The Parasexual Lifestyle of *Candida albicans*

Richard J. Bennett*
Department of Molecular Microbiology and Immunology, Brown University, 171 Meeting St, Providence, RI 02912

Abstract

*Candida albicans* is both a prevalent human commensal and the most commonly encountered human fungal pathogen. This lifestyle is dependent on the ability of the fungus to undergo rapid genetic and epigenetic changes, often in response to specific environmental cues. A parasexual cycle in *C. albicans* has been defined that includes several unique properties when compared to the related model yeast, *Saccharomyces cerevisiae*. Novel features include strict regulation of mating via a phenotypic switch, enhanced conjugation within a sexual biofilm, and a program of concerted chromosome loss in place of a conventional meiosis. It is expected that several of these adaptations co-evolved with the ability of *C. albicans* to colonize the mammalian host.

Introduction

Sexual reproduction is a ubiquitous property in eukaryotic cells, having arisen in the common ancestor to this lineage. It involves an alternation between ploidy states, often between that of haploid and diploid; mating (or gamete fusion) leads to a doubling of the...
genetic material in the cell, whereas meiosis leads to a halving of the DNA content in the cell. Fungal species exhibit highly diverse sexual cycles, from the relatively simple modes of sex seen in the model hemiascomycete \textit{S. cerevisiae}, to the more complex reproductive cycles seen in the mushroom-forming basidiomycetes (for recent reviews on fungal sex see [1–5]). In the case of \textit{C. albicans}, this important opportunistic pathogen is capable of causing both debilitating mucosal and life-threatening invasive infections, with the potential to infect almost every organ in the human body. \textit{Candida} species were originally defined as species that can form pseudophyphae or true hyphae but lacked any form of sexual reproduction [6]. However, experiments have now deciphered an unusual parasexual mating cycle in \textit{C. albicans} that shows several adaptations to that found in model yeasts.

\textbf{Regulation of cell type}

The sexual cycle in \textit{S. cerevisiae} has served as the primary model for studies into mating and meiosis in yeast. In this species, mating occurs between \textit{a} and \textit{α} cells and is regulated by genes encoded at a single genetic locus called the mating-type or \textit{MAT} locus. Three transcription factors encoded at the \textit{MAT} locus, \textit{a}1, \textit{α}1, and \textit{α}2, determine whether cells behave as \textit{a}, \textit{α}, or \textit{a}/\textit{α} cells (Figure 1A). \textit{MATa}-containing cells express the \textit{a}1 gene, \textit{MATα} cells express \textit{α}1 and \textit{α}2 genes, and \textit{MATa}/\textit{α} cells express all three genes. In \textit{a} cells, \textit{α}1 is responsible for the induction of \textit{α}-specific genes, whereas in \textit{α} cells, \textit{a}-specific genes are constitutively expressed. However, \textit{a}-specific genes are not expressed in \textit{α} cells due to repression by \textit{α}2 together with the Mcm1 transcription factor [7]. \textit{MATa} and \textit{MATα} cells both express “haploid-specific” genes that include pheromone-signaling factors that promote mating [8,9]. Meiosis occurs only in \textit{MATa}/\textit{α} cells, as a complex between \textit{a}1 and \textit{α}2 turns off haploid-specific genes including \textit{RME1}, which is a repressor of meiosis [7]. Thus, the combinatorial control of cell-type expression by \textit{MAT} transcription factors regulates both mating and meiosis in \textit{S. cerevisiae}.

\textbf{Mating in \textit{Candida albicans} and rewiring of cell type}

\textit{C. albicans} is a hemiascomycete yeast related to \textit{S. cerevisiae}, although these species diverged more than 900 million years ago, and are approximately as divergent as humans and fish [10]. Natural isolates of \textit{C. albicans} are diploid, and it was originally thought that this species could not undergo any form of sexual reproduction. However, diploid \textit{a} and \textit{α} strains of \textit{C. albicans} were engineered from a clinical \textit{a}/\textit{α} isolate and shown to be capable of mating to form tetraploids, both \textit{in vitro} and in a murine model of systemic infection [11,12]. Subsequent experiments established that \textit{C. albicans} mating shows parallels with that in \textit{S. cerevisiae}; both species secrete sex-specific pheromones that induce mating responses in cells of the opposite sex, leading to cell-cell conjugation and karyogamy [13–17]. Despite these similarities, the transcriptional circuits regulating mating have diverged since these two species last shared a common ancestor. In particular, where there is negative regulation of the \textit{a}-specific genes in \textit{S. cerevisiae} \textit{α} cells via the \textit{α}2 protein, there is positive regulation of the \textit{a}-specific genes in \textit{C. albicans} via an additional transcription factor, \textit{a}2, that is absent from \textit{S. cerevisiae} (Figure 1A and B)[18]. Analysis of multiple yeast species revealed that the transcriptional circuit in \textit{C. albicans} is the ancestral circuit, whereas that in \textit{S. cerevisiae} is a derived circuit [19]. Strikingly, some hemiascomycete species (e.g. \textit{K}.}
C. albicans' have retained a hybrid form of the circuit in which α-specific genes are both positively regulated by α2 and negatively regulated by α2 (Figure 1B) [20]. The hybrid state has been resolved by different routes in the lineages giving rise to modern yeast species; in some species regulation reverted to the ancestral state, while in others, such as S. cerevisiae, the ancestral mode of regulation was lost and only the derived mode of regulation was retained [20].

**Mating in C. albicans is regulated by the white-opaque switch**

Initial studies of *C. albicans* mating revealed that mating efficiency was low both *in vitro* and *in vivo*. However, a major breakthrough came with the discovery that mating is regulated not only by the mating locus, but also by an epigenetic switch between phenotypic states. Certain isolates of *C. albicans* had long been recognized to undergo phenotypic switching between “white” and “opaque” states. Cells in the white state are round and give rise to bright, dome-shaped colonies whereas cells in the opaque state are elongated and give rise to darker, flatter colonies (see images in Figure 2)[21]. Miller and Johnson revealed two key attributes of the switch. First, that switching to the opaque state occurred in α or α cells, but not α/α cells, due to repression by the α1/α2 complex. Second, that mating between opaque cells occurs a million times more efficiently than that between white cells [22]. These findings revolutionized the field, enabling mechanistic studies of *C. albicans* mating for the first time.

Regulation of the white-opaque switch requires the transcription factor Wor1, whose expression is necessary and sufficient for formation of opaque cells [23–25]. Wor1 acts as part of a complex network of interacting transcription factors to regulate bistable switching in *C. albicans* [26,27]. A similar white-opaque switch was subsequently uncovered in the related *Candida* species *C. dubliniensis* and *C. tropicalis* [28–30]. It therefore appears that the white-opaque switch evolved in the common ancestor to these three species, all of which are opportunistic human pathogens.

One curious aspect of the white-opaque switch is that *C. albicans* opaque cells are generally unstable at 37°C and switch back to the white state *en masse* [31]. It was therefore envisaged that mating takes place outside of the mammalian host, and conjugation events were observed during opaque cell colonization of the skin [32]. However, mating on the skin rarely results in productive daughter cell formation [33], and low mating frequencies were recently reported using this infection model [34]. Other niches may therefore hold greater promise for mating in the host, particularly given that host cues can stabilize opaque cells at body temperature. For example, N-acetyl glucosamine, CO2, anaerobiosis, and cellular stress all promote formation of the opaque state [35–38], and some of these cues can even induce white-to-opaque switching in α/α isolates [39]. Furthermore, at least in one clinical isolate white-to-opaque switching was observed in a murine model of gastrointestinal colonization [40], and occasional mating was also reported from this niche [35]. It therefore appears that mating, at least at low frequency, can occur in multiple sites in animal models of infection.
Concerted chromosome loss

In most eukaryotes, sexual reproduction involves a meiotic program in which DNA replication is followed by two successive DNA divisions, thereby halving the genetic material in the cell. The \textit{C. albicans} genome contains many conserved genes that function in meiosis in other species \cite{41,42}, including some that are functional when heterologously expressed in \textit{S. cerevisiae} \cite{43}. However, a conventional meiosis has not been described in \textit{C. albicans}. In its place, tetraploid mating products return to a diploid, or near-diploid state, by a parasexual mechanism of concerted chromosome loss (Figure 3) \cite{44,45}. During parasex, cells undergo limited recombination, which is dependent on the ‘meiotic’ recombinase, Spo11 \cite{46}. This indicates an intriguing mechanistic link between the programs of meiosis and parasexual chromosome loss. Several steps in the parasexual cycle are stimulated by environmental stress, suggesting that parasex might be particularly advantageous under conditions where genetic variation is beneficial \cite{47}. Parasexual chromosome loss is thought to involve chromosome non-disjunction during mitotic divisions, leading to unstable cells of intermediate ploidy, which then further reduce their ploidy back to diploid or near diploid \cite{45,48}. A similar parasexual cycle was observed in \textit{C. tropicalis}, implying that meiosis is also absent (or at least cryptic) in this species \cite{49}.

Despite the absence of a conventional meiosis in \textit{C. albicans}, the products of the parasexual cycle exhibit diverse genotypes and phenotypes \cite{46}. Diversity is due to a combination of mechanisms, including shuffling of whole chromosome homologs, genetic recombination, and the presence or absence of supernumerary chromosomes (Figure 3). Given that aneuploidy plays a widespread role in generating phenotypic variants and antifungal drug resistance \cite{50–52}, it is clear that parasex could generate potentially important traits relevant to fungal pathogenesis.

Recently it was shown that \textit{C. albicans} not only undergoes a diploid-tetraploid-diploid cycle but is capable of transitioning between diploid and haploid cell types. Haploid forms of the species were thought to be inviable due to the presence of multiple recessive lethal alleles, but rare haploid cells were obtained both \textit{in vitro} and following infection of an animal host \cite{53}. Haploid cells were formed by a parasexual mechanism of concerted chromosome loss similar to that observed in tetraploid cells. Furthermore, haploid \textit{a} and \textit{α} cells could switch to the opaque state and undergo mating to regenerate \textit{a}/\textit{α} diploid cells, or could form \textit{MTL} homozygous diploids by spontaneous auto-diploidization \cite{53}. While viable, all haploid cells showed marked fitness defects that currently limit their use in studying pathogenesis.

Why did \textit{C. albicans}, as well as closely related species, apparently lose the ability to undergo a conventional meiosis? One possibility is that the formation of sexual spores accompanying a traditional meiosis would be a hindrance to commensal species, as spores are likely to be highly immunogenic and targeted by host defenses \cite{54}. In addition, parasex is capable of generating progeny that are extremely diverse due to cells existing in multiple ploidy states (Figure 3). The plethora of aneuploidies produced by parasex could be important for generating a pool of genetically diverse isolates upon which selection could then act.
It is worth noting that, in contrast to *C. albicans*, several *Candida* clade species have retained a full sexual cycle that includes meiosis and sexual sporulation [41]. For example, *Candida lusitaniae* undergoes meiosis despite containing a similar repertoire of ‘meiosis-specific’ genes to that of *C. albicans* [55]. However, whereas Ime1 is the master transcriptional regulator of meiosis in *S. cerevisiae*, an ortholog of this gene is missing in *C. lusitaniae* and other *Candida* clade species [41]. Instead, it appears that the transcription factor Ste12 regulates both mating and meiosis in *C. lusitaniae*, indicating fusion of the regulatory programs controlling these two pathways [56]. These studies reveal that genomic analysis alone cannot determine whether a cryptic meiosis still remains to be discovered in *C. albicans*.

**Unisexual reproduction**

Several yeast species, including *S. cerevisiae* and *S. pombe*, undergo mating-type switching in which cells alternate between α and α cell types. Mating-type switching evolved independently in these species, and in both cases involves copying of one of two silent cassettes encoding mating type information to the active expression site [57,58]. Recent experiments suggest that this mode of mating-type switching evolved from an ancestral two-locus system, in which switching occurred using a simpler flip-flop inversion mechanism [59]. These studies also suggest that mating-type switching was lost in *C. albicans* and other *Candida* clade species; these species do not contain silent mating-type cassettes or undergo mating-type switching, potentially limiting self-fertility [41]. Surprisingly, however, *C. albicans* was found to be capable of same-sex mating under the appropriate conditions. This result was first observed using strains that lack the barrier protease, Bar1, that inactivates α pheromone [60]. *C. albicans* opaque α strains express both α and α pheromone; Bar1 normally degrades secreted α pheromone, but in the absence of Bar1 an autocrine feedback loop involving this pheromone promotes fusion between opaque α cells. Same-sex α-α mating also occurs in mixed cultures of wildtype opaque α and α cells, in which α pheromone produced by α cells overwhelms Bar1 activity from α cells [60]. Subsequent experiments revealed that activation of pheromone signaling is sufficient for driving unisexual mating, and even that pheromones from other *Candida* species can induce same-sex mating in *C. albicans* [61]. More recently it was shown that white cells can also be induced to secrete pheromone which can promote both opposite-sex and same-sex mating of *C. albicans* opaque cells [34].

Same-sex mating has been observed in another prevalent human pathogen, *Cryptococcus neoformans* [62,63], suggesting that unisexual reproduction may be beneficial for diverse fungal pathogens. Unisexual mating could have advantages for a species, including the fact that sex can occur in the absence of an opposite sex partner, and that the costs associated with unisexual reproduction are significantly less than those accompanying bisexual mating [4].

**Pheromone signaling drives biofilm formation**

*C. albicans* infections are often a consequence of biofilm formation, in which communities of fungal cells adhere both to one another and to a biological or inert substrate. Biofilm
growth is particularly troublesome on implanted medical devices such as catheters, pacemakers, heart valves, or prosthetic joints, as biofilms are resistant to antifungal therapy or immune clearance [64,65]. *C. albicans* forms biofilms under a variety of culture conditions, and the ability to undergo filamentation is generally associated with biofilm maturation [66,67].

The Soll group revealed that pheromone signaling could act as a novel environmental cue driving biofilm formation in *C. albicans*. White α or ω cells, although unable to mate, responded to pheromones produced by opposite-sex opaque cells by adhering to an inert surface [68]. Furthermore, formation of these ‘sexual’ biofilms could support mating between opaque α and ω cells by stabilizing pheromone gradients between opposite-sex opaque partners [68,69]. Studies of sexual biofilms reveals parallels with ‘conventional’ biofilms, with both structures consisting of a basal layer of yeast cells upon which filamentous cells and extracellular matrix develop [68–70].

The transcriptional regulation of sexual biofilm remains controversial. Experiments in our group showed that pheromone signaling in white cells is via the canonical MAPK pathway and its transcription factor, Cph1 (ortholog of ScSte12) [71]. In fact, deletion of Cph1 abolished the complete transcriptional response of white cells to a pheromone challenge [71]. In contrast, the Soll group found that Cph1 was not involved in the response to pheromone in white cells, and instead Tec1 was the key transcription factor mediating sexual biofilm formation [72]. A third independent study showed that Cph1 was critical for MAPK signaling in white cells, but that Tec1 was also required for MAPK-signaling induced adhesion by white cells [73]. It therefore remains to be seen how exactly Cph1/Tec1 promote adherence and biofilm formation in response to pheromone signaling. The role of sexual biofilms in nature has also yet to be addressed; most clinical isolates are α/α strains [74,75] and are able to form conventional biofilms but are not capable of forming pheromone-induced biofilms. In these strains, it is the presence of the α1/α2 complex that (directly or indirectly) inhibits the expression of pheromone, pheromone receptor, pheromone processing and MAPK signaling genes, thereby blocking both the secretion of, and response to, sexual pheromones [18,34,76].

**Future perspectives**

Identification of a parasexual cycle in *C. albicans* has raised as many questions as it has answered. Discovery of this unusual cycle reveals an elegant mechanism for mating and genetic rearrangement, and yet population structure analyses suggests that recombination between isolates is rare in nature [75,77]. Sex could be restricted in *C. albicans* to limit the disruption of genotypes that are already well adapted for commensal growth [54]. However, sex or parasex has now been documented in most *Candida* clade species [41], indicating that these species, originally labeled as *Fungi Imperfecti*, have maintained (para)sexual reproduction over millions of years. The retention of facultative sexuality is compelling, and suggests that sex continues to play important roles in these species. Determining the role(s) of sex in *C. albicans* will therefore continue to shed light on cryptic modes of sex in fungal species, as well as adaptations to this ancestral program that can enhance the commensal or pathogenic lifestyle.
Acknowledgments

The author would like to acknowledge Iuliana Ene, Matthew Hirakawa, and Christine Scaduto for comments on the manuscript. Funding in the author’s laboratory is supported by the NIH (RO1AI081704, R21AI081560) and a PATH award from the Burroughs Wellcome Fund. Scanning electron micrograph images of *C. albicans* cells were provided by Matthew Hirakawa.

References


22. Miller MG, Johnson AD. White-opaque switching in Candida albicans is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell. 2002; 110:293–302. This work demonstrated that the white-opaque switch was a key mechanism for regulation of mating in C. albicans. [PubMed: 12176317]


Curr Opin Microbiol. Author manuscript; available in PMC 2016 December 01.


<table>
<thead>
<tr>
<th>Highlight Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>• <em>C. albicans</em> was long considered to be an obligate asexual species</td>
</tr>
<tr>
<td>• An unusual parasexual cycle has now been uncovered in <em>C. albicans</em></td>
</tr>
<tr>
<td>• Mating competency is dependent on cells undergoing a white-to-opaque switch</td>
</tr>
<tr>
<td>• Both heterothallic and homothallic modes of mating exist</td>
</tr>
<tr>
<td>• In place of meiosis cells undergo concerted chromosome loss</td>
</tr>
</tbody>
</table>
Figure 1. Cell type regulation in *S. cerevisiae* and *C. albicans*

(A) Schematics show transcription factors regulating α-specific genes, α-specific genes, and haploid-specific genes in *S. cerevisiae*. The transcription factors α1, α1, α2, Mcm1, and Ste12 act coordinately to regulate cell type specificity in *S. cerevisiae*.

(B) Rewiring of α-specific gene regulation between *S. cerevisiae* and *C. albicans*. The ancestral mode of regulation involves the α2 transcription factor acting in concert with Mcm1 to regulate α-specific genes. This mode of regulation has been retained in *C. albicans*. In some yeast lineages, expression of the α-specific genes also came under negative control of the α2 transcription factor. In *S. cerevisiae*, the ancestral form of regulation by α2 was lost, whereas in *K. wickerhamii* the hybrid form of regulation (involving both α2 and α2) has been retained.
Figure 2. Mating in *C. albicans*
Outline of the steps during heterothallic a-α mating in *C. albicans*. White cells switch to the mating-competent opaque form, and then undergo pheromone signaling between cells of opposite sexes. Mating projections fuse to form a mating zygote within which karyogamy (nuclear fusion) occurs. The nucleus in the tetraploid mating product undergoes replication, with one tetraploid nucleus entering the budding daughter cell. Continued replication and budding of tetraploid cells occurs. Scanning electron micrograph images of white, opaque and zygote cells are shown (scale bar, 2.5 μm, images courtesy of Matthew Hirakawa).
Figure 3. Genetic rearrangements during the parasexual cycle in *C. albicans*

Diploid (2N) a and α cells switch from the white state to the mating-competent opaque state, and subsequently undergo mating to form tetraploid (4N) a/α cells. Tetraploid cells can continue to divide as stable tetraploids or can be induced to undergo concerted chromosome loss, producing diploid a, α, and a/α cells. Chromosome loss is accompanied by genetic recombination and results in large numbers of aneuploid cells (e.g., 2N+1 and 2N+2 cells) as well as diploid cells with different *MTL* configurations. For clarity, only one of the eight *C. albicans* chromosomes is shown in each cell.