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Microbe Profile: *Cryptococcus neoformans* species complex

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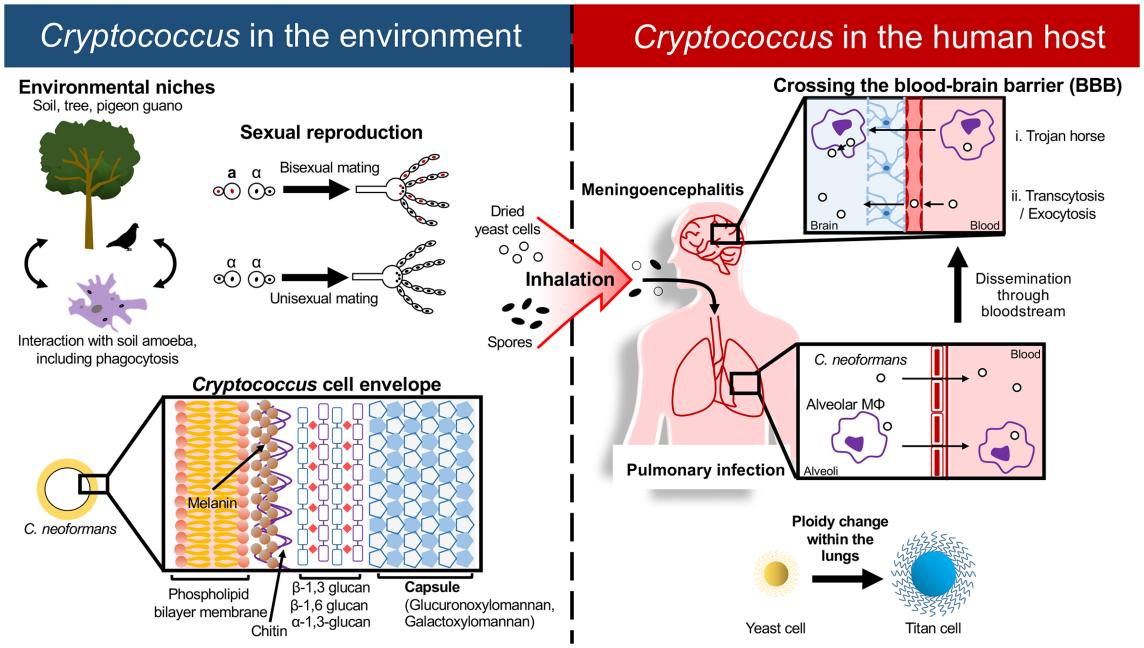

Graphical abstract

Abstract

Cryptococcus neoformans is a lethal fungus disguised in a polysaccharide coat. It can remain dormant in the host for decades prior to reactivation, causing systemic cryptococcosis in humans and other mammals. *Cryptococcus* deploys a multitude of traits to adapt to and survive within the host, including immunosuppression, an ability to replicate intra- and extra-cellularly in phagocytes, changes in morphology and ploidy, a predilection to infect the CNS, and the capacity to utilize neurotransmitters and unique carbon sources available in the brain. These pathogenic strategies displayed by this fungus might have evolved through its interactions with microbial predators in the environment.

TAXONOMY

Domain *Eukaryota*, kingdom *Fungi*; phylum *Basidiomycota*; class *Tremellomycetes*; order *Tremellales*; family *Cryptococcaceae*; genus *Cryptococcus*; species *Cryptococcus neoformans* and *Cryptococcus gattii*. The '*Cryptococcus neoformans*' and '*Cryptococcus gattii*' species complexes (collectively pathogenic *Cryptococcus* species) encompass at least seven

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Abbreviations: CNS, central nervous system; MAT, mating type locus.

distinct biological and phylogenetic species, with potentially additional cryptic species to be defined.

PROPERTIES

Cryptococcus species are aerobic mesophilic fungi phylogenetically more closely related to mushrooms than to the baker's yeast. *Cryptococcus* species are found on trees or in soil contaminated with bird guano. They grow as budding yeast cells (~5 µm in diameter) under nutrient rich conditions. A characteristic feature of *Cryptococcus* is the polysaccharide capsule, whose production is stimulated by conditions that mimic human physiology, including high CO_2, low iron, neutral/alkaline pH and serum. The ability to utilize various phenolic substrates, including the neurotransmitter dopamine, to produce melanin enhances its survival in the host and distinguishes it from other pathogenic yeasts.

GENOME

The pathogenic *Cryptococcus* species are haploid, although naturally occurring diploid strains resulting from inter-lineage mating or endoreplication have been frequently recovered from certain geographical regions. The haploid genome is about 20 Mb in size, organized into 14 chromosomes with regional centromeres enriched with transposable elements and their remnants. The overall GC content of the genome is about 48%. Cryptococcal genome encodes approximately 7000 protein-coding genes, as well as more than 1000 genes encoding potential long non-coding RNAs. The transcription landscapes of *Cryptococcus* resemble those of higher eukaryotes and are unusually complex. For instance, >98% of its genes contain intron(s), with five introns per gene on average [1]. Untranslated regions (UTRs), alternative splicing or alternative transcription start and stop sites are prevalent and likely biologically important. The *Cryptococcus* mating-type locus (*MAT*) is notably one of the largest among those that have been characterized, spanning over 100 kb and encompassing more than 20 genes [2]. *MAT* defines a cryptococcal cell to be either **a** or α mating type. The mitochondrial genomes vary between 20–40 kb largely due to differences in the presence of introns and the size of the intergenic regions. Mitochondrial DNA is typically inherited from the *MAT***a** parent during bisexual reproduction.

PHYLOGENY

Sequence analyses revealed two large clusters within the pathogenic *Cryptococcus* species complex, corresponding to the *C. neoformans* and *C. gattii* species complexes, which diverged from a common ancestor more than 40 million years ago. These subcomplexes further contain two and five distinct pathogenic species, respectively [3]. Significant divergence has accumulated among these seven species, including nucleotide polymorphisms, chromosomal rearrangements, as well as chromosomal translocations mediated by transposons located within the centromeres. While *C. neoformans* spp. are distributed worldwide and cause systemic cryptococcosis in mostly immunocompromised patients, species in the *C. gattii* complex are often found in tropical and subtropical regions and affect immunocompetent individuals. Nonetheless, compared to the closely related non-pathogenic sibling clades, the pathogenic *Cryptococcus* species form a monophyletic clade, suggesting a single origin of pathogenicity potential. Disease manifestations may differ slightly among the *Cryptococcus* species, but diagnosis and treatment are largely the same regardless of the species involved [4].

KEY FEATURES AND DISCOVERIES

First described in the 1890s, *Cryptococcus* only caused diseases sporadically prior to the HIV/AIDS pandemic and advanced medical treatments (cancer chemotherapy, organ transplantation, long-term corticosteroid use, chronic leukaemia and lymphomas, and sarcoidosis) [5]. Now a major threat to public health, *Cryptococcus* claims hundreds of thousands of lives each year and is responsible for ~15% of deaths among HIV/AIDS patients. *Cryptococcus* does not transmit from humans to humans. Environmental exposure is common and asymptomatic in the general population as the fungus is either cleared or becomes dormant [6]. Fatal systemic cryptococcosis, partly attributable to reactivation of dormant infections, occurs commonly in immunocompromised individuals. The mortality rates of cryptococcal meningitis with current antifungal treatments range from 10–70%, with the highest death rates observed in sub-Saharan regions of Africa. Currently there is no vaccine against cryptococcosis.

Cryptococcus received its classification as a basidiomycete when its **a**-α bisexual reproduction was discovered in the 1970s. Subsequently, unisexual reproduction between cells of the α mating type was discovered, which may have contributed to the preponderance of α isolates (>99%) in natural populations. Both sexual reproduction models involve morphological differentiation, ploidy changes and meiotic recombination [7].

The unusual features of the cryptococcal cell envelope have attracted considerable attention. The polysaccharide capsule is attached to the cell wall but often shed copiously into the extracellular milieu. Capsule enhances *Cryptococcus* evasion of host detection, inhibits phagocytosis and suppresses host immune functions. The cryptococcal cell wall is enriched with chitosan, the deacetylated form of the universal fungal component chitin. Chitosan also contributes to cryptococcal host evasion. Melanin anchored in the chitin layer of the cell wall protects the fungus from biotic and abiotic assaults, including radiation and reactive oxygen species. *Cryptococcus* synthesizes melanin from varying phenolic substrates, including the neurotransmitter dopamine. These features collectively contribute to *Cryptococcus* as a stealth pathogen.

Investigation into cryptococcal biology has accelerated greatly after the 1990s. With advanced genetic and biochemical tools and resources, more facets of its virulence attributes are being discovered [8], including key pathways (e.g. calcineurin, cAMP-PKA and HOG) involved in virulence and stress

response, extracellular vesicles that can deliver virulence factors over relatively long distances, changes in cell shape, cell size and ploidy (e.g. titan cells) in coordination with disease progression, an exceptional ability to withstand high levels of CO_2 and hypoxia as an obligate aerobe, heteroresistance to antifungals through genome alterations, production of surface proteins that dictate its neurotropism and more. *Cryptococcus* species have become model systems to study microbial pathogenicity, sexual reproduction, as well as general eukaryotic biology.

OPEN QUESTIONS

(1) How does *Cryptococcus* exist in a dormant state and what triggers reactivation?
(2) How does regulation of the cryptococcal life cycle or cell cycle affect the yeast population and control disease progression in the host?
(3) How can we identify immune-protective fungal antigens that can prevent cryptococcal infection/reactivation in immunocompromised hosts?
(4) How to identify anti-cryptococcal targets and develop selective drugs that are fungicidal with minimal side effects?
(5) How microevolution affects cryptococcal adaptation to the host or antifungal drug treatment?

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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