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Yeast cell differentiation: Lessons from pathogenic and non-pathogenic yeasts

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ABSTRACT

Yeasts, historically considered to be single-cell organisms, are able to activate different differentiation processes. Individual yeast cells can change their life-styles by processes of phenotypic switching such as the switch from yeast-shaped cells to filamentous cells (pseudohyphae or true hyphae) and the transition among opaque, white and gray cell-types. Yeasts can also create organized multicellular structures such as colonies and biofilms, and the latter are often observed as contaminants on surfaces in industry and medical care and are formed during infections of the human body. Multicellular structures are formed mostly of stationary-phase or slow-growing cells that diversify into specific cell subpopulations that have unique metabolic properties and can fulfill specific tasks. In addition to the development of multiple protective mechanisms, processes of metabolic reprogramming that reflect a changed environment help differentiated individual cells and/or community cell constituents to survive harmful environmental attacks and/or to escape the host immune system. This review aims to provide an overview of differentiation processes so far identified in individual yeast cells as well as in multicellular communities of yeast pathogens of the *Candida* and *Cryptococcus* spp. and the *Candida albicans* close relative, *Saccharomyces cerevisiae*. Molecular mechanisms and extracellular signals potentially involved in differentiation processes are also briefly mentioned.

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1. Introduction

Cell differentiation and the formation of tissues composed of specialized cells that gain specific properties, fulfill specific tasks and mutually interact is a prerequisite for the formation of multicellular organisms, including humans. However, single cell organisms such as yeasts are also capable of differentiation and forming specialized cell-types that exist either as individuals or as constituents of organized multicellular populations. Cell differentiation to opposite mating types and switching from yeast form to filamentous form (hyphae or pseudohyphae) are examples of individual yeast cell differentiation. Both processes have been investigated using different yeast species, and they can contribute to the virulence and invasiveness of pathogenic yeast [1,2]. In addition, yeast multicellular structures, particularly biofilms and colonies, have been intensively studied and a number of different cell-types have been identified therein. Differentiated cell-types are usually specifically localized within the structure; they can perform specific tasks and can even mutually interact. Hence, yeast multicellular structures are considered primitive multicellular organisms composed of differentiated cells that are organized into primitive tissues [2–6]. The spatial positioning of cells within the structure allows the formation of gradients of metabolites, signaling molecules and waste products, which can all participate in cell diversification and specialization. Additional mechanisms exist that allow the cell-constituents to more efficiently adapt to changes in the environment and/or to be involved in structure protection against environmental attack. These mechanisms are particularly important in host infections, during which yeast cells must adapt to a particular host niche and resist the immune system and drugs during the therapy treatments. For all of these reasons, populations of yeast biofilms are usually more successful in host infections than planctonic yeasts [7]. Yeasts of different species such as *Candida* spp. and *Cryptococcus* spp. have been identified as important opportunistic pathogens in humans, particularly in immunocompromised patients. However, since the end of the last century, even *Saccharomyces cerevisiae* has begun to be considered as a possible opportunistic pathogen [8–10]. Clinical isolates of *S. cerevisiae* usually differ from the domesticated strains used in laboratories and from those used for centuries in baking, brewing, distilling and wine making. Clinical *S. cerevisiae* isolates are usually more resistant to copper [10] and to oxidative stress [11] with transcription factor Rds2p contributing to survival under oxidative stress conditions [12]. Another factor that could be associated with the clinical appearance of *S. cerevisiae* is its relatively high resistance to azoles; which can open up new ecological niches to *S. cerevisiae* in an azole-treated host [9]. In addition, various processes potentially involved in pathogenicity are paralleled in *S. cerevisiae* and its close relative *Candida albicans*. For this reason, identification of molecular mechanisms involved in these processes using even non pathogenic *S. cerevisiae* strains can help to identify similar mechanisms in *C. albicans* or other pathogenic yeast. In this review, we therefore summarize current knowledge of differentiation processes identified in *Candida* spp., *Cryptococcus neoformans* and *S. cerevisiae*.

2. Architecture and cell differentiation within yeast multicellular structures

When growing on solid, semisolid or even liquid surfaces, yeasts of different species form structures such as colonies, biofilms, flors, mats, flocs, fingers and stalks. Analyses of the internal architecture and cell differentiation have led to the identification of several prominent features that characterize different types of colonies and biofilms. These features indicate that different developmental programs exist, leading to the formation of structures with considerably different architecture, which may reflect a particular yeast life-style under certain conditions.

2.1. Structured biofilm colonies

Candida and *Cryptococcus* spp. often form remarkably structured colonies (Fig. 1A) that are composed of cell-shapes that range from oval cells to filamentous cell-shapes, including pseudohyphae and true unconstricted hyphae. Different methods of scanning electron microscopy (SEM) showed that cell-types differing in morphology are localized in different colony areas [13–15]. *S. cerevisiae* strains from natural environments also create structured biofilm colonies (Fig. 2) [16,17] that morphologically resemble those formed by *Candida* or *Cryptococcus* species. Specialized cell subpopulations are formed within these *S. cerevisiae* biofilm colonies, which provide colonies with multiple protective mechanisms against environmental attacks, including drug treatment [18]. Biofilm colonies are composed of two major parts: the aerial part, which is composed of oval cells that are assembled around the cavity that is free of the cells, and the subsurface “roots” that are formed by pseudohyphae invading the agar [18]. Stationary-phase cells that are more resistant to the environment are formed early during colony development in surface layers of the aerial region, whereas cells inside the colony and the tips of the roots are dividing, even in older colonies. Cell layers with the active multidrug resistance (MDR) transporters Pdr5p and Snq2p are localized over the whole colony, providing additional protection to cells on the colony surface and the tips of the roots. Cells inside the colony are embedded within an abundant extracellular matrix (ECM) that blocks the penetration of different chemical species. The structured colony architecture is strengthened by extracellular fibers that connect the cells; the formation of these fibers is dependent on Flo11p adhesin [18]. Similar fibers have been found among *Candida albicans* cells [19] (Fig. 1A). A filamentous network of extracellular polysaccharides forms large capsule of *Cryptococcus neoformans* cells [20,21].

2.2. Yeast biofilms

Similar to yeast colonies, yeast biofilms that are grown on different supports are also composed of different cell layers [5,22–24]. Biofilm development usually begins with cell adhesion to a solid surface; the adhered cells then divide and form basal polylayers, from which pseudohyphae/hyphae are grown. Hence, the mature biofilm of *C. albicans* is often composed of two main components: the basal layers of yeast-shaped cells that anchor the biofilm to the support and an upper layer that is formed predominantly by vertically oriented hyphae that deposit an ECM in this area of the biofilm (Fig. 3A) [5,25,26]. In addition to MDR pumps, which contribute to the drug resistance of biofilms (usually at early stages of their formation), later changes in sterol composition can play an important role in biofilm resistance to some antifungals, such as amphotericin B and the azoles [7]. ECM, which is supposed to be able to sequester drugs [27,28], and other mechanisms, such as the activation of stress-response pathways that enable cells to cope with the diverse stresses [29] can also be involved in *C. albicans* biofilm resistance. The formation of *C. neoformans* biofilm also comprises phases of surface attachment, microcolony formation, ECM production and biofilm maturation. However, encapsulated yeast-shaped cells predominantly form the biofilm. Antibodies specific to polysaccharides of the capsule block the adhesion of *C. neoformans* cells to the surface and biofilm formation [30].

In some *Candida* biofilms, another cell-type called persisters was found as a small cell-fraction (~0.01–1%) that is highly tolerant to drugs. These persisters are cells that are completely invulnerable to amphotericin B and chlorhexidine and can repopulate the biofilm when majority of cells is eliminated by drugs [31].