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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 *C. albicans* close relative, *Saccharomyces cerevisiae*. Molecular mechanisms and extracellular signals potentially involved in differentiation processes are also briefly mentioned.

Keywords: pathogenic yeasts; biofilms and colonies; cell differentiation; phenotypic switching; *Candida*, *Cryptococcus* and *Saccharomyces* spp.

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 *C. 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 (Figure 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 (Figure 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 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] (Figure 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 (Figure 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]. Proteomic profiling showed that metabolic activities (such as glycolysis, tricarboxylic acid cycle and protein synthesis) are lowered in the persisters but that proteins that are involved in virulence and the stress response are upregulated in these cells [32]. As shown recently, antifungal-tolerant persisters are produced mainly during the surface adhesion phase of biofilm formation, and this adhesion is necessary for the emergence and maintenance of persister cells [33].

2.3. Other multicellular structures of yeasts

In addition to colonies and biofilms, yeasts can form more obscure multicellular structures. Structures of approximately 3-mm-long fingers are formed by *C. albicans* on low-density agar under conditions that can be found in the gastrointestinal tract, i.e., 20% CO_2 and 37°C (Figure 1B) [34]. According to the model scheme based on SEM and light microscopy, the finger is composed mainly of unbudded compacted yeast cells forming a bulb that is partially embedded inside the agar. From the bulb, there is likely an outgrowth of single core non-branched hypha, from which yeast-shaped cells are derived, and, together with hypha, become components of the finger structure [34]. The finger formation is regulated by the Ras1p-cAMP-Tec1p pathway that is involved also in the regulation of hyphae formation, stimulation of white to opaque switching and a/α biofilm formation (3.1.). Because the interface between the finger and the bulb is mechanically fragile, the authors suggested that the finger might function as a dispersal mechanism in host niches with high levels of CO_2. The fingers partially resemble stalks that are formed by *S. cerevisiae*, *C. albicans* and *Schizosaccharomyces pombe* (Figure 1C) [35]. *S. cerevisiae* stalks are 5-30 mm long and are formed from cells that fall into small cavities within the agar under conditions where the majority of plated cells are killed by UV irradiation. The stalks are composed of a central core containing yeast-shaped cells and asci with spores and are surrounded by a shell structure composed of vacuolated cells with thick cell walls together with dying cells [36]. A detailed comparison of stalks and fingers showed that they are distinct structures [34].

3. Cell differentiation during phenotypic switching

Phenotypic switching between two or more different cell-forms in response to different environmental signals is a typical feature of yeasts. Distinct switching cell-forms can differ in their metabolism, virulence, environmental resistance and other features, including the morphological characteristics of their populations. Specific cell-forms can be found either as individuals (for example, spread in the host organism) or as specifically localized constituents of multicellular populations.

The most studied example of phenotypic switching is dimorphic transition from yeast-form to filamentous cell forms that are often capable of invading and penetrating solid substrates such as tissues. Dimorphic transition is common both to yeast of *Candida* sp. and to wild *S. cerevisiae* strains. Hyphal forms play important roles during *C. albicans* infections; hyphae can invade epithelial and endothelial cells, cause damage to tissues by releasing hydrolytic enzymes and escape from macrophages when formed from engulfed yeast cells [37]. Current knowledge of the widely studied molecular mechanisms and regulations involved in pseudohyphal/hyphal growth in different yeast species has been summarized in recent reviews [29, 37, 38], therefore, we focus mostly on those examples when dimorphic switch contributes to the development of yeast multicellular populations.

3.1. Opaque-white switching of C. albicans and biofilm formation

The widely studied white-opaque switching system of *C. albicans* (Figure 3B) includes two major cell-forms: white cells that are round and form white colonies and opaque cells that are bean-shaped, larger than white cells and form large flat gray colonies. White and opaque cells differ in their metabolism and virulence. Opaque cells express genes that are related to oxidative metabolism including fatty acid oxidation [39], which may correlate with the fact that opaque cells better colonize the skin [40]. White cells, presumably with fermentative metabolism, are more virulent in the blood stream and cause systemic infections [41].

White-opaque switching is tightly linked to the mating state of cells. White cells that are heterozygous in their MTL locus (a/α) are usually not able to switch to an opaque state because the gene encoding the Wor1p transcription factor that is important for the switch is repressed by the a/α repressor. Thus, usually only those white cells that are homozygous at the MTL locus (a/a, α/α, a, α) can switch to opaque homozygotes that are able to mate and subsequently form white heterozygotes. In contrast to opaque homozygotes, white homozygotes do not release pheromones or form shmoo in response to pheromone and are not able to mate [42, 43]. However, white cells still produce pheromone receptors [44]. The white-to-opaque switch is regulated on different levels, including transcriptional and post-transcriptional regulation and chromatin remodeling, and affected by environmental factors such as temperature, oxygen level and CO_2 level [45].

Two major types of biofilms, the formation of which is associated with white/opaque *C. albicans* cells, have been described. The conventional biofilms are formed mostly by *MTL*a/α heterozygous white cells. The structure of these biofilms is composed of a basal layer of round cells and a thick layer of hyphae/pseudohyphae embedded in ECM [26] (see 2.2.). These biofilms are usually impermeable to different molecules, impenetrable by leukocytes and resistant to drugs. The more recently discovered sexual biofilms are composed predominantly of homozygous (a/a or α/α) white cells together with a minority of opaque cells. The architecture of sexual biofilms is similar to conventional biofilms, but sexual biofilms are more permeable to different compounds, can be penetrated by leukocytes and are susceptible to drugs [46]. Six major transcription regulators (Efg1p, Tec1p, Bcr1p, Brg1p, Rob1p and Ndt80p) potentially regulate the formation of conventional biofilms, causing changes in the expression of approximately 15% of genes [47]. Four of these transcription

factors (Tec1p, Bcr1p, Brg1p, and Rob1p) are also involved in the formation of sexual biofilms [48]. In addition, Cph1p (ortholog of *S. cerevisiae* Ste12p) is a key regulator of sexual biofilms. Upstream signaling includes the Ras-cAMP pathway in conventional biofilms, which activates Efg1p and the MAPK pathway in sexual biofilms that activate Cph1p [48-50] or Tec1p [51] transcription regulators.

Pheromone communication between white and opaque cells seems to be important for opaque cell mating and for the formation of sexual biofilms [44]. Pheromones that are produced by homozygous opaque cells (minor population) stimulate the adhesiveness of white cells, which contributes to the formation of the biofilm structure. The biofilm itself then provides an environment that allows for a more efficient formation of pheromone gradients between opaque cells; these cells consequently form oriented mating tubes and mate more efficiently than they do when present in the cell monolayer [44]. These findings reveal the complexity of the relationship between opaque and white cells that co-exist within the biofilm, which allows the use of different strategies for mating and thus sexual reproduction, which has adaptive benefits over asexual reproduction [50].

Two other described phenotypic states of *C. albicans* cells, i.e., gray and gut cells, differ from opaque and white cells in both cell and colonial morphology (Figure 3B). Gray cells that have been identified in some clinical isolates are metabolically different from both white and opaque cells, produce more extracellular proteins, including Sap proteinases, and are the most successful in cutaneous infections. The switching among white, opaque and gray cells is regulated by Wor1p and Efg1p regulators [52]. Gut cells overexpress the *WOR1* gene and are highly competitive in colonizing the gastrointestinal tract [53]. Thus, different cell-types can be formed during phenotypic switching of *C. albicans*, which are affected by different environments and acquire properties that allow the yeast to occupy and propagate in various niches.

3.2. Phenotypic switching of C. neoformans

In the natural environment, *C. neoformans* exists mainly in the yeast-shaped form but undergoes either mating with cells of the opposite mating type or unisexual diploidization to produce filamentous forms and generate infectious basidiospores [54]. Interestingly, in host tissues, yeast forms of *C. neoformans* predominate over pseudohyphae/hyphae, which are the forms that seem to be more important for cryptococcal fitness in the environment. *C. neoformans* possess a polysaccharide capsule that contributes significantly to the virulence and release of carbohydrates in the host and can cause deleterious changes to the immune system [55]. The capsule size changes according to conditions: it is relatively small in cells grown under laboratory conditions but is highly enlarged during pulmonary infection [56]. Different types of yeast-shaped forms have been isolated from different tissues of an infected organism. Titan/giant cells with a 900-fold enlarged body have been identified in the lungs [57]. Titan cells possess a compact capsule that is formed by highly cross-linked polysaccharides, a 2- to 3-μm-thick cell wall, and multiple intracellular vesicles of unknown function. These cells contain ~16 times more DNA because of continuing replication without cell division, accumulate melanin pigment in the cell wall, are highly resistant to y-irradiation, organic solvents and oxidative stress and do not undergo phagocytosis [21]. Titan cell formation is stimulated by the presence of cells with an opposite mating type and is dependent on the MAPK pathway [57]; the formation is also dependent on cAMP and independent of Ras1p [21].

Interestingly, increased proportion of titan cells opposes high inflammation and virulence [57]. Hence, in contrast to the enlargement of cells via an increased volume of the capsule, which contributes to *C. neoformans* virulence, the formation of highly resistant but avirulent titan cells seem to be more related to the survival strategy of *C. neoformans* within the host.

Fries et al [13] described the phenotypic switching of hypovirulent *C. neoformans* cells (SM) that form smooth colonies into two more virulent cell-types that form structured colonies (Figure 1A). One type of structured colonies was composed of pseudohyphae (PH cells, the more virulent form), the other one of round cells with an enlarged capsule (WR cells, the most virulent form). The amount and composition of the capsule, particularly the structure of glucuronoxylomannan, differed among the three cell-types. The structured colony architecture was caused by a different organization of either pseudohyphae (PH colonies) or yeast-shaped cells embedded in a high amount of extracellular polysaccharides (WR colonies) compared with the SM cells of smooth colonies. The structured architecture of *C. neoformans* colonies thus can be formed independently of the presence of filamentous cell-forms, similarly to the formation of *S. cerevisiae* biofilm colonies. The typical architecture of biofilm colonies (2.1.) can be formed exclusively by yeast-shaped cells that are differently organized in different colony areas, including in roots that are composed of cell-chains invading the agar [58, 59].

3.3. Domestication of wild S. cerevisiae strains

S. cerevisiae wild strains are able to perform phenotypic switching leading to changes in cell physiology and formation of colonies of distinct morphotypes. For example, the process of domestication, during which wild *S. cerevisiae* strains forming biofilm colonies reprogram their metabolism and begin to form smooth colonies that are similar in morphology, differentiation and other properties to colonies of laboratory strains, i.e., the strains that have existed for a long time under plentiful conditions [16]. Domestication and the switch back to a feral phenotype are accompanied by global expression reprogramming, thus leading to switch off/on features that are typical of biofilm colonies, including the production of ECM and Flo11p [58-60].

4. Extracellular signals involved in yeast cell differentiation

Different regulatory proteins and signaling pathways participate in different types of phenotypic switching and/or formation of multicellular structures of different yeast species (some examples are mentioned above). Nevertheless, most of these regulators/pathways have pleiotropic functions and participate in multiple cellular processes. The identification of upstream signals that specify the function of a particular regulator/pathway under distinct environmental conditions is therefore crucial. Examples of extracellular signals that are involved in phenotypic switching and/or the development of multicellular populations are shown below.

4.1. Cell wall adhesins and ECM

The presence of cell wall adhesins and ECM composed of carbohydrates and extracellular proteins is one of the typical characteristics of biofilms and biofilm colonies formed by different species. ECM composition

differs among species and strains and is affected by the yeast life-style as demonstrated by differences in ECM composition between planktonic and biofilm *C. albicans* cells [61, 62]. Hundreds of different proteins, α-1,2-branched α-1,6-mannans associated with β-1,6-glucans, glycerolipids and DNA of random non-coding sequences are components of the *C. albicans* ECM [63]. In mammals, ECM proteins participate in cell-cell communication and cell differentiation [64]. The signaling function(s) of ECM proteins in yeast are still rather fragmentary. Recently identified Znf2p transcription factor-controlled Cfl1p adhesin (a constituent of the cell wall as well as secreted protein; [65]) of *C. neoformans* contributes to the regulation of the yeast-to-pseudohyphae cell differentiation and the formation of aerial hyphae and biofilm colonies [66]. The production of Cfl1p, a fraction of which is cleaved and released from cells, is facilitated by autoinduction either inter- or intracolonially in a paracrine manner [65]. Similarly, mucin Msb2p, which is released from the cell wall to ECM in *S. cerevisiae*, contributes to the activation of the MAPK pathway and is involved in filamentous growth in *S. cerevisiae* [67] and in *C. albicans* [68]. *S. cerevisiae* mucin-like protein Flo11p/Muc1p can also be released from the cell wall to the ECM and affects the adherence properties and filamentous growth of cells [69]. Flo11p is also essential for the formation of biofilm colonies [58, 60, 70].

4.2. Ammonia/um signal in cell differentiation

Differences in the level of nitrogen source, particularly of ammonia/ammonium, are often considered a factor that regulates phenotypic switching, pseudohyphal/hyphal growth and colony development. First, yeast of different genera including *C. neoformans* and *S. cerevisiae*, can begin to form filamentous cells under conditions of low ammonium, which is dependent on the presence of ammonium permeases; the formation of filamentous cells often results in the formation of structured colonies [71, 72]. Second, during the early stages of biofilm colony formation, wild *S. cerevisiae* strains form pseudohyphae that grow in the direction of a source of volatile ammonia that is produced by neighboring colony (Figure 4A) [60]. The application of volatile methylammonia evokes the same cellular response, which indicates that a change in pH can be involved in this type of dimorphic switch [60].

Third, colonies of different species, including *Candida* sp., *Cryptoccocus* sp. and *S. cerevisiae*, pass through the phases of acidification and alkalization of the medium [73, 74]. Alkalization is accompanied by production of volatile ammonia that functions as a signal that is involved in colony development, metabolic reprogramming and cell differentiation [4, 74-76]. The transition of *C. mogii* colonies from an acidic to alkali phase is accompanied by expressive changes in colony and cell morphology, during which pseudohyphae that form relatively smooth acidic-phase colonies decompose to oval cells that reorganize to wrinkled structured colonies that produce high levels of ammonia (Figure 4C) [73]. Ammonia signaling in *S. cerevisiae* smooth colonies leads to prominent cell differentiation into two distinct cell-types localized to upper (U cells) and lower (L cells) colony areas (Figure 4B), which gain specialized properties and functions [75, 77]. U cells are vital, metabolically active and stress-resistant cells that produce three Ato proteins, putative ammonium exporters [76], which play important roles in ammonia production, whereas stressed L cells activate mechanisms that are

involved in the release of nutrients to feed long-lived U cells [75, 78]. Production of Ato proteins is controlled by mitochondrial retrograde signaling [79].

Fourth, ammonia directly contributes to the pathogenesis of *C. albicans*. *C. albicans* cells release ammonia and thus alter the pH of the environment and induce a cell switch to the hyphal form (Figure 4D) [80]. Thus, ammonia that is released by phagocytosed *C. albicans* cells could cause the pH neutralization of the macrophage phagosome, and the neutral pH induces a yeast-to-hyphae switch of phagocytized cells and consequent hyphae escape. The expression of *C. albicans ATO*-gene-homologues is induced by phagocytosis. Proteins of Ato family identified originally in *S. cerevisiae* colonies [76], thus seem to be key mediators of metabolic changes that enable *C. albicans* cells to overcome the macrophage innate immunity barrier. Another alkali signal of unspecified origin that is sensed through the Rim101 pathway has been implied to play a regulatory role in areas of *S. cerevisiae* colonies in which sporulation takes place [81].

4.3. CO_2 in yeast cell differentiation

Changes in the level of CO_2 in the atmosphere can influence the formation and architecture of yeast colonies (2.3.). Carbonic anhydrase is the key enzyme that significantly increases the spontaneous rate of hydration of CO_2 to HCO_3^-, which is the substrate for fundamental carboxylation reactions in yeast cellular metabolism. *C. albicans* and *S. cerevisiae* (as well as other fungi) require carbonic anhydrase when growing at atmospheric CO_2 concentrations. Two subpopulations that differentially produce carbonic anhydrase are formed in glucose-grown colonies under atmospheric conditions: the upper cell layers produce high amounts of carbonic anhydrase and cells in the lower colony areas do not produce this enzyme [82].These findings indicate a CO_2 flux and related metabolic adaptation inside the colonies.

5. Conclusions and perspectives

The examples described above indicate that yeasts can differentiate under changing environmental conditions to multiple cell forms that differ in their physiology, metabolism and regulation. These forms are then better adapted to particular conditions for survival, stress defense and reproduction. Observed changes between particular cell-types are often reflected in a different virulence and in the ability to occupy, infect or persist in different niches, including different tissues of the mammalian body. Differences that are observed among different species, which occasionally show that a process that is important for infectivity in one organism could be less important or even unimportant in another organism, imply the existence of different strategies used by different yeasts.

Yeast infections are often associated with the colonization of cell surfaces in the host and the formation of highly resistant biofilms, followed by the release and dispersal of cells from the biofilm and the occupation of new niches. The infections associated with the presence of a biofilm often lead to higher host mortality, as compared to infections caused by planktonic yeast [25]. The identification of mechanisms that are involved in the development of yeast multicellular structures is therefore crucial for designing anti-fungal strategies in the future. Further progress in understanding the differentiation of colonies, biofilms and other yeast populations

formed within a mammalian organism will require the development of new methods of in situ cell-analysis within the structure and/or after the separation of specific subpopulations. Although eminent progress has been made in such technologies over the last several years, particularly in the field of fluorescence and confocal microscopy of native colonies and biofilms [5, 18], the more efficient utilization of OMICS techniques, for example, could be important.

In summary, significant progress in studies of yeast cell differentiation has been made. Different types of colonies of different species, particularly the structured biofilm colonies that exhibit many features similar to those of biofilms, have been established as an excellent model system for the investigation of yeast differentiation and formation of different cell-types. Regardless the progress in knowledge of yeast cell differentiation, information on the properties, functions and particularly on molecular regulations involved in formation of specific yeast cell subpopulations are still rather fragmentary. Many questions in this field thus remain for further investigation.

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Figure legends

Figure 1: Morphology and ultrastructure of yeast multicellular structures. A. The morphology and cell organization of colonies formed by *C. neoformans*; fibers on the *C. neoformans* cell surface and fibers connecting *C. albicans* cells. B. *C. albicans* fingers, the pathway regulating finger formation and the scheme of finger architecture. C. *C. albicans* stalks and the architecture of *S. cerevisiae* stalks. Republished with permission of ASM, Elsevier and Oxford Univ. Press from [13, 19, 20] (A); and ASM from [34-36] (B, C); permission conveyed through Copyright Clearance Center.

Figure 1

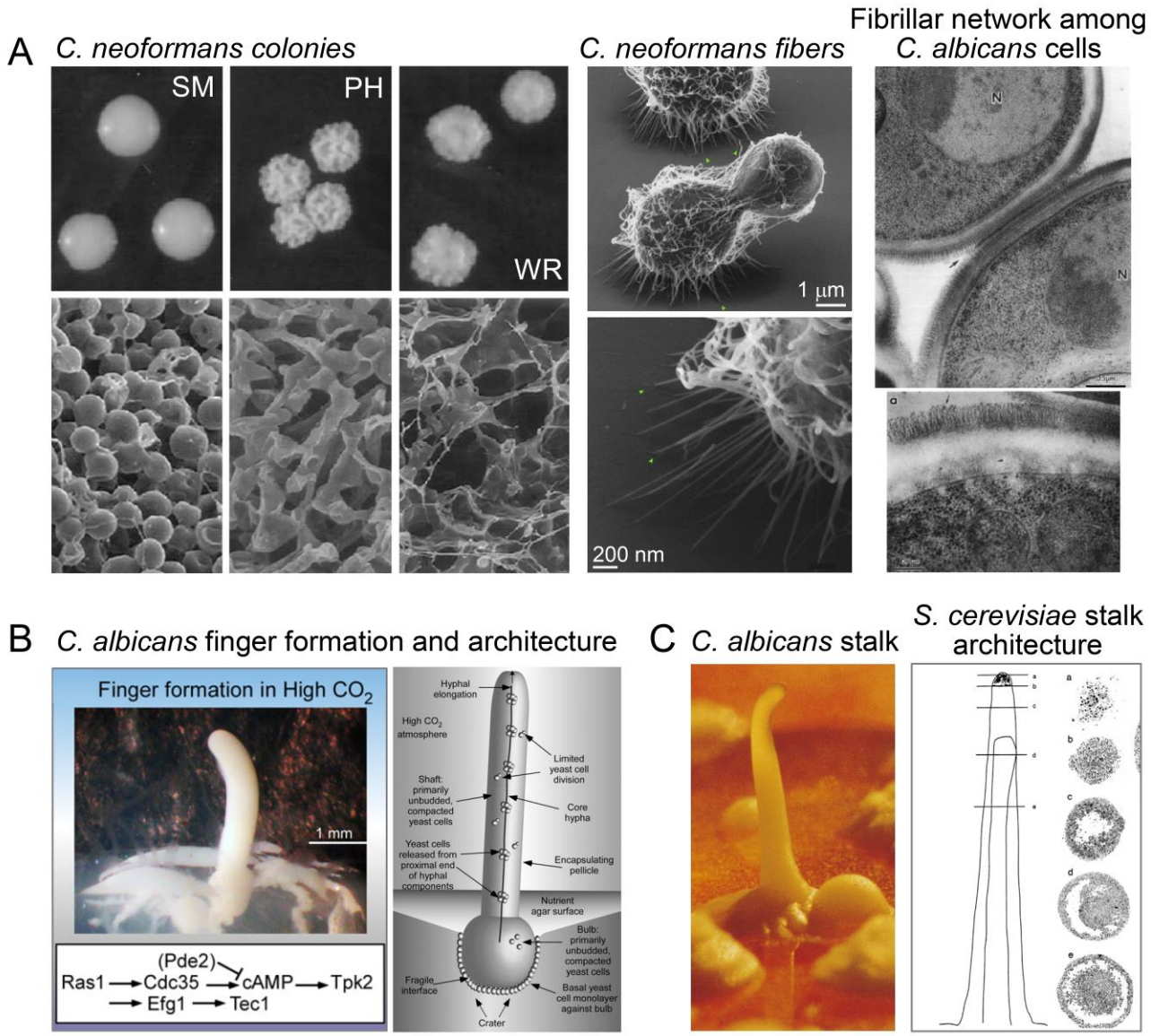

Figure 2: Characteristic features of *S. cerevisiae* biofilm colonies. A. Colony morphology and cell organization in smooth colonies of a domesticated strain and biofilm colonies of a wild strain containing ECM (indicated by arrows) [16]. B. Fibers connecting cells of biofilm colonies. C. MDR pumps are active in green cells and inactive in green/red cells of biofilm colonies. D. The localization of stationary-phase cells in the upper colonial layers (red arrows); dividing cells are in the internal areas (white arrows). E. Morphology of cells within biofilm colonies; yeast-shaped cells are in the aerial area (inset e1) and pseudohyphae are in the subsurface area (inset e2). F. Localization of semipermeable ECM (red area); in green, cells without ECM. Parts B-F are modified from [18].

Figure 2

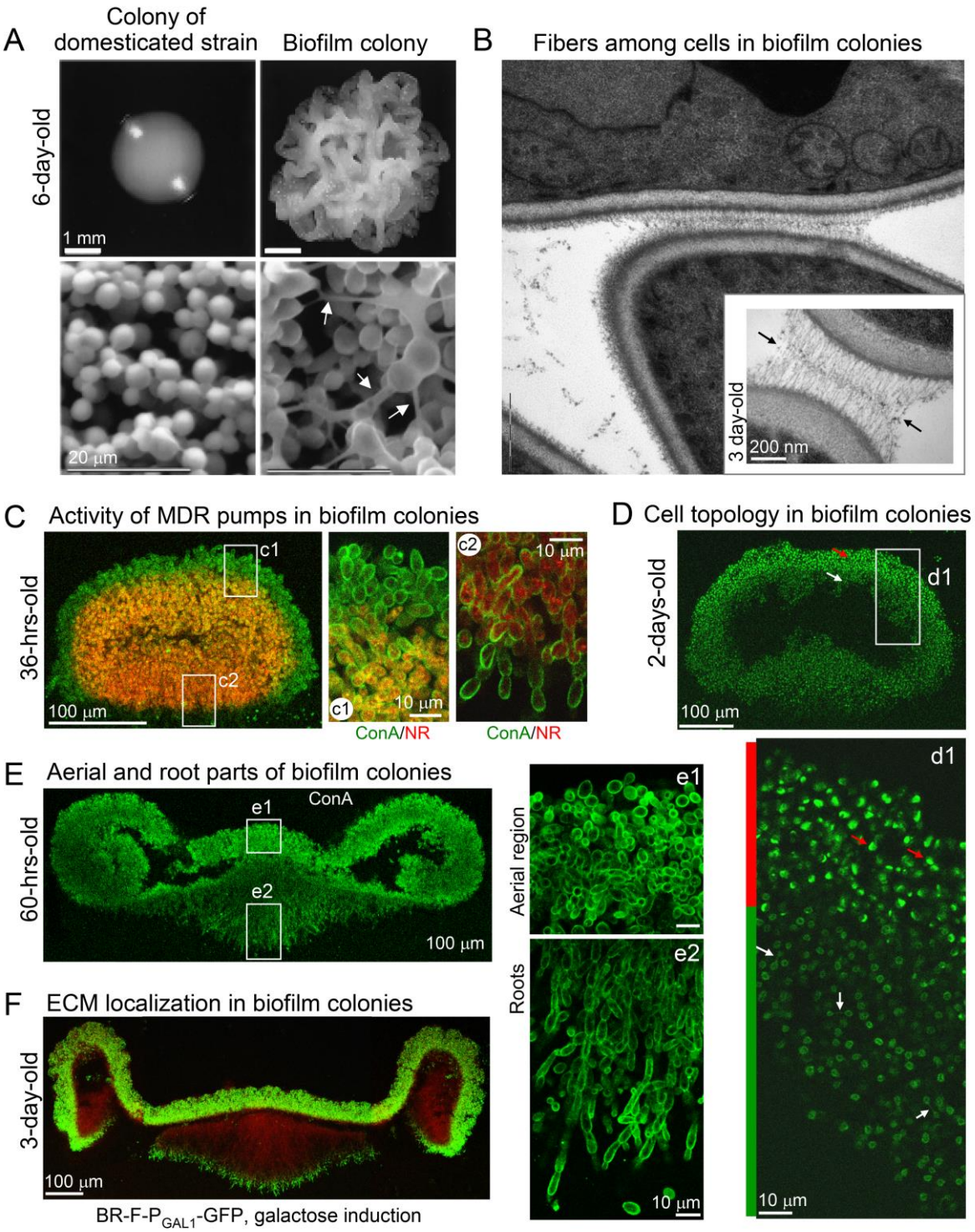

Figure 3: Biofilm architecture and regulations of phenotypic switching. A. Architecture of conventional biofilms using confocal (left) and scanning electron (center) microscopy, and a biofilm model-scheme (right); from [5] (with permission of ASM). B. Regulation of white-opaque-gray-gut cell switching and biofilm formation. Images of colonies (stained by phloxine B) and cells of a particular cell-type are from [52], SEM images of opaque and gut cells are from [53] (with permission of Nature Publishing Group) and of white cells from [2]. Permission conveyed through Copyright Clearance Center.

Figure 3

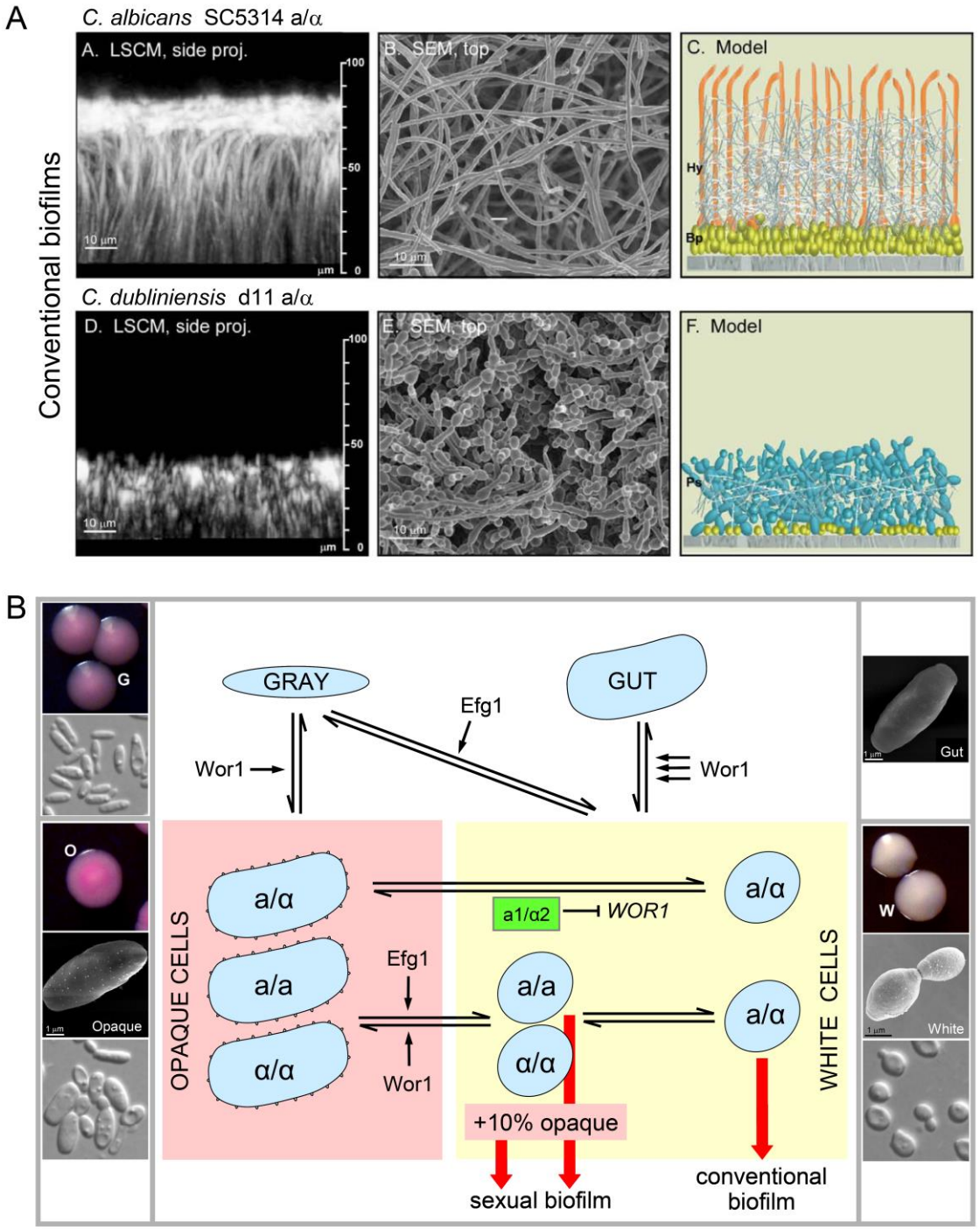

Figure 4: Ammonium/ammonia regulations in yeast colonies. A. Ammonia emitted by microcolonies induces pseudohyphal growth in the direction of adjacent microcolonies that are formed by haploid (left) or diploid (right) *S. cerevisiae* strains [60]. B. Ammonia signaling among smooth *S. cerevisiae* colonies (upper left) leads to cell differentiation into U cells and L cells (right). U cells, but not L cells, produce Ato1p-GFP as shown in the vertical colony cross-section (bottom left) [77]. C. Change in the morphology and cell differentiation in the *C. mogii* colony induced by ammonia from the right side [73]. D. Ammonia-dependent alkalization of the medium, colony morphology and filamentous cell formation in colonies of *C. albicans* grown on different carbon sources [80].