B, Heitman J, Xue C. Nutrient and stress sensing in pathogenic yeasts. Front Microbiol. 2019;10:442. doi:10.3389/fmicb.2019.00442.
42. Cao C, Xue C. More than just cleaning: Ubiquitin-mediated proteolysis in fungal pathogenesis. Front Cell Infect Microbiol. 2021;11:774613. doi:10.3389/fcimb.2021.774613.
43. Hong MJ, Kim DY, Seo YW. SKP1-like-related genes interact with various F-box proteins and may form SCF complexes with Cullin-F-box proteins in wheat. Mol Biol Rep. 2013;40(2):969-81. doi:10.1007/s11033-012-2139-1.
44. Zhou P, Howley PM. Ubiquitination and degradation of the substrate recognition subunits of SCF ubiquitin–protein ligases. Mol Cell. 1998;2(5):571-80. doi:10.1016/S1097-2765(00)80156-2.
45. Komander D. The emerging complexity of protein ubiquitination. Biochem Soc Trans. 2009;37(5):937-53. doi:10.1042/BST0370937.
46. Thompson LL, Rutherford KA, Lepage CC, McManus KJ. The SCF complex is essential to maintain genome and chromosome stability. Int J Mol Sci. 2021;22(16):8544. doi:10.3390/ijms22168544.
47. Nguyen KM, Busino L. The biology of F-box proteins: The SCF family of E3 ubiquitin ligases. In: Sun Y, Wei W, Jin J, editors. Cullin-RING ligases and protein neddylation: Biology and therapeutics. Singapore: Springer Singapore; 2020. p. 111-22. doi:10.1007/978-981-15-1025-0_8.
48. Kipreos ET, Pagano M. The F-box protein family. Genome Biol. 2000;1(5):reviews3002.1. doi:10.1186/gb-2000-1-5-reviews3002.
49. Kuroda H, Takahashi N, Shimada H, Seki M, Shinozaki K, et al. Classification and expression analysis of Arabidopsis F-box-containing protein genes. Plant Cell Physiol. 2002;43(10):1073-85. doi:10.1093/pcp/pcf151.
50. Skaar JR, Pagan JK, Pagano M. Mechanisms and function of substrate recruitment by F-box proteins. Nat Rev Mol Cell Biol. 2013;14(6):369-81. doi:10.1038/nrm3582.
51. Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW. F-Box Proteins Are Receptors that Recruit Phosphorylated Substrates to the SCF Ubiquitin-Ligase Complex. *Cell.* 1997;91(2):209–19. https://doi.org/10.1016/S0092-8674(00)80403-1
52. Ponts N, Yang J, Chung D-WD, Prudhomme J, Girke T, et al. Deciphering the Ubiquitin-Mediated Pathway in Apicomplexan Parasites: A Potential Strategy to Interfere with Parasite Virulence. *PLOS ONE.* 2008;3(6):e2386. https://doi.org/10.1371/journal.pone.0002386
53. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 2014;43(D1):D447–52. https://doi.org/10.1093/nar/gku1003
54. Gonzalez LE, Keller K, Chan KX, Gessel MM, Thines BC. Transcriptome analysis uncovers Arabidopsis F-BOX STRESS INDUCED 1 as a regulator of jasmonic acid and abscisic acid stress gene expression. *BMC Genomics.* 2017;18(1):533. https://doi.org/10.1186/s12864-017-3864-6
55. Venancio TM, Balaji S, Geetha S, Aravind L. Robustness and evolvability in natural chemical resistance: identification of novel systems properties, biochemical mechanisms and regulatory interactions. *Mol BioSyst.* 2010;6(8):1475–91. https://doi.org/10.1039/c002567b
56. Chow EWL, Pang LM, Wang Y. From Jekyll to Hyde: the yeast-hyphal transition of *Candida albicans*. *Pathogens.* 2021;10(7):859. https://doi.org/10.3390/pathogens10070859
57. Hong MJ, Kim J-B, Seo YW, Kim DY. F-box genes in the wheat genome and expression profiling in wheat at different developmental stages. *Genes.* 2020;11(10):1154. https://doi.org/10.3390/genes11101154
58. Wu T, Fan C-L, Han L-T, Guo Y-B, Liu T-B. Role of F-box Protein Cdc4 in Fungal Virulence and Sexual Reproduction of

Cryptococcus neoformans. *Front Cell Infect Microbiol.* 2022;11:806465. https://doi.org/10.3389/fcimb.2021.806465
59. Rivera A, Hohl TM, Collins N, Leiner I, Gallegos A, et al. Dectin-1 diversifies *Aspergillus fumigatus*-specific T cell responses by inhibiting T helper type 1 CD4 T cell differentiation. *J Exp Med.* 2011;208(2):369–81. https://doi.org/10.1084/jem.20100906
60. Rivera A, Ro G, Van Epps HL, Simpson T, Leiner I, et al. Innate immune activation and CD4+ T cell priming during respiratory fungal infection. *Immunity.* 2006;25(4):665–75. https://doi.org/10.1016/j.immuni.2006.08.016
61. Rivera A, Van Epps HL, Hohl TM, Rizzuto G, Pamer EG. Distinct CD4+-T-cell responses to live and heat-inactivated *Aspergillus fumigatus* conidia. *Infect Immun.* 2005;73(11):7170–9. https://doi.org/10.1128/IAI.73.11.7170-7179.2005
62. Olson MR, McDermott DS, Varga SM. The Initial Draining Lymph Node Primes the Bulk of the CD8 T Cell Response and Influences Memory T Cell Trafficking after a Systemic Viral Infection. *PLOS Pathog.* 2012;8(12):e1003054. https://doi.org/10.1371/journal.ppat.1003054
63. Traynor TR, Herring AC, Dorf ME, Kuziel WA, Toews GB, et al. Differential roles of CC chemokine ligand 2/monocyte chemotactic protein-1 and CCR2 in the development of T1 immunity. *J Immunol.* 2002;168(9):4659–66. https://doi.org/10.4049/jimmunol.168.9.4659
64. Huffnagle GB, McNeil LK. Dissemination of *C. neoformans* to the central nervous system: role of chemokines, Th1 immunity and leukocyte recruitment. *J Neurovirol.* 1999;5(1):76–81. https://doi.org/10.3109/13550289909029748
65. García-Barbazán I, Trevijano-Contador N, Rueda C, de Andrés B, Pérez-Tavárez R, et al. The formation of titan cells in *Cryptococcus neoformans* depends on the mouse strain and correlates with induction of Th2-type responses. *Cell Microbiol.* 2016;18(1):111–24. https://doi.org/10.1111/cmi.12488
66. Zaragoza O, Alvarez M, Telzak A, Rivera J, Casadevall A. The relative susceptibility of mouse strains to pulmonary *Cryptococcus neoformans* infection is associated with pleiotropic differences in the immune response. *Infect Immun.* 2007;75(6):2729–39. https://doi.org/10.1128/IAI.00094-07
67. Carroll SF, Lafferty EI, Flaczyk A, Fujiwara TM, Homer R, et al. Susceptibility to progressive *Cryptococcus neoformans* pulmonary infection is regulated by loci on mouse chromosomes 1 and 9. *Infect Immun.* 2012;80(12):4167–75. https://doi.org/10.1128/IAI.00417-12
68. Guillot Lc, Carroll SF, Homer R, Qureshi ST. Enhanced innate immune responsiveness to pulmonary *Cryptococcus neoformans* infection is associated with resistance to progressive infection. *Infect Immun.* 2008;76(10):4745–56. https://doi.org/10.1128/IAI.00341-08
69. Tallerico R, Todaro M, Di Franco S, Maccalli C, Garofalo C, et al. Human NK cells selective targeting of colon cancer-initiating cells: a role for natural cytotoxicity receptors and MHC class I molecules. *J Immunol.* 2013;190(5):2381–90. https://doi.org/10.4049/jimmunol.1201542
70. Herring AC, Lee J, McDonald RA, Toews GB, Huffnagle GB. Induction of interleukin-12 and gamma interferon requires tumor necrosis factor alpha for protective T1-cell-mediated immunity to pulmonary *Cryptococcus neoformans* infection. *Infect Immun.* 2002;70(6):2959–64. https://doi.org/10.1128/IAI.70.6.2959-2964
71. Zhang Y, Wang F, Tompkins KC, McNamara A, Jain AV, et al. Robust Th1 and Th17 immunity supports pulmonary clearance but cannot prevent systemic dissemination of highly virulent *Cryptococcus neoformans* H99. *Am J Pathol*. 2009;175(6):2489–500. https://doi.org/10.2353/ajpath.2009.090530
72. Feretzaki M, Hardison SE, Wormley FL Jr, Heitman J. *Cryptococcus neoformans* hyperfilamentous strain is hypervirulent in a murine model of cryptococcal meningoencephalitis. *PLoS One*. 2014;9(8):e104432. https://doi.org/10.1371/journal.pone.0104432
73. Jain AV, Zhang Y, Fields WB, McNamara DA, Choe MY, et al. Th2 but not Th1 immune bias results in altered lung functions in a murine model of pulmonary *Cryptococcus neoformans* infection. *Infect Immun*. 2009;77(12):5389–99. https://doi.org/10.1128/IAI.00809-09
74. Murdock BJ, Huffnagle GB, Olszewski MA, Osterholzer JJ. Interleukin-17A enhances host defense against cryptococcal lung infection through effects mediated by leukocyte recruitment, activation, and gamma interferon production. *Infect Immun*. 2014;82(3):937–48. https://doi.org/10.1128/IAI.01477-13
75. Innan H, Kondrashov F. The evolution of gene duplications: classifying and distinguishing between models. *Nat Rev Genet*. 2010;11(2):97–108. https://doi.org/10.1038/nrg2689
76. Zhang J. Evolution by gene duplication: an update. *Trends Ecol Evol*. 2003;18(6):292–8. https://doi.org/10.1016/S0169-5347(03)00033-8
77. Lechner E, Achard P, Vansiri A, Potuschak T, Genschik P. F-box proteins everywhere. *Curr Opin Plant Biol*. 2006;9(6):631–8. https://doi.org/10.1016/j.pbi.2006.09.003
78. Nakayama K, Nagahama H, Minamishima YA, Miyake S, Ishida N, et al. Skp2-mediated degradation of p27 regulates progression into mitosis. *Dev Cell*. 2004;6(5):661–72. https://doi.org/10.1016/S1534-5807(04)00131-5
79. Reed SI. Ratchets and clocks: the cell cycle, ubiquitylation and protein turnover. *Nat Rev Mol Cell Biol*. 2003;4(11):855–64. https://doi.org/10.1038/nrm1246
80. Bai C, Sen P, Hofmann K, Ma L, Goebl M, et al. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell*. 1996;86(2):263–74. https://doi.org/10.1016/S0092-8674(00)80098-7
81. Ou C-Y, Pi H, Chien C-T. Control of protein degradation by E3 ubiquitin ligases in Drosophila eye development. *Trends Genet*. 2003;19(7):382–9. https://doi.org/10.1016/S0168-9525(03)00146-X
82. Ho MS, Tsai P-I, Chien C-T. F-box proteins: the key to protein degradation. *J Biomed Sci*. 2006;13:181–91.
83. Liu T-B, Wang Y, Stukes S, Chen Q, Casadevall A, et al. The F-Box protein Fbp1 regulates sexual reproduction and virulence in *Cryptococcus neoformans*. *Eukaryot Cell*. 2011;10(6):791–802. https://doi.org/10.1128/EC.00004-11
84. Stempinski PR, Gerbig GR, Greengo SD, Casadevall A. Last but not yeast-The many forms of *Cryptococcus neoformans*. *PLoS Pathog*. 2023;19(1):e1011048. https://doi.org/10.1371/journal.ppat.1011048
85. Kono K, Al-Zain A, Schroeder L, Nakanishi M, Ikui AE. Plasma membrane/cell wall perturbation activates a novel cell cycle checkpoint during G1 in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*. 2016;113(25):6910–5. https://doi.org/10.1073/pnas.1523824113
86. Niederhuber JE. *Clinical Immuno-Oncology*. Chapter: Book Name. 2023 of publication; Elsevier Health Sciences.
87. Liu T-B, Xue C. The ubiquitin-proteasome system and F-box proteins in pathogenic fungi. *Mycobiology*. 2011;39(4):243–8. https://doi.org/10.5941/MYCO.2011.39.4.243
88. Fan C-L, Han L-T, Jiang S-T, Chang A-N, Zhou Z-Y, et al. The Cys2His2 zinc finger protein Zfp1 regulates sexual reproduction and virulence in *Cryptococcus neoformans*. *Fungal Genet Biol*. 2019;124:59–72. https://doi.org/10.1016/j.fgb.2019.01.002

89. Abd-Hamid N-A, Ahmad-Fauzi M-I, Zainal Z, Ismail I. Diverse and dynamic roles of F-box proteins in plant biology. *Planta*. 2020;251:1–31. https://doi.org/10.1007/s00425-020-03356-8
90. Wang Y, Chuhao L, Yan S, Bingwei Y, Gan Y, et al. Genome-Wide Analysis and Characterization of Eggplant F-Box Gene Superfamily: Gene Evolution and Expression Analysis under Stress. *Int J Mol Sci*. 2022;23(24):16049. https://doi.org/10.3390/ijms232416049
91. Recio R, Perez-Ayala A. *Cryptococcus neoformans* meningoencephalitis. *N Engl J Med*. 2018;379(3):281. https://doi.org/10.1056/NEJMicm1801051
92. Sangalli-Leite F, Scorzoni L, da Silva JF, de Oliveira HC, de Lacorte Singulani J, et al. Synergistic effect of pedalitin and amphotericin B against *Cryptococcus neoformans* by in vitro and in vivo evaluation. *Int J Antimicrob Agents*. 2016;48(5):504–11. https://doi.org/10.1016/j.ijantimicag.2016.07.025
93. Donlin MJ, Zunica A, Lipnicky A, Garimallaprabhakaran AK, Berkowitz AJ, et al. Troponoids can inhibit growth of the human fungal pathogen *Cryptococcus neoformans*. *Antimicrob Agents Chemother*. 2017;61(4):e02574-16. https://doi.org/10.1128/AAC.02574-16
94. Shi W, Chen Z, Chen X, Cao L, Liu P, et al. The combination of minocycline and fluconazole causes synergistic growth inhibition against *Candida albicans*: an in vitro interaction of antifungal and antibacterial agents. *FEMS Yeast Res*. 2010;10(7):885–93. https://doi.org/10.1111/j.1567-1364.2010.00664.x
95. Fragkou PC, Poulakou G, Blizou A, Blizou M, Rapti V, et al. The role of minocycline in the treatment of nosocomial infections caused by multidrug, extensively drug and pandrug-resistant *Acinetobacter baumannii*: a systematic review of clinical evidence. *Microorganisms*. 2019;7(6):159. https://doi.org/10.3390/microorganisms7060159
96. Okagaki LH, Strain AK, Nielsen JN, Charlier C, Baltes NJ, et al. Cryptococcal cell morphology affects host cell interactions and pathogenicity. *PLoS Pathog*. 2010;6(6):e1000953. https://doi.org/10.1371/journal.ppat.1000953
97. Zaragoza O, Garcia-Rodas R, Nosanchuk JD, Cuenca-Estrella M, Rodríguez-Tudela JL, et al. Fungal cell gigantism during mammalian infection. *PLoS Pathog*. 2010;6
98. Dambuza IM, Drake T, Chapuis A, Zhou X, Correia J, et al. The Cryptococcus neoformans Titan cell is an inducible and regulated morphotype underlying pathogenesis. PLoS pathogens, (2018); 14(5): e1006978. https://doi.org/10.1371/journal.ppat.1006978
99. Kozubowski L, Heitman J. Profiling a killer, the development of Cryptococcus neoformans. FEMS microbiology reviews, (2012); 36(1): 78-94 https://doi.org/10.1111/j.1574-6976.2011.00286.x