PEARLS

Cellular plasticity of pathogenic fungi during infection

Kenya E. Fernandes, Dee A. Carter

School of Life and Environmental Sciences and the Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, Australia

* dee.carter@sydney.edu.au

Introduction

A widespread trait amongst fungi is their ability to alter their morphology in response to environmental stimuli. The type and degree of alteration, which commonly includes changes in cell size and shape, can vary between strains and even between individual cells within genetically uniform populations, providing many levels of variation within species. As well as enhancing their ability to survive in different environmental niches, this variation plays an important role in the ability of human fungal pathogens to survive and cause disease in the host. Some well-known examples are the thermally dimorphic fungi, which grow as moulds at 22 °C–25 °C in the soil and covert into yeasts at 37 °C when in a mammalian host [1]. Some fungi, rather than switching between 2 distinct forms in different niches, produce a variety of forms in the host that appear to have roles in the infection process. This phenomenon has not been as extensively studied as thermal dimorphism but may be important for understanding disease progression and outcome. Here, we review selected examples of pathogenic fungi that produce distinct morphological forms when living in and on host tissues that may be linked to infection and virulence.

White-to-opaque and yeast-to-hyphae transitions in Candida albicans

C. albicans is a commensal of humans that colonises the mucosal surfaces of most healthy individuals but can cause life-threatening infections in immunocompromised hosts [2]. The ability to thrive in different niches within the host is crucial for survival, and C. albicans possesses an array of morphological forms that are thought to aid it in this process (Table 1; Fig 1A). During commensal growth, C. albicans exhibits a range of morphologies that appear to be suited to various host niches [3]. White, opaque, grey, and gastrointestinal-induced transition (GUT) yeast cell types have been described, and C. albicans can switch between these, enabling it to adapt rapidly to changes in its environment. White cells are smooth and round, whereas opaque cells are elongated, with more vacuoles and cell surface protuberances, and grey cells are the smallest cell type and are elongated with no protuberances [4, 5]. Opaque cells have been found to mate more efficiently than white cells and, along with grey cells, are more virulent and capable of faster proliferation on epithelial surfaces, whilst white cells are more virulent in systemic bloodstream infection models [6–8]. Opaque cells also possess increased resistance to macrophages and neutrophils because, unlike white cells, they do not secrete a
chemoattractant, and it has been suggested that the switch to opaque cells may be a mechanism to escape the immune system [9]. GUT cells are morphologically similar to opaque cells but do not possess cell surface protuberances, are unable to mate, and seem to be specialised for commensalism in the mammalian gastrointestinal tract, displaying superior fitness to other cell types in models of this niche [10].

With host immunosuppression, \textit{C. albicans} can become an opportunistic pathogen, and this is accompanied by transitions from the unicellular yeast form to hyphal and pseudohyphal cell types [3]. \textit{Candida} hyphae are long, filamentous, and multicellular [11], whilst pseudohyphal are elongated ovals that have features of both yeasts and hyphae. Depending on environmental factors, these 3 cell types can either stably proliferate to maintain their cell type or transition to the other cell types, and the ability to do so is a crucial determinant of virulence in \textit{C. albicans}. Hyphal and pseudohyphal forms are invasive and are thought to have an increased ability to penetrate into host tissues and internal organs and cause damage, whilst yeast cells may aid dissemination through the bloodstream because of their small size [15, 16]. Mutants that cannot interchange between these cell types are typically defective in infection models, and isolates taken from patients with disseminated candidiasis generally contain both yeast and hyphal forms [12, 13]. Biofilms, which are complex and densely packed communities of cells with increased resistance to host defences and antifungal drugs, also typically contain all 3 cell types [14].

\textbf{Yeast-to-hyphae transition and pleomorphism in \textit{Malassezia} spp.}

\textit{Malassezia} spp. are lipid-dependent yeasts that are a major component of the normal skin mycobiome of humans and other animals, although metagenomic sequencing studies have

\begin{table}
\centering
\caption{The variety of morphological forms produced by pathogenic fungi that may play a role during the infection process.}
\begin{tabular}{llll}
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Fungus & Phylum & Morphological Form & Description \\
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\textit{C. albicans} & Ascomycota & hyphae & long, tube-shaped filaments, multicellular \\
 & & pseudohyphae & elongated ellipsoids, multicellular \\
 & & white cells & small, round-to-oval \\
 & & grey cells & small, elongated ovals \\
 & & opaque cells & larger elongated ovals, more vacuoles, surface protuberances \\
 & & GUT cells & larger elongated ovals \\
\textit{Malassezia} spp. & Basidiomycota & hyphae & elongated, 10–25 $\mu$m in length \\
 & & regular yeasts & round, 8 $\mu$m in diameter \\
 & & ovoid cells$^*$ & ovoid, 2.5–6 $\mu$m in length \\
 & & cylindrical cells$^*$ & short variants 1.5–3 $\mu$m in length, long variants 6 $\mu$m in length \\
\textit{Coccidioides} \textit{immitis} and \textit{C. posadasii} & Ascomycota & spherules & spherical, 30–80 $\mu$m in diameter, containing 100–300 endospores \\
 & & endospores & spherical, 2–7 $\mu$m in diameter \\
 & & arthroconidia$^*$ & can be highly variable, including spherical, triangular, and barrel-shaped \\
 & & fungal ball$^*$ & spheroid mass of highly branched hyphae \\
\textit{Cryptococcus} \textit{neoformans}/\textit{gattii} complex & Basidiomycota & regular yeasts & spherical, 4–7 $\mu$m in diameter \\
 & & capsule enlargement & cells with large polysaccharide capsules \\
 & & titan cells & greater than 15 $\mu$m in diameter, thickened cell walls, larger vacuoles \\
 & & micro cells & smaller than 1 $\mu$m in diameter \\
 & & irregular cells$^*$ & elongated ellipsoids or tapered and egg-shaped \\
 & & pseudohyphae$^*$ & elongated ellipsoids, multicellular \\
\hline
\end{tabular}
\end{table}

*Less well-characterised morphological forms with currently unknown implications for virulence.

Abbreviations: GUT, gastrointestinal-induced transition.

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found *Malassezia* DNA in various terrestrial and marine habitats, suggesting some species may inhabit a wider ecological niche than was previously assumed [17–19]. Under certain
conditions that induce fungal overgrowth or through host immunocompromisation, commensal Malassezia species can become opportunistic pathogens, and cause a variety of dermatological conditions. In host tissues, Malassezia can reversibly switch between yeasts of around 8 μm in diameter and hyphae or pseudohyphae of 10–25 μm in length (Table 1; Fig 1B), resulting in a characteristic “spaghetti and meatballs” appearance in scrapings from infected skin or lesions [20]. The yeast form of Malassezia has many known virulence attributes, including the ability to either up-regulate or suppress the immune response [21]. Hyphal forms are difficult to produce in vivo and are therefore less well understood, but it is thought that they can penetrate keratinised skin cells, gaining access to deeper nutrient-rich layers where they can revert to the yeast form and proliferate to replace cells being shed at the epidermal surface [15]. Beyond these commonly known forms, considerable other variations in the morphology of Malassezia have been observed, including ovoid and cylindrical cells and hyphae of varying size [22] (Table 1). To date, these morphologies have received little attention, and they may play distinct and potentially important roles in infection.

Spherule formation and hyphal polymorphism in Coccidioides immitis and C. posadasii

C. immitis and C. posadasii are soil-dwelling pathogenic fungi that cause pulmonary infection via airborne arthroconidia, which can develop into life-threatening disseminated disease in at-risk individuals [23]. Although originally thought to be an accidental pathogen and opportunist, recent studies indicate that the infection of small animals such as bats and armadillos may form part of the life cycle of Coccidioides and that it has evolved specific adaptations for host interaction [24]. Central to its pathogenicity during human infection is the production of spherules and endospores. Spherules are formed from a gradual enlargement and transformation of inhaled arthroconidia into a structure that is typically 30–80 μm in diameter and contains around 100–300 endospores [25] (Table 1; Fig 1C). Upon rupture of the spherule, the endospores are released into host tissues, where each can produce hyphal growth or develop into a new spherule, repeating the growth cycle [26]. In addition to temperature and CO₂ levels, there is evidence that the transition to spherules can be stimulated by contact with neutrophils, and reversion of arthroconidia to hyphal forms has occasionally been observed in infected lung cavities with no neutrophils [23]. Other, less well-characterised morphotypes have also been observed clinically, but their role in infection is not well understood (Table 1) [27, 28].

Variation in capsule and cell size in Cryptococcus

Cryptococcus neoformans and members of the C. gattii species complex are encapsulated yeasts that cause severe respiratory and cerebral disease, primarily amongst immunocompromised individuals [29]. Although the pathogenic Cryptococcus species are considered to be environmental fungi, adaptations for survival during interactions with environmental predators such as amoebae, insects, and other short-lived organisms are thought to explain their broad host range and possession of various pathogenic traits [30]. Cryptococcus cells are typically spherical and 4–7 μm in diameter, but during human infection, the appearance of forms of varying size is common (Table 1; Fig 1D). The polysaccharide capsule possessed by Cryptococcus cells is highly dynamic and undergoes substantial enlargement during human infection [31]. Cells with enlarged capsules have been shown to be more resistant to oxidative stress, antimicrobial peptides, and phagocytosis and are generally associated with more severe pathology [32]. Both C. neoformans and members of the C. gattii complex can produce highly enlarged “titan” cells, which are greater than 15 μm in diameter and have been seen to reach up to 100 μm. Titan
cells have unique characteristics, including thickened cell walls, dense capsules, large vacuoles, and polyploidy [33]. These traits appear to contribute to survival in the host, with their capsular properties increasing resistance to oxidative, nitrosative, and other stresses and their massive size preventing phagocytosis and elimination by macrophages [34]. When replicating, titan cells produce regular-sized progeny; hence, they are always part of a heterogeneous mixture of cell types [35].

At the other end of the spectrum, C. neoformans can produce “micro” cells, a subpopulation of cells that are smaller than 1 μm in diameter [36]. Whilst much less research has been done on micro cells, they are a cell type that has been seen during human infection, and it is thought that they may cross biological barriers more readily because of their small size, aiding dissemination of the pathogen to other body sites [37, 38]. Micro cells are seen in different C. neoformans varieties and genotypes but have not been observed in species of the C. gattii complex [39]. Recent studies have further identified cells with unusual, irregular morphologies in some Cryptococcus strains. These can be tapered and egg-shaped or elongated and of a more pseudo-hyphal form. Their presence in clinical populations has been associated with increased antifungal tolerance but decreased virulence, which suggests they may promote persistence in the host [38, 39]. Like titan cells, micro and irregular cell types always appear as part of a mix of different cell types.

Conclusions

Diverse fungal morphologies are increasingly being recognised as key traits associated with virulence, aiding pathogens in various ways, including adhesion to physiological barriers, dissemination through the body, and manipulation of host immune responses [40]. Whilst some morphological forms have well-characterised implications for virulence and pathogenesis, there is still much to learn about the capacity of fungi for morphogenesis during disease progression. This knowledge may ultimately help inform disease diagnosis and prognosis, with implications for treatment strategies.

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