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Phenotypic Switching in Fungi

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Abstract

Over the past three decades new fungal diseases have emerged that now constitute a major threat, especially for patients with chronic diseases and/or underlying immune deficiencies. Despite the epidemiologic data, the emergence of stable drug-resistant or hyper-virulent fungal strains in human disease has not been demonstrated as seen in emerging viral and bacterial infections. Fungi are eukaryotic microbes that capitalize on a sophisticated built-in ability to generate phenotypic variability. This successful strategy allows them to undergo rapid adaptation in response to environmental challenges, such as individual body locations that may exhibit drastic differences in temperature and pH. Rapid microevolution can also confer drug resistance and protect them from the host's immune response. This review explores phenotypic switching in pathogenic fungi, including *Candida* spp and *Cryptococcus* spp, and how phenotypic switching contributes to the pathogenesis of fungal diseases.

Introduction

High-frequency phenotypic switching has been observed in prokaryotic and eukaryotic microbes, including pathogenic fungi. Phenotypic switching enables micro-organisms to undergo rapid microevolution and to adapt to a constantly changing microenvironment; it also facilitates pathoadaptation in the host. For fungi, switching was first described more than 20 years ago in *Candida albicans* [1,2]. In more recent years, phenotypic switching also has been demonstrated in other fungi, including *Candida glabrata* and *Cryptococcus neoformans* [3–8].

Phenotypic switching in fungi is an in vitro reversible phenomenon that is defined as the spontaneous emergence of colonies with altered colony morphology at rates higher than somatic mutation rates [9]. (Phenotypic switching in molds may occur, but diagnosis would be difficult because plating of many single colonies is technically not feasible.) Phenotypic switching in fungi can occur at any time during colony growth, which results in sectorized colonies. The switch often is associated with a diverse array of changes on the cellular level, resulting in complex phenotypes. What distinguishes phenotypic switching from other phenotypic changes (eg, the yeast-hyphal transition or capsule induction) is that switching only occurs in a small percentage of the population; it also is reversible but the colony morphologies are stably passed on for many generations after the initial switching event. Fungi have evolved multiple regulatory pathways that recognize a range of environmental changes, including pH, temperature, and the presence of serum. These environmental signals can promote yeast-hyphal transition or capsule induction, which are coordinated morphological changes of the entire fungal population. At the same time, phenotype variability may occur randomly at a low frequency, and altered phenotypes that have biological advantages in certain settings can then

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be selected. This allows these fungi to rapidly adapt to different host microenvironments with both heritable and nonheritable mechanisms, which helps to promote tissue invasion and evasion of the host immune system. Thus, phenotypic switching represents one of several different mechanisms by which “phenotype heterogeneity” can be achieved in a fungal population.

Phenotypic Switching in the Human Host

Of the estimated 1.5 million fungal species, fewer than 200 species cause disease in the human host because many fungi cannot grow above 37°C. Thus, the host represents a microenvironment of particular interest for fungal pathogens and commensals. Most *Candida* spp are commensals and naturally colonize certain niches in the human host. Colonization of the host occurs without damage to the host, but under certain circumstances when the host is weakened, the commensal can dominate the microbiome and become a pathogen that causes disease or death. The environmental fungus *C. neoformans* is ubiquitous in the environment and thus is more of an “accidental” pathogen, although its main virulence factor, the polysaccharide capsule, is upregulated in the host. Both exposure and usually an immunocompromised host are required for this fungus to become an opportunistic human pathogen. It is important to acknowledge that the boundaries of commensal, opportunistic, and primary pathogens are not well defined and in part depend on our ability to recover the fungus from the host.

Regardless of the particular classification of the fungus, phenotypic switching within the colonized or infected host most certainly can affect the host-pathogen relationship. Phenotypic switching can either augment damage to the host (and thus change a commensal into a pathogen) or augment baseline virulence of an opportunistic pathogen, which may facilitate persistence. Classic examples of how phenotypic switching can alter the host-pathogen relationship are provided by 1) *Trypanosoma cruzi*, in which phenotypic switching generates antigenically different variants that can escape recognition by specific antibody [10], and 2) *Neisseria meningitidis*, in which phenotypic phase variation is pivotal in determining whether the microorganism is a commensal or an invasive pathogen [11].

Most fungal infections are chronic, and even though switching may occur at a low frequency, there is ample time for switch variants to emerge and evade the immune response. Studies addressing the phenotypic diversity of an isogenic population can be difficult to undertake because it is hard to examine a pathogen population in vivo. This review presents findings on phenotypic switching of human fungal pathogens. Extensive work has been done with the human commensal and opportunistic pathogen *C. albicans* and with the human pathogen *C. neoformans*.

Phenotypic Switching in *Candida albicans*

Candida albicans is a commensal that colonizes the human host in various niches. In 1985, it was first demonstrated that common strains of *C. albicans* switched reversibly at high frequency between a number of variant phenotypes [1,12]. *Candida* strains exhibited different variant phenotypes in their switching repertoires, but switching in all strains showed common characteristics [9]. Two switching *C. albicans* strains have been intensely investigated (Table 1). The first *Candida* model switching strain, the “3153A switching system,” is quite heterogeneous and includes many variant phenotypes. Cells switch between seven general phenotypes, including “original smooth,” “star,” “irregular wrinkle,” “ring,” “mottled,” “fuzzy,” and “revertant smooth” [1,9,13]. In recent years, most research has been performed on the white-opaque switching strain because the phenotypes are numerous and variable. The W0-1 switching strain switches between a white colony phenotype and an opaque colony phenotype. Switching in the white-to-opaque direction occurs at a frequency of 10^4 to 10^5 per

cell generation, and switching from the opaque to white phase occurs at the slightly higher frequency of 5×10^4 per cell generation. However, appearances of white/opaque-sectored colonies are common in switching. This strain also has a clearly distinguishable phenotype at a microscopic level (Fig. 1). The white colony phenotype is composed of round budding cells, and the opaque colony phenotype contains large, elongated, asymmetrical budding cells [2, 14,15].

Phenotypic switching in *Candida albicans* is important for mating

The ability of a *C. albicans* strain to undergo white-opaque switching is regulated by the mating-type-like (MTL) locus. Opaque-phase a^- and α -type *Candida* cells mate 10^6 times more efficiently than white-phase a and α cells. In contrast, white-phase cells appear to be more robust and thus survive better within a mammalian host. Approximately 3% and 7% of clinical *C. albicans* isolates can undergo white-opaque switching. Switching *Candida* strains that are found in nature are homozygous a/a or α/α strains. *Candida* strains that are heterozygous (a/α) at the MTL locus cannot undergo white-opaque switching [16,17]. Opaque cells have been isolated from inside mammalian hosts, and elegant studies in mice have confirmed that mating can actually occur in the host [18]. Certain signals from the host can impact the expression of *MTL* genes, and other signals may also exist that stabilize the opaque-phase in specific locations within the host. Taken together, white-to-opaque switching represents a critical step in the mating process; the opaque cells are the mating-competent form of *C. albicans*, whereas the white cells are better suited to survive within the mammalian host environment but can generate mating-competent cells when needed.

Effects of phenotypic switching in *Candida albicans* on host-pathogen interaction

Switching involves the transcriptional regulation of a number of phase-specific genes, which are regulated by phase-specific trans-acting factors [19–21]. Several of these genes have been implicated in virulence [19,22–24]. Kvaal et al. [25] extensively studied the role of white-opaque switching with respect to virulence, demonstrating that opaque-phase cells were more virulent in newborn mouse cutaneous models of infection and that white-phase cells were more virulent in an intravenous systemic infection model [25]. Opaque-phase cells better colonize the skin and mate on the skin of newborn mice (surface temperature 31.5°C) in a highly efficient manner; up to 40% of opaque a and α cells can fuse during a 24-hour period [26]. Furthermore, switching can affect other virulence traits, including the bud-hyphal transition [27], the yeast's sensitivity to neutrophils and oxidants [28], antigenicity [27], adhesion [29], secretion of proteinase [22,30], drug susceptibility, and phagocytosis by macrophages [31]. All of these altered traits could potentially affect the survival within the mammalian host.

Molecular mechanisms and regulation of *Candida albicans* switching

In the white-opaque phenotypic switching strain, more than 373 genes are differentially expressed in the switch variants. Analysis determined that 221 genes were expressed at a higher level in opaque cells and 152 were more highly expressed in white cells [32]. These genes represent diverse functions, including metabolism, adhesion, cell surface composition, stress response, signaling, mating type, and virulence. Approximately one third of the differences among cell types are related to metabolic pathways. Opaque cells expressed a transcriptional profile consistent with oxidative metabolism, and white cells expressed a transcription profile that was consistent with a fermentative metabolism. Metabolic specialization of switch phenotypes may enhance selection in relation to the nutrients available at different anatomical sites, especially because the transcriptional bias was obtained regardless of carbon source. Zordan et al. [33] demonstrated that an interlocking feedback loop network is responsible for stably maintaining the epigenetic state of switch variants through many cell divisions. *MTLa1*, *MTLa2*, *WOR1*, *CZF1*, *WOR2*, and *EFG1* were found to constitute a genetic circuit

that regulates the white-opaque switch. This circuit, formed from a network of interlocking positive feedback loops, appears to be distinct for *C. albicans* and is not present in closely related fungi. It was proposed that because *EFG1* and *CZF1*, for example, have other key functions in the *Candida* life cycle, their involvement in white-opaque switching could be a recent adaptation that occurred in the warm-blooded host [33,34••].

Candida glabrata

Candida glabrata is the second most prevalent *Candida* spp that colonizes humans [35]. *Candida glabrata* is more closely related to *Saccharomyces cerevisiae* than to *C. albicans*, and it employs mechanisms different from those of *C. albicans* to generate phenotypic plasticity during pathogenesis. *Candida glabrata* switches between a number of phenotypes distinguished best by graded colony coloration on agar containing 1 mM CuSO₄. This phenomenon is referred to as *core switching* [36], and the core phenotypes are white (Wh), light brown (LB), dark brown (DB), and very dark brown (vDB). A core phenotype can spontaneously and reversibly switch to an irregular wrinkle (IW_r) phenotype, a transition referred to as *irregular wrinkle switching*. The graded brown coloration of the core phenotypes is thought to be the result of graded levels of conversions of Cu²⁺ to Cu¹⁺ and the associated reduction of SO₄²⁻ to S¹⁻. It is important to note that CuSO₄ does not induce switching per se. Switch-colony variants can be best identified on CuSO₄ supplemented agar, which stains Wh, LB, DB, and vDB colonies in an intensity order that is reversed from the pink-graded colors on phloxine B-containing agar. The coloration of the IW_r phenotype is white on CuSO₄-containing agar, regardless of the core phenotype from which the IW_r strain arose. However, IW_r exhibits a propensity for switching back to the core phenotype from which it arose, suggesting that although it expresses characteristics of Wh, it retains, or “remembers,” its original core phenotype. A microscopic analysis revealed that during the first 3 days of colony development, cells in the centers of Wh, LB, DB, and vDB colonies expressed almost exclusively the budding yeast phenotype; on day 3, pseudohyphae and cells extending tubes accumulated, so that by day 7 the proportions of these two cellular phenotypes reached 40% to 50% and 10% to 20%, respectively. In contrast, IW_r colonies were composed almost exclusively of pseudohyphae in the first 6 days and then accumulated both budding yeast cells and tubes. Core switching, pseudohyphae formation, and tube formation occurred in the majority of 62 tested clinical *C. glabrata* strains, suggesting that core switching is a general characteristic of most *C. glabrata* strains. Data from transcriptional profiling showed that the majority of genes upregulated in DB (78%) were involved in copper assimilation, sulfur assimilation, and stress responses; these genes also were differentially regulated in the various phenotypes. DB is the most commonly expressed phenotype at sites of host colonization. With respect to differences in host-pathogen interaction among the various phenotypes, little is known to date and future studies will have to delineate how upregulation of these genes in the context of phenotypic switching may play a role in colonization and virulence.

Candida lusitanae

Candida lusitanae is an emerging human pathogen that, unlike other fungal pathogens, either has intrinsic resistance or frequently develops resistance to the commonly used antifungal agent amphotericin B. It is infrequently found as a human commensal (skin, respiratory, gastrointestinal, and genitourinary tracts) and less than 1% of yeasts recovered from hospitalized patients are *C. lusitanae* [37]. The mechanism(s) of this resistance to amphotericin B is not well understood [38]. Studies have shown that *C. lusitanae* undergoes phenotypic switching when plated on yeast peptone dextrose (YPD) agar with 1 mM CuSO₄. Three colony colors are observed: LB to DB to W, which are similar to *C. glabrata*. The study showed that phenotypic switching affected amphotericin B resistance. High resistance (minimum inhibitory concentration [MIC] of 256 µg/mL) was associated with W, whereas LB and DB colonies

exhibited lower MICs of 2 to 8 µg/mL and 2 to 16 µg/mL, respectively. Filamentation (pseudo-hyphae) was associated with DB colonies. All phenotypes occurred spontaneously with greater frequency (10^{-2} to 10^{-4}) than spontaneous mutations, and all phenotypes were reversible, fulfilling the two phenotypic-switching criteria. High amphotericin B MICs were always associated with W colonies but not all W colonies exhibited high MICs. Data suggest that phenotypic switching in *C. lusitaniae* could represent a key strategy that confers a selective advantage during chronic infection in a host that is treated with amphotericin B.

Trichosporon asahii

Infections with *Trichosporon* spp have been recognized with increasing frequency over the past two decades [39,40]. These non-*Candida* yeasts have a broad spectrum of clinical manifestations, from self-limiting cutaneous infections to life-threatening invasive disease in the immunocompromised host. A recent study investigated phenotypic switching in 61 clinical isolates of *T. asahii*, producing four different morphological phenotypes on Sabouraud dextrose agar (SDA). These are white farinose (69%), white pustular (18%), yellowish white (10%), and white cerebriform (3%). Strains of the three major types (white farinose, white pustular, and yellowish white) produced two to five colony types when cultured on SDA at 37°C. The frequency of switching among colony types was 10^2 to 10^4 , similar to *C. albicans* and *C. neoformans*. The colonies switch to the smooth type—perhaps irreversibly—at frequencies of 10^2 to 10^3 . The enzymatic activity of beta-N-acetyl-hexosaminidase in the smooth type was significantly greater than that of the parent type in all strains. This could potentially confer enhanced virulence, but more data would be required to determine this.

Phenotypic Switching in Various *Cryptococcus* Species

Cryptococcus neoformans var *neoformans* and *Cryptococcus neoformans* var *grubii* are encapsulated pathogenic yeasts that can be found in soil worldwide and can cause chronic meningoencephalitis, pneumonia, and disseminated disease syndromes predominantly in immunocompromised patients. *Cryptococcus neoformans* var *gattii* causes a similar disease syndrome and used to be found mainly in subtropical climates like Australia, but more recently, out-breaks have been described in the northwestern part of the United States [41]. Phenotypic switching has been reported in *C. neoformans* var *grubii*, which are serotype A strains (SB4 and J32) [4], in *C. neoformans* var *neoformans*, which are serotype D strains (24067A, RC-2) [6], and in the sibling species *C. neoformans* var *gattii* [42]. In these strains, colonies with altered morphology arise spontaneously at a frequency of about 1 in 10^{-4} to 10^{-5} , and reversion to the parent smooth colony type occurs at a comparable frequency (approximately 1 in 10^{-3} to 10^{-5}) (Table 2). The smooth colonies (S and SM) of SB4, 24067, and NP-1 are round with a smooth dome surface and smooth edges. The mucoid (M and MC) colonies are round with smooth edges and a shiny and mucoid-appearing colony surface. They represent the parent colony morphology in the *C. neoformans* var *gattii* strain (NP-1). Wrinkled (WR), serrated (C), and pseudohyphal (PH) colonies exhibit an irregular dome surface with or without serrated margins. Both smooth and mucoid colonies represent standard colony types common to many *C. neoformans* strains, whereas the WR, C, S, and PH colony types of SB4 and 24067A are rarely observed in clinical isolates. Phenotypes and switching frequencies are most stable in the RC-2 switching strains; therefore, most of the in vivo work has been done with that strain.

Phenotypic switch variants of *Cryptococcus* exhibit distinct phenotypes with altered polysaccharide capsule

All switching strains in *C. neoformans* exhibit changes in the polysaccharide capsule, which is important because it may affect phagocytosis and rapid destruction by macrophages [43, 44]. For RC-2, the MC capsule is larger, and excessive production of viscous exo-polysaccharide results in a shiny surface (Fig. 1). Careful analysis of newly generated MC

switch variants demonstrates that other phenotypic characteristics are also changed. The MC switch variant also more slowly and exhibits increased sensitivity to lysing enzymes when compared with the SM parent. In a similar fashion, the SB4, 24067A, and NP1 variants exhibit changes in polysaccharide capsule that affect virulence. Glucuronoxylomannan (GXM) is the predominant capsular polysaccharide and is composed of (1→3)-linked linear α -D-mannopyranan with β -D-xylopyranosyl (Xylp) and β -D-glucopyranosyluronic acid (Glc pA) residues added to the mannose at various positions. Six structural reporter groups (SRGs) are defined based on the amount of 2-0-linked, 4-0-linked Xylp residues, and 2-0-linked Glc pA residues [45]. In *C. neoformans* strains SB4 and 24067A, phenotypic switching resulted in significant changes of the biochemical composition of GXM [5]. The GXMs of the C colony type of SB4 are composed of mixtures of SRGs (M2 and M3 for the C colony), whereas SB4-SM exhibits predominately SRG M2. In a similar fashion, the PH and WR colonies of 24067A exhibit a mix of SRGs (M1 and M5), whereas the SM parents are predominately M1 and M2. The addition of a Xylp group at the 4-0 position in M3 and M5 most likely requires a different enzyme than linkage to the 2-0 position. M3 SRGs are traditionally thought to be present only in the GXM of *C. neoformans* var *gattii* isolates (serotype B and C) and not in GXM of *C. neoformans* var *neoformans* isolates (serotype A and D). In RC-2, phenotypic switching alters the biophysical and biochemical properties of GXM [46]. Viscosity data in solutions of different ionic strength suggest that the spacing of Glc pA along the mannose backbone differs between the GXM of the SM and that of the MC strain. Because NMR measures an average repeat unit, no differences between SM and MC of RC-2 were detected.

Phenotypic switch variants exhibit difference in virulence in animal infection models

In all the “switching” *C. neoformans* var *neoformans* and *C. neoformans* var *gattii* strains studied so far, phenotypic switching results in variants that exhibit altered virulence in murine infection models (Table 2). For RC2, the MC variant is consistently more virulent than the SM parent in murine intratracheal infection models, intravenous infection models, and intracisternal infection models. Similar effects are seen for 24067A, in which the WR variant is more virulent, and for SB4, in which the serrated and wrinkled variants are more virulent. For *C. neoformans* var *gattii* strain NP1, MC was also found to be more virulent in the pulmonary model, but only the switched strain NP1-SM was able to elicit central nervous system infection. This suggests that switching in this case was required for crossing of the blood-brain barrier. Studies also indicate that RC-2 MC (but not the RC-2 SM variant) can promote intracranial pressure in a rat model of cryptococcal meningitis. In humans, elevated intracranial pressure is the leading cause of high morbidity and mortality in patients with chronic cryptococcosis; these studies suggest phenotypic switching may contribute to the development of intracranial pressure [47]. Antifungal interventions do not enhance phenotypic switching per se, and antifungal susceptibility is not affected by phenotypic switching. However, in mice infected with a switching strain (RC2-SM), both antifungal drug therapy and administration of anticapsular monoclonal antibody promoted the emergence of RC2-MC variants in chronic murine infection models [48]. These findings are important because they further our understanding on how phenotypic switching may affect the outcome of infection in chronically infected hosts who are treated. Phenotypic switching may contribute to the frequent treatment failures observed in immunocompromised hosts because treatment affects selection in the host.

Molecular mechanism of phenotypic switching

The molecular mechanisms mediating phenotypic switching in *C. neoformans* are currently not understood. Although karyotype instability was observed in strain 24067A and SB4 [4, 49] similar to the switching *C. albicans* strain 3153A, it could not consistently be correlated with phenotypic variability and was irreversible. Investigations involving differential display of RC2-SM and RC2-MC messenger RNA (RC-2 strain) demonstrated that phenotypic

switching was associated with downregulation of genes in the MC switch variant relative to the SM switch variant. The functions of most of these genes are still unknown; however, some may represent immunogenic epitopes. Future studies are directed at determining the specific function of the individual genes.

Conclusions

Phenotypic switching has been described for several pathogenic fungi, and it enables these pathogens to adapt to changing environments. Such environments include different hosts that will employ sophisticated immunological tools to rid themselves of the pathogen and variable environmental niches where microbes can be subjected to many types of selection pressures. This process allows the fungus to be resilient while maintaining the ability to generate diversity. Data from *C. albicans* emphasize the relevance of phenotypic switching for key biological functions such as mating, in which phenotypic switching can essentially protect a vulnerable phenotype of a species and thereby maintain its ability to mate. Data from chronic infection models of *C. neoformans* demonstrate how phenotypic switching in a fungus during experimental infection could potentially alter outcome and promote persistence.

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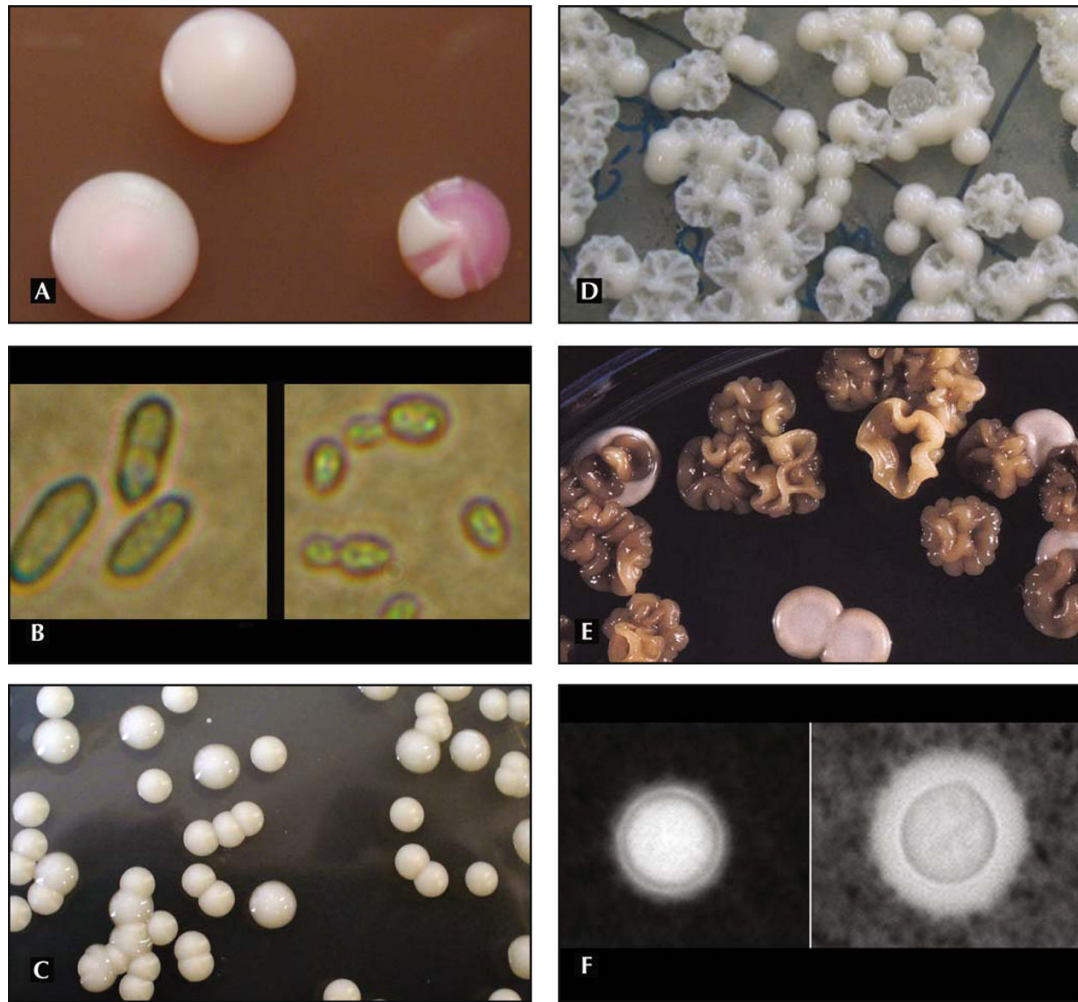


Figure 1. Colony morphology of switch variants in *Candida* species and *Cryptococcus neoformans*. *Candida* spp: (A) white-opaque switch variants and sectored colonies; and (B) microscopic phenotype differences of the white and opaque variant. *Cryptococcus neoformans*: (C) RC-2 smooth and mucoid colonies; (D) SB4 SM and wrinkled colonies; (E) differences in melanization of SM and WR switch variants of 24067A; and (F) capsule differences of SM and MC switch variants.

Table 1
 Characteristics of switching *Candida* and *Trichosporon* strains

Species	Switching strain	Parent	Variants	Switching frequency
<i>Candida albicans</i>	3/55A	Smooth	Star, ring, irregular wrinkled, stopple, hat, and fuzzy	1.4×10^{-4} (all variants)
	WO1	White (W)	Opaque (O)	4.6×10^{-4} to 4.4×10^{-3} (W→O)
	Strain 1001	Smooth (S)	Rough (R)	2.1×10^{-2} to 1.3×10^{-1} (O→W) $< 10^{-4}$ (S→R) $< 9 \times 10^{-4}$ (R→S)
<i>Candida glabrata</i>	NUM 961	Smooth (S)	Semi-rough (SRT-1)	3.5×10^{-3} (sectored colony)
	35B11	Dark brown (DB)	White (Wh)	3×10^{-5} (S→SRT-1)
			Light brown (LB)	2×10^{-3} (DB→Wh)
			Very dark brown (vDB)	9×10^{-3} (DB→LB)
<i>Candida lusitanae</i>			Irregular wrinkled (IW _r)	8×10^{-3} (DB→vDB)
	JHH Clr-3B	Light brown (LB)	White (W)	1×10^{-4} (DB→IW _r)
			Light brown (LB)	1×10^{-4} (LB→W)
			Dark brown (DB)	6.6×10^{-2} (LB→DB)
<i>Trichosporon asahii</i>		White farinose (WF)	White, radial, fissure (WRF)	1.4×10^{-2} (DB→LB)
		White pustular (WP)	White farinose (WF)	2×10^{-4} (W→LB)
		Yellowish white (Y)	White (W); yellowish white (Y); smooth (S); radial fissure (RF)	10^{-2} to 10^{-4} (all variants)

Table 2

Characteristics of switching *Cryptococcus* strains

Switching strain	Serotype	Phenotype	Switching frequencies	Virulence (in murine models)	Infl ammatory response	GXM SRG
SB-4	A	Smooth (S) Serrated (C)	2.2×10^{-3} to 9×10^{-6}	WR > S > C	C > S > WR in rats*	ND
J32	A	Wrinkled (WR) Smooth (NIM) Mucoid (MC)	3.4×10^{-3} to 4.1×10^{-5}	ND	ND	ND
24067A	D	Smooth (SM) Mucoid (MC)	2.2×10^{-3} to 6×10^{-6}	WR persists; SM and PH are cleared in rats	WR > PH > SM in rats [†]	M1, M2, M5, M6
RC-2	D	Pseudohyphal (PH) Wrinkled (WR #0) Smooth (SM) Mucoid (MC)	10^{-4} to 0.5×10^{-4}	MC > SM in mice	MC > SM in mice [‡]	M1, M5 M1, M5, M6 ND
NP1	B	Smooth (NP1-SM) Mucoid (NP1-MC)	MC to SM >> SM to MC Range (10^{-3} to 10^{-5})	MC > SM (mice)	MC > SM in mice	M3, M1, M6 M3, M1, M6

* Minimal infl ammation in wrinkled (WR)-infected rats in contrast to intense caseous necrosis in serrated (C)-infected rats.

[†] No infl ammation in smooth (SM)-infected rats in contrast to extensive granuloma formation in wrinkled (WR)-infected rats.

[‡] Organized granuloma formation in smooth (SM)-infected mice in contrast to extensive and destructive infl ammatory response in mucoid (MC)-infected mice.

GXM—glucuronoxylomannan; ND—not done; SRG—structural reporter group.