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Review

Cryptococcus neoformans mutant screening: a genome-scale's worth of function discovery

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ABSTRACT

Described in humans in 1894, *Cryptococcus neoformans*, a medically-important basidiomycete fungus, has since been studied in order to identify the factors that enable it to cause disease. Large-scale collections of mutants have been created, and mutant strains deposited in biorepositories. Studying these collections provides deeper functional insights for genes controlling biological processes, and ultimately complements a wealth of genomics and transcriptomics information. Therefore, to many laboratories use of these resources is profoundly insightful to study the molecular basis of cryptococcal pathobiology. The available collections of *C. neoformans* mutants also aid the molecular target identification and drug discovery efforts. To facilitate access to information, large-scale gene discoveries made from screening *C. neoformans* collections for over the past decade and a half are hereby compiled in a single document, and their key findings presented in brief. *Cryptococcus neoformans* is potentially the only basidiomycete yeast with extensively analyzed collections of mutants, and this makes it a plausible model for the creation of important collections for other basidiomycete fungi. Indeed, the genome-wide collections discussed in this present review represent a large sum of genes with associated phenotypes in virulence of this species on which prospective screens can rely as a reference point. Therefore, it is desired that studies underway will draw from these genome-wide screens to identify management strategies against fungal diseases.

1. Introduction

Cryptococcus neoformans is a basidiomycete yeast residing in various environmental sources including *Eucalyptus* (Elhariri *et al.* 2016) and mopane trees (Desjardins *et al.* 2017; Litvintseva *et al.* 2011), as well as in bird excreta (Abulreesh *et al.* 2015; Canónico-González *et al.* 2013; Soltani *et al.* 2013) (Fig. 1). Despite being an environmental fungus, it can induce mycoses (or cryptococcoses) in mammals that are noncommunicable between infected hosts. The infection leading to these diseases occurs when airborne spores and/or desiccated yeasts inhaled from the environment make contact with a host, typically a human or animal host (Heitman *et al.* 2010). The infections are particularly life-threating if inhaled spores come into contact with an immunocompromised individual who has been exposed to the human immunodeficiency virus

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(HIV) or has undergone organ transplantation. Interestingly, infections observed in the 'non-HIV non-transplant individuals' found among groups of individuals affected by *Cryptococcus* infections have been recorded. This has sparked great interest to study *C. neoformans* infection pathways in persons showing no apparent immune system disorders (Capoor *et al.* 2007; Coelho and Casadevall, 2016; Chen et al. 2008; Kwon-Chung and Saijo 2015; Lui *et al.* 2006; Murakami *et al.* 2016; Pappas, 2013; Suchitha *et al.* 2012).

A healthy immune system can typically clear *C. neoformans* spores (Fig. 1a–b), or it can be affected by mild or latent infections due to these spores (Fig. 1a, c). This is contrary to an immunocompromised host where the spores may proliferate beyond latency to infect the pulmonary system as well as other body tissues (Fig. 1c–g). Cryptococci from *C. neoformans* also possess a remarkable neurotropism for the central nervous system, often resulting in fatal cryptococcal meningoencephalitis (CM). For many years it has been understood that the ability of cryptococci to cross the blood–brain barrier is linked to secretion of digestive enzymes and leveraging of phagocytes as transit 'Trojan horses' (Fig. 1g–j). But recent analyses show that far more detailed mechanisms promote CM disease development (Na Pombejra *et al.* 2017; Santiago-Tirado *et al.* 2017; Sorrell *et al.* 2016; Vu *et al.* 2014). Understanding the mechanisms leading to CM development can yield more effective treatment procedures for the disease. The standard virulence factors – capsule, melanin and growth at high temperatures (Fig. 1) – are also pivotal for the success of *C. neoformans* to cause infections that manifest into various disease symptoms (Cordero and Casadevall, 2017; Nosanchuk and Casadevall, 2003, 2006; O'Meara and Alspaugh, 2012; Perfect, 2006). However, less well-characterized virulence factors are becoming worrisome as they may serve as alternative means to increase the severity of cryptococcoses (Fig. 1). Thankfully, there are ongoing investigations that are unpacking the mechanisms controlling these virulence factors before they become a common medical concern (Alspaugh, 2015; Okagaki and Nielsen, 2012; Okagaki *et al.* 2010; Taylor-Smith and May, 2016; Wang *et al.* 2012; Zaragoza *et al.* 2010).

In the early 2000's the focus of genetic analyses largely prioritized large-scale functional analyses for genes controlling capsule and melanin formation (Fig. 2). It was around 2008 and following this year that much was done in terms of large-scale gene discoveries other than screening for capsule and melanin genes. Perhaps heralding this is the study by Liu *et al.* (2008), providing the very first extensive collection ever to saturate the *C. neoformans* genome with mutations, single-handedly covering 20 % of protein coding genes. The

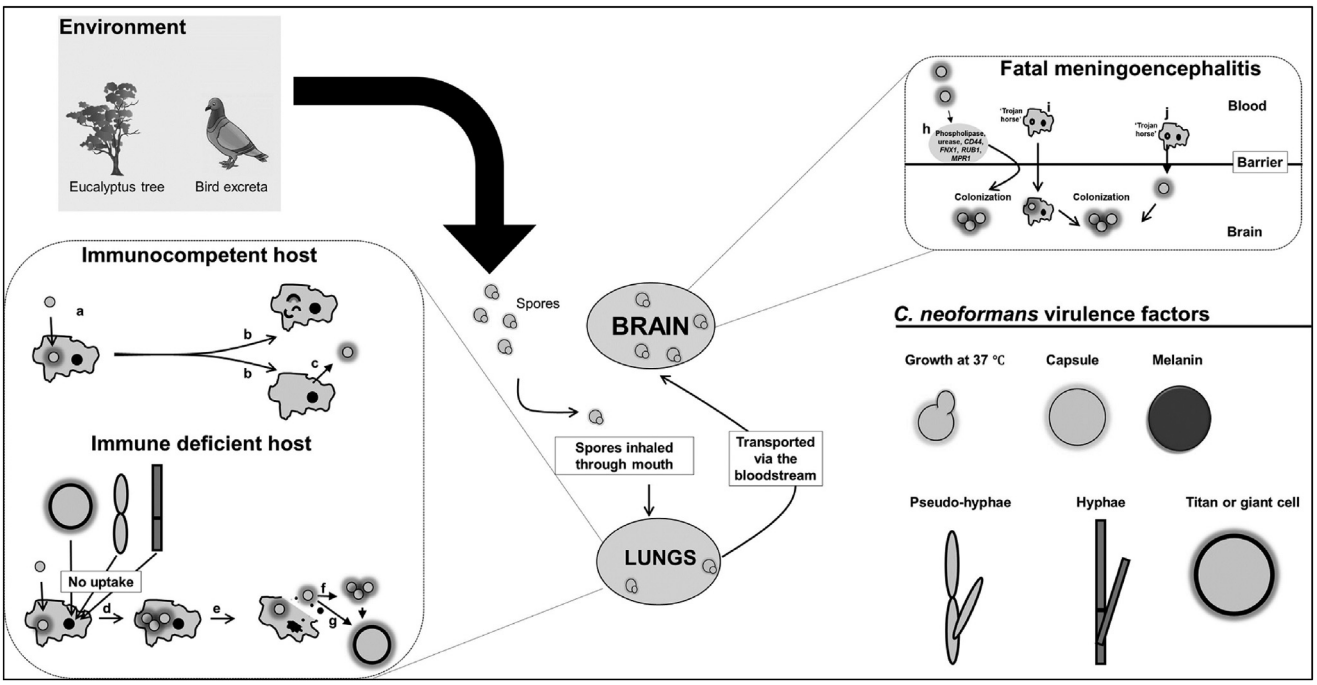

Fig. 1 – The link between the environment within which *C. neoformans* is found, the host and cryptococses development. Yeast spores are inhaled by the host from the environment and interact with mammalian phagocytes (e.g. lung macrophages). Interaction in an immunocompetent host results in phagocytes swallowing yeast cells (a). Contact made by these cells triggers phagosomal conditions which either lead to the destruction or latency of the yeast cells (b). During latency non-lytic discharge of yeast cells occurs (c). Spores can also attack an immune deficient host. Here yeast cells can alter phagocytic conditions in their favor, and multiply (d), leading to rupture of the phagocyte (e). Further multiplication outside the phagocyte can lead to disseminated cryptococcoses such as bloodstream infections (fungemeia) or reinfection of healthy phagocyes (f). Yeast cells may switch to giant cells (g), or hyphae. Because of the large size of these cells, internalization by phagocytes is prevented, and this may results in further infections in other locations of the host. Fatal meningoencephalitis develops when *C. neoformans* cross the blood brain barrier (BBB) following secretion of degradative enzymes (h), or by using phagocytes as transit molecules to cross the barrier (i). Phagocytes close enough to the BBB can excrete yeast cells which finally colonize the brain surface (j).

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