Yeast, Filaments and Biofilms in Pathogenesis of Candida albicans

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Introduction

The yeast *Candida albicans* is an opportunistic human pathogen, affecting immunocompromised people, mostly in hospital environments. People at risk include those suffering from HIV, cancer and intensive care unit patients, for example, those undergoing major surgery and organ transplants. *Candida* infections are the fourth most common cause of hospital-acquired infections in the United States, and the mortality is huge, around 40%.

Essential for pathogenesis of *C. albicans* is its ability to change cellular morphology and grow as either ovoid, yeast form cells or as elongated filaments (**Fig. 1**, reviewed in reference 1). Filaments can be either pseudohyphae or true hyphae, differing in morphology and several aspects of the cell cycle (1).

The current view is that it is the ability to switch between the two forms that underpins the success of *C. albicans* as a pathogen. Both filaments and yeast cells are observed in infected tissues (2) and *C. albicans* mutants compromised in either yeast or filamentous form display reduced virulence in animals (3-6).

Simplistically, because of their morphology and polarised growth, filaments are thought to be important for tissue invasion, whereas yeast form cells are used for dissemination in the host. However, there is more to the morphological transitions than invasion and dissemination. Yeast and filaments differ in their cell surface properties and interact differently with host immunity (7, 8). Furthermore, the switch to filamentous morphology is co-regulated with expression of genes encoding virulence-promoting functions and those required for acquisition of essential nutrients (9, 10).

In addition to growth as yeast or filaments, *C. albicans* can also switch to a multicellular form, growing as biofilms. These multicellular communities of yeast and filamentous cells are surrounded by a coat of extracellular matrix (11, see **Fig. 1B**). Biofilms of *C. albicans* form on attachment to surfaces and are a huge issue in clinical settings. Biofilms readily form on implanted medical devices, such as catheters, pacemakers, heart valves and prosthetic devices, enabling the fungus access to the bloodstream for dissemination and life-threatening systemic infections. Moreover, biofilm-borne cells display high resistance to antifungal drugs – they can be up to 1,000-fold more resistant than planktonic cells (11). Currently, it is very hard to treat biofilm-associated infections.

Here we review genetic, molecular and virulence aspects of the morphogenetic switch between yeast and filamentous forms, and the development of drug-resistant biofilms of *C. albicans.*

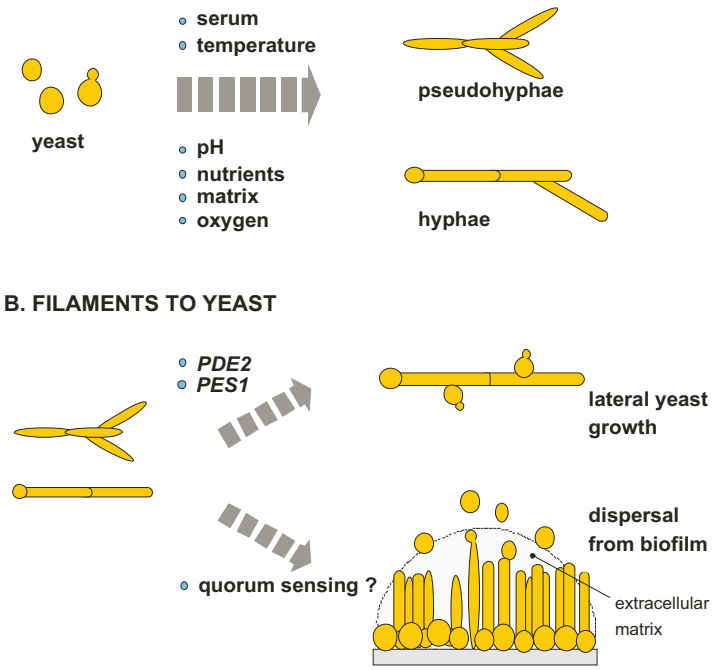

Fig. 1. Morphological transitions in *Candida albicans*.

A. The yeast-to-filaments morphogenetic switch is initiated by multiple signals, such as serum, temperature, pH and nutrients. Pseudohyphae and hyphae form, differing in morphology and the cell cycle. Pseudohyphae show constrictions between mother and daughter cells, whereas hyphae do not. Unlike in pseudohyphae, cell walls are parallel throughout the length of the hyphae.

B. Yeast cells form from filaments on sub-apical fragments, resulting in lateral yeast growth on filaments. The signals regulating this transition are not known and only two genes are known to be required, *PES1* and *PDE2*. The filament-to-yeast transition might also occur in biofilms, facilitating dispersal. Quorum sensing molecules, such as farnesol, are candidate regulators of this transition.

The Yeast-to-Hyphae Morphogenetic Switch and the Importance of Filaments in Virulence

In *C. albicans*, the transition from yeast to filaments is regulated by multiple signals, including the presence of serum, high temperature, starvation for nutrients, pH, embedded growth and lower oxygen levels (**Fig. 1A**).

Signal transduction cascades are activated by these signals, resulting in modulation of downstream transcription factors and changes in gene expression (1). The signal transduction pathways involved in filamentation include the cAMP-PKA pathway, the MAP kinase pathway, the pH-dependent Rim101 pathway and a matrix-sensing pathway (1). The effectors of these pathways differ. For example cAMP-PKA acts through the transcription factor Efg1, whereas the MAP kinase pathway regulates the activity of Cph1. Early work showing that the 'yeast-locked' double *cph1/efg1* deletion mutant is avirulent in mice laid the foundation for understanding the role of filaments in virulence of *C. albicans* (3).

The main argument in support of filamentous morphology being required for pathogenicity is that the inability to filament reduces the virulence of *C. albicans* in animal models. The closest relative of *C. albicans*, *Candida dubliniensis*, is less virulent, a property that is explained by a reduced ability to undergo filamentous differentiation (12). Interpreting experiments with mutants in transcription factors regulating morphogenetic programs (such as *efg1/cph1*) is not always direct because of the pleiotropic effects of the studied genes on gene expression. Two recent studies partially circumvented this problem by using tetracycline-regulated alleles of either Nrg1 (a repressor of filamentation) or Ume6 (an activator of filamentation) to enable switching between the two forms at different stages of infection. Yeast cells colonised tissues, but transition to filaments increased virulence and was required for killing of mice by *C. albicans* (4, 5).

Filaments of *C. albicans* are believed to have a number of roles in pathogenesis. Filaments are required for tissue damage and escape of the fungus from macrophages (3). Host immunity deals differently with yeast cells and with filaments, presumably due to different cell surface composition. For example, yeast form cells induce production of T-helper type 1 (Th1) cells, which have a protective role, whereas interaction of dendritic cells with *C. albicans* filaments results in differentiation of Th2 cells, which are associated with disease states (7).

The transition to filamentous growth is co-regulated with expression of genes promoting virulence, such as cell wall adhesins and hydrolytic enzymes (9). Another cellular pathway related to filamentous morphology is acquisition of the essential nutrient iron. The clue to this relationship comes from studies showing that Als3, a filament-induced cell surface adhesin, is the receptor for the iron source ferritin (10). Finally, the ability to undergo the yeast-to-hyphae morphogenetic switch is essential for development of mature biofilms, which are at the heart of fatal infections with *Candida* (11).

Yeast Morphology and Virulence of *C. albicans*

Mutants that are constitutively filamentous are less virulent *in vivo* (6), suggesting that yeast form cells play a role in pathogenesis. The compact morphology of yeast cells is more suitable than filaments for dissemination in systemic infections and from biofilms. Furthermore, the abovementioned studies with the tetracycline-regulated NRG1 allele (4) showed that when *C. albicans* is kept in yeast form, it can establish infection of organs, meaning that yeast cells are involved in invasion of organs early in the infection (contrary to the dogma that filaments are required for tissue invasion). Also, yeast cells provoke a different immunological response than hyphae; for example, they inhibit differentiation of monocytes into dendritic cells (13).

The yeast form is maintained partly through repression of the filamentous program by the transcriptional repressors Tup1, Nrg1 and Rfg1 (14). This repression is lifted upon induction of filamentation (14). What is much less clear is how filamentous cells switch back to yeast form, and what the relevance of this switch is during infections (**Fig. 1B**). Yeast form cells grow on the sub-apical fragments of filaments during standard filamentous growth, and this is called 'lateral yeast growth'. Two genes are known to be required for this transition: *PES1*, a homologue of the ribosome biogenesis factor Pescadillo (15), and *PDE2*, a cyclic nucleotide phosphodiesterase that affects cAMP signaling (16). Mutants in either *PES1* or *PDE2* are compromised for virulence in animal models, supporting the idea that the ability to switch from filaments to yeast is important for pathogenesis (15, 16).

Another situation that might require cells to switch from filamentous to yeast morphology is in biofilms (**Fig. 1B**). Mature biofilms consist of predominantly filamentous cells, and dispersal might be enhanced by a filament-to-yeast transition in the biofilm. Recent work showed that modulating the expression levels of the filament-specific transcription factor Ume6 drives a reversible transition from yeast to filaments in the absence of inducers (5), providing proof that a single molecular event can drive morphogenesis to filaments and back to yeast. This transition could be regulated by quorum-sensing molecules such as farnesol, which is produced in dense cultures and inhibits filament formation. Quorum-sensing molecules could act by modulating levels of key regulators of morphogenesis. In support of this model, farnesol causes increased expression levels of the Tup1 repressor of filamentation (17).

Biofilms of *C. albicans* – Yeast, Filaments and Drug Resistance

Biofilms of *C. albicans* are multicellular communites of yeast, pseudohyphae and hyphae, which form upon adherence to substrates (**Fig. 2**, reviewed in 11). Genetic analysis has shown that most mutants unable to form wildtype biofilms are affected in either cell wall structure (required for adherence to the substrate and to other cells) or the yeast-to-filament differentiation program (reviewed in 11).

Biofilm maturation also requires production of extracellular matrix, which consists of sugars, proteins, phosphorus, and other components. How extracellular matrix production is regulated is poorly understood. Recently, a gene affecting biofilm matrix production was found (18). It encodes the transcription factor Zap1, which regulates the expression of cell surface glucoamylases (presumably affecting shredding of glucans from the wall into the matrix) and alcohol dehydrogenases that might act by producing quorum-sensing alcohols. Finally, dispersal of cells from the biofilm is required for sustaining the infection. As mentioned above, quorum-sensing mechanisms and the filament-to-yeast transition might be involved in dispersal.

Biofilms are not just a mixture of yeast and filamentous cells. A biofilm is a distinct developmental state, which differs phenotypically from planktonic cells. For example, metabolism is different in biofilms, and specific gene expression programs are activated during biofilm formation. One of the biofilm-specific features that is of high concern in clinical settings is high resistance to antifungal drugs (**Fig. 2**).

Why are biofilms resistant to antifungal drugs? This question drives current research in our lab. Physical and physiological properties appear to contribute, such as upregulation of efflux pumps, formation of extracellular matrix, persister cells, upregulation of oxidative stress responses, changes in membrane sterol composition and increased cell density (**Fig. 2**, reviewed in 11). What triggers the development of these features and what the relationship is between the different resistance mechanisms is largely unknown. One metabolic feature that could be driving some of these changes is the limited availability of oxygen to cells in the biofilm (19). Limiting oxygen presumably results in lower mitochondrial function, and this could impact on ergosterol biosynthesis (because of requirements for heme) and regulation of oxidative stress responses. In the model yeast *Saccharomyces cerevisiae*, mitochondrial dysfunction leads to upregulation of multidrug resistance pumps (20), suggesting increased expression of efflux pumps in biofilms could be caused by changes in mitochondrial metabolism. An integrative understanding of biofilm drug resistance will be required for development of efficient treatments against life-threatening infections with *C. albicans*.

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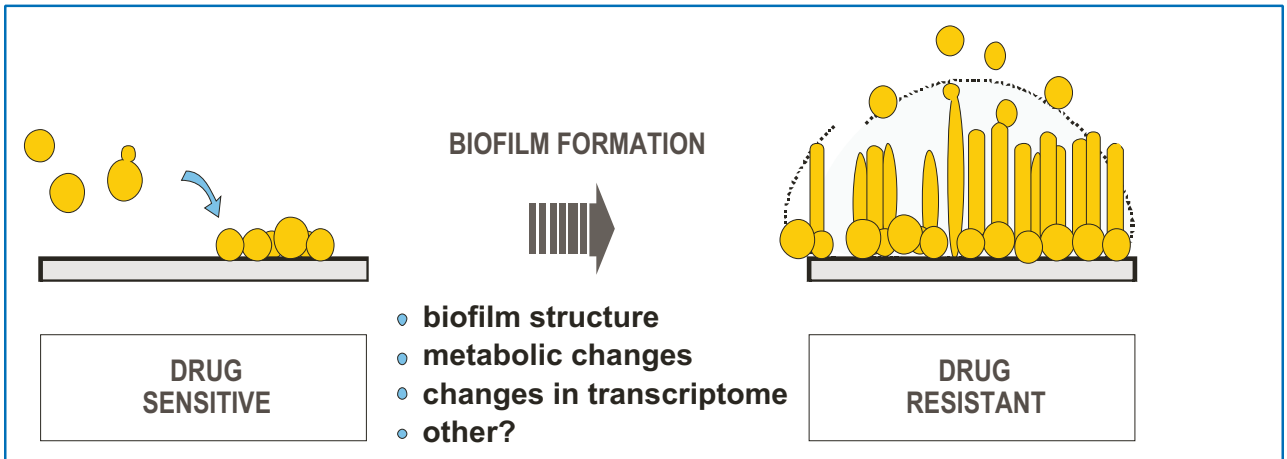

Fig. 2. Drug resistance of *Candida albicans* biofilms.
Biofilms form after adherence of *C. albicans* cells to a substrate. Maturation of biofilms is accompanied by development of drug resistance. What triggers drug resistance is not fully understood, but it is likely a result of physical properties of the biofilm, metabolic and gene expression changes. Upregulation of efflux pumps, appearance of persister cells (which are insensitive to drugs due to an unknown mechanism), extracellular matrix formation (components of which can bind antifungals), changes in membrane composition (the membrane being the target of antifungal treatments), as well as density of the cell population in a biofilm, all contribute to drug resistance.

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