Published in final edited form as:

Curr Opin Microbiol. 2009 December ; 12(6): 650–654. doi:10.1016/j.mib.2009.09.010.

White-opaque switching in *Candida albicans*

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Summary

The human commensal yeast *Candida albicans* undergoes an epigenetic switch between two distinct types of cells, referred to as white and opaque. These two cell types differ in many respects, including their cell and colony morphologies, their metabolic states, their mating behaviors, their preferred niches in the host, and their interactions with the host immune system. Each of the two cell types is heritable for many generations and switching between them appears stochastic; however, environmental cues can significantly alter the frequency of switching. We review recent work on white-opaque switching, including the establishment of the transcriptional circuit underlying this switch, the identification of environmental signals that affect switching rates, newly discovered differences between the two types of cells, and the involvement of white-opaque switching in biofilm formation. We also review recent speculation on the evolution and adaptive value of white-opaque switching.

Introduction

Candida albicans, the most common human fungal pathogen, resides asymptotically in the gastrointestinal tract of most healthy humans. However, when the host immune system is compromised due to age, disease, or medical treatment, *C. albicans* can cause a range of medical problems; including disseminated blood-born infections with mortality rates exceeding 40% [1]. *C. albicans* differs from the great majority of fungal species (and even from some very closely related species) by possessing the ability to switch between two distinctive types of cells, white and opaque. Each cell type is heritable for many generations and switching occurs without a change in the DNA sequence of the genome (hence our use of the term 'epigenetic's) [2•–6]. Under standard laboratory conditions switching is rare (approximately every 10⁴ cell divisions) although, as will be discussed, environmental factors can strongly influence the switching rate [7]. White and opaque cell types have distinct properties which result largely from the differential regulation of approximately 400 genes, about seven percent of the genome [8,9]. Metabolic preferences [8], environmental responses [10], biofilm interactions [11••], host-cell immune interactions [12,13], and the ability to mate [14••] show significant differences between white and opaque cells. It is an attractive

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hypothesis that white-opaque switching evolved during *C. albicans*' long association with its warm-blooded host and that it plays a crucial role in the pathogen-host relationship.

White-opaque switching is closely linked with mating

White-opaque switching was discovered in 1987 by David Soll and colleagues [2•]. Its key role in the mating cycle of *C. albicans* was established some 15 years later [reviewed in 15, 16]. In brief, *C. albicans*' mating is controlled by transcriptional regulators encoded at the Mating Type Like (MTL) locus. There are two versions of this locus, referred to as **a** and α , which contain transcriptional regulators specifying **a** and α -type mating respectively. In cells containing both versions of this locus, referred to as **a**/ α cells, two homeodomain proteins (one from the **a** locus and one from the α locus) form the **a**1– α 2 heterodimer which represses mating functions as well as white-opaque switching. In order to mate, an **a** cell must encounter an α cell but both cells must be in the opaque form for mating to culminate. These observations help to explain why *C. albicans* mating was so difficult to detect: more than 95% of clinical *C. albicans* isolates are **a**/ α strains [17] (and hence are mating and switching incompetent) and, in order to mate, **a** and α cells both have to undergo the rare switch to the opaque state [Figure 1].

Multiple feedback loops contribute to stability of the two cell types

One key characteristic of the white and opaque cell types is their stability over thousands of generations under normal laboratory conditions; that is, upon cell division, white cells almost always give rise to white cell progeny and opaque cells to opaque progeny. It has been proposed that this behavior results from the topology of the transcriptional circuit underlying the switch; this circuit consists largely of interlocking positive feedback loops [Figure 2a] [18•].

At the core of this circuit is a protein called WOR1, the first major regulator of the opaque state to be identified [19–21]. WOR1 expression is required to switch from the white to the opaque state and ectopic expression of WOR1 can drive an entire population of white cells to the opaque state. WOR1 expression produces a direct positive feedback loop by binding its own promoter and turning on its own expression [19–21]. Activation of this feedback loop produces a forty-fold increase in WOR1 transcript levels in opaque cells compared to white cells. The WOR1 promoter is directly repressed by the **a**1– α 2 heterodimer, thus explaining the inability of **a**/ α cells to switch from white to opaque [Figure 2b] [9,14•,20].

Three additional transcriptional regulators, EFG1, WOR2, and CZF1, complete the known regulatory circuit [Figure 2a] [18•,22–25•], and it has been proposed that this circuit can account for the major characteristics of the white-opaque switch. According to this model, the circuit is largely inactive in the white state; this is the default state [Figure 2c]. Switching occurs when the circuit becomes excited; because of the series of positive feedback loops, the circuit can remain excited for many generations [Figure 2d]. It has been hypothesized that inheritance of the opaque state results from molecules of the regulators being passed on to daughter cells following cell division; the concentrations of these regulators in the daughter cells would then be sufficiently high to re-excite the circuit and retain the opaque state. What triggers white-to-opaque switching in the first place? It has been proposed that when components of the switching circuit reach a critical threshold concentration, the circuit becomes excited. In principle, this could occur through random fluctuations in the levels of a critical molecule such as WOR1. The reverse process, opaque-to-white switching, would then occur when insufficient quantities of the regulators are passed on to a daughter cell and the circuit would wind down.

Layered on this core transcriptional circuit are several chromatin modifying factors whose presence also affects switching. For example, deletion of the histone deacetylase HDA1 slightly increases switching rates from white to opaque while deletion of RPD3, another histone

deacetylase, increases switching in both directions [26]. Recently, the acetyltransferase NAT4 and histone deacetylases HST2, SET3, and HOS2 have also been implicated in regulation of switching. Deletion of each of these genes reduced rates of white-to-opaque switching and all but HST2 increased switching from opaque to white [25••]. It is not yet clear precisely what role these chromatin remodeling factors play in the switch; one simple model is that they serve to reinforce the characteristics of the transcriptional circuit described above.

Growth rate affects switching frequency

Although the circuit described above can explain much of the behavior of the two cell types, it does not readily account for how environmental cues can affect the rate of switching. It has long been known that oxidative stress increases switching from white to opaque [10] while elevated temperatures drive switching from opaque to white [7]. Recent studies have identified several new signals that affect switching frequencies and have led to new hypotheses linking these signals to the regulatory circuit

Exposure to the DNA damaging agent methyl methane sulfonate (MMS) and deletions of the DNA repair genes RAD51 and RAD52 both increase switching from white to opaque [27•]. Hydroxyurea (HU), which delays cells in S phase of the cell cycle, also increased the white-to-opaque switching rate. These results suggest a direct link between progression through the cell cycle and white-opaque switching. According to one model, an extended cell cycle in slow growing cells would facilitate switching by allowing WOR1 to accumulate to higher levels before being diluted by cell division; this would result in a greater proportion of cells with sufficient WOR1 to excite the regulatory circuit of Figure 2. In support of this hypothesis, depletion of the mitotic cell cycle regulator CLB4 slows cell growth and also produces increased switching from white to opaque [27•].

Anaerobic growth stabilizes the opaque cell type

Recent work has also revealed new insights into the interplay between temperature and white-opaque switching. The observation of *in vivo* mating of *C. albicans* in a mouse host [28] had long presented a paradox. *C. albicans* must be opaque in order to mate [14••] and yet opaque cells were not believed to be stable at the internal body temperature of 37°C. However, it has recently been discovered that opaque cells are stable and capable of mating at 37°C if also exposed to anaerobic conditions [29]. Further work revealed that anaerobic conditions not only stabilize opaque cells but even stimulate white to opaque switching [30••]. Studies in the mouse support this view: when feces were collected from mice injected with a switching competent white strain, it was discovered that between 4 and 10 percent of cells were in the opaque form. Thus white-to-opaque switching can indeed occur at 37°C in a mouse model.

What is the basis for anaerobic growth stimulating white-to-opaque switching? Growth under anaerobic conditions leads to ergosterol depletion, and it has been hypothesized that this metabolic cue may signal cells to switch. In support of this view, addition of the ergosterol biosynthesis inhibitors lovastatin or ketoconazole produced an increase in switching to opaque similar to that seen under anaerobic conditions [30••].

An independent set of experiments has shown that elevated CO₂ levels can also stimulate white-to-opaque switching and can stabilize opaque cells at 37°C [31•]. Because CO₂ levels vary dramatically among different tissues of the host, this observation strongly suggests that rates of white-opaque switching are tuned to different niches within the host.

Opaque cells may evade the innate immune system

Differences between white and opaque cells significantly affect *C. albicans*' interactions with the host. For example, white cells appear better suited to internal infections while opaque cells thrive in skin infections [32–34]. Opaque and white cells also differ in their interactions with specific components of the host immune system. For example, opaque cells, unlike white cells, do not secrete a chemoattractant for leukocytes [12]. As a possible consequence of this difference, opaque cells are significantly less susceptible than white cells to phagocytosis by macrophage cell lines *in vitro* [13]. This ability to avoid the innate immune system may help *C. albicans* colonize internal environments where opaque cells are particularly stable. It should be stressed, however, that we know relatively little about the complete relationship between the two cell types and the host.

White cells respond to pheromone by forming biofilms

In response to the α mating pheromone, opaque **a** cells up-regulate several hundred genes, many of which are involved in preparing the cells for the subsequent steps of mating [35]. Although they appear unable to mate, white **a** cells also respond to the α pheromone [11•, 36]. Both white and opaque **a** cells sense α -factor through the same α -factor receptor, STE2 [37], although white cells use a novel signaling loop not required for detection in opaque cells [38].

The response of white cells to mating pheromone is markedly different from the response observed in opaque cells. Many fewer genes are induced and, rather than preparing to mate, white cells undergo a different type of response: they form a biofilm [11•]. What could be the purpose of this response? One hypothesis is that biofilm formation facilitates subsequent mating. According to this idea, detection of pheromone allows white cells to recognize the presence of a small population of opaque cells, and by forming a biofilm, concentrates cells of opposite mating types to promote mating. Regardless of its precise role, the observation that white and opaque cells respond very differently to mating pheromone is a particularly fascinating difference between the two types of cells.

Conservation of the switch components

Although white-opaque switching appears unique to *C. albicans* and its very close relatives [39], the components that regulate the switch are conserved among many fungal species. For example, WOR1, the central regulator of white-opaque switching, is a member of a large family of fungal proteins distinguished by a highly conserved N-terminal region [21]. Although nearly every species of fungi for which a genome sequence is available has a WOR1 homolog, the structure and biochemical function of this conserved region is not known. In all species where they have been studied, the WOR1 homologs appear to play some role in regulating morphological changes. For example, the *Histoplasma capsulatum* WOR1 ortholog RYP1 is a key regulator of the yeast-to-mycelial switch. RYP1 is differentially expressed in the two types of cells, binds its own promoter, and is necessary for establishment and maintenance of the yeast cell type [40•]. At this point, we can only guess how these deeply conserved transcriptional regulators became “wired-up” to catalyze white-opaque switching in *C. albicans*.

Conclusion

Despite many advances in our understanding of the regulation and role of white-opaque switching in *Candida albicans*, we still possess only a tentative grasp of many important aspects of this switching system. It remains to be determined whether random fluctuations in regulator levels can explain the stochastic nature of switching. The role of opaque cells in the host and

their interaction with the innate immune system remain among the most pressing questions in this field. Specific host niches that stabilize opaque cells or promote switching from white to opaque remain largely unidentified. The exact role of biofilm formation in facilitating *in vivo* mating remains to be determined, as does the *in vivo* role of anaerobic growth and CO₂. Finally, the selective pressures under which the white-opaque switch evolved remain obscure.

Acknowledgments

The authors thank Chris Cain, Sarah Foss, Aaron Hernday, and Oliver Homann for valuable comments on this manuscript. Work of the authors was supported by grants from the National Institutes of Health (AI49187) and the Ellison Foundation (ID-SS-0628-04).

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Figure 1.

Outline of the parasexual cycle of *C. albicans*. **a** cells mate with α cells, but both types of cells must be in the opaque form for this to happen. *C. albicans* is diploid so mating produces a tetraploid a/α cell. This cell can lose chromosomes to return to the diploid state; a conventional meiosis has not been observed in *C. albicans*.

Figure 2.

Model of the white-opaque regulatory circuit and its activity in different cell types. (a) White boxes represent factors that are enriched in white cells compared with opaque cells, and yellow boxes represent opaque-enriched factors. Lines with arrows represent positive control and lines with bars, negative control. (b) White *a/α* cell, with the *a1/α2* heterodimer repressing WOR1. This cell is locked in the white form. (c) White *a* or *α* cell. (d) Opaque *a* or *α* cell. WOR1, WOR2, and CZF1 are up-regulated relative to white cells, and EFG1 is down regulated. In panels b–d, down-regulated genes and inactive interactions are shown in gray while up-regulated genes are shown in black and active interactions are shown in red. Adapted from Zordan et al 2007 [18•].