HHS Public Access

Author manuscript

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

Published in final edited form as:

Curr Opin Microbiol. 2015 December ; 28: 10–17. doi:10.1016/j.mib.2015.06.017.

The Parasexual Lifestyle of *Candida albicans*

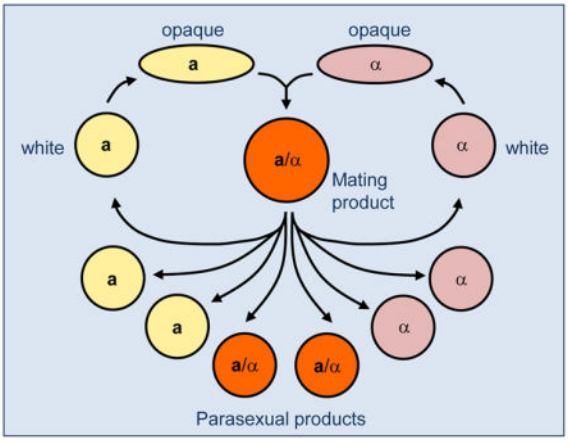
Richard J. Bennett*

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.

Graphical Abstract



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

*corresponding author: Richard_Bennett@brown.edu.

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 *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 *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. *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 *C. albicans* that shows several adaptations to that found in model yeasts.

Regulation of cell type

The sexual cycle in *S. cerevisiae* has served as the primary model for studies into mating and meiosis in yeast. In this species, mating occurs between **a** and α cells and is regulated by genes encoded at a single genetic locus called the mating-type or *MAT* locus. Three transcription factors encoded at the *MAT* locus, **a**1, α1, and α2, determine whether cells behave as **a**, α, or **a**/α cells (Figure 1A). *MAT***a**-containing cells express the **a**1 gene, *MAT*α cells express α1 and α2 genes, and *MAT***a**/α cells express all three genes. In α cells, α1 is responsible for the induction of α-specific genes, whereas in **a** cells, **a**-specific genes are constitutively expressed. However, **a**-specific genes are not expressed in α cells due to repression by α2 together with the Mcm1 transcription factor [7]. *MAT***a** and *MAT*α cells both express “haploid-specific” genes that include pheromone-signaling factors that promote mating [8,9]. Meiosis occurs only in *MAT***a**/α cells, as a complex between **a**1 and α2 turns off haploid-specific genes including *RME1*, which is a repressor of meiosis [7]. Thus, the combinatorial control of cell-type expression by *MAT* transcription factors regulates both mating and meiosis in *S. cerevisiae*.

Mating in *Candida albicans* and rewiring of cell type

C. albicans is a hemiascomycete yeast related to *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 *C. albicans* are diploid, and it was originally thought that this species could not undergo any form of sexual reproduction. However, diploid **a** and α strains of *C. albicans* were engineered from a clinical **a**/α isolate and shown to be capable of mating to form tetraploids, both *in vitro* and in a murine model of systemic infection [11,12]. Subsequent experiments established that *C. albicans* mating shows parallels with that in *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 **a**-specific genes in *S. cerevisiae* α cells via the α2 protein, there is positive regulation of the **a**-specific genes in *C. albicans* via an additional transcription factor, **a**2, that is absent from *S. cerevisiae* (Figure 1A and B)[18]. Analysis of multiple yeast species revealed that the transcriptional circuit in *C. albicans* is the ancestral circuit, whereas that in *S. cerevisiae* is a derived circuit [19]. Strikingly, some hemiascomycete species (e.g. *K.*

wickerhamii) have retained a hybrid form of the circuit in which **a**-specific genes are both positively regulated by **a**2 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 **a** or α cells, but not **a**/α cells, due to repression by the **a**1/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, CO_2, 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 **a**/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 *C. albicans* genome contains many conserved genes that function in meiosis in other species [41,42], including some that are functional when heterologously expressed in *S. cerevisiae* [43]. However, a conventional meiosis has not been described in *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) [44,45]. During parasex, cells undergo limited recombination, which is dependent on the 'meiotic' recombinase, Spo11 [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 [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 [45,48]. A similar parasexual cycle was observed in *C. tropicalis*, implying that meiosis is also absent (or at least cryptic) in this species [49].

Despite the absence of a conventional meiosis in *C. albicans*, the products of the parasexual cycle exhibit diverse genotypes and phenotypes [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 [50–52], it is clear that parasex could generate potentially important traits relevant to fungal pathogenesis.

Recently it was shown that *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 *in vitro* and following infection of an animal host [53]. Haploid cells were formed by a parasexual mechanism of concerted chromosome loss similar to that observed in tetraploid cells. Furthermore, haploid **a** and α cells could switch to the opaque state and undergo mating to regenerate **a**/α diploid cells, or could form *MTL* homozygous diploids by spontaneous auto-diploidization [53]. While viable, all haploid cells showed marked fitness defects that currently limit their use in studying pathogenesis.

Why did *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 [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 **a** 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 **a** strains express both **a** 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 **a** cells. Same-sex **a**-**a** mating also occurs in mixed cultures of wildtype opaque **a** and α cells, in which α pheromone produced by α cells overwhelms Bar1 activity from **a** 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 **a** or α 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 **a** and α 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 *Sc*Ste12) [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 **a**/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 **a**1/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

1. Ene IV, Bennett RJ. The cryptic sexual strategies of human fungal pathogens. Nat Rev Microbiol. 2014; 12:239–251. [PubMed: 24625892]
2. Ni M, Feretzaki M, Sun S, Wang X, Heitman J. Sex in fungi. Annu Rev Genet. 2011; 45:405–430. [PubMed: 21942368]
3. Lee SC, Ni M, Li W, Shertz C, Heitman J. The evolution of sex: a perspective from the fungal kingdom. Microbiol Mol Biol Rev. 2010; 74:298–340. [PubMed: 20508251]
4. Heitman J, Sun S, James TY. Evolution of fungal sexual reproduction. Mycologia. 2013; 105:1–27. [PubMed: 23099518]
5. Heitman J, Carter DA, Dyer PS, Soll DR. Sexual reproduction of human fungal pathogens. Cold Spring Harb Perspect Med. 2014:4.
6. Reedy, JL.; Heitman, J. Evolution of *MAT* in the *Candida* species complex: sex, ploidy, and complete sexual cycles in *C. lusitaniae*, *C. guilliermondii*, and *C. krusei*. In: Heitman, J., editor. Sex in Fungi. ASM Press; 2007. p. 235-245.
7. Johnson AD. Molecular mechanisms of cell-type determination in budding yeast. Curr Opin Genet Dev. 1995; 5:552–558. [PubMed: 8664541]
8. Mitchell AP, Herskowitz I. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature. 1986; 319:738–742. [PubMed: 3513021]
9. van Werven FJ, Neuert G, Hendrick N, Lardenois A, Buratowski S, van Oudenaarden A, Primig M, Amon A. Transcription of two long noncoding RNAs mediates mating-type control of gametogenesis in budding yeast. Cell. 2012; 150:1170–1181. [PubMed: 22959267]
10. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, De Montigny J, Marck C, Neuveglise C, Talla E, et al. Genome evolution in yeasts. Nature. 2004; 430:35–44. [PubMed: 15229592]
11. Hull CM, Raisner RM, Johnson AD. Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. Science. 2000; 289:307–310. [PubMed: 10894780]
12. Magee BB, Magee PT. Induction of mating in *Candida albicans* by construction of *MTLa* and *MTLalpha* strains. Science. 2000; 289:310–313. [PubMed: 10894781]
13. Bennett RJ, Miller MG, Chua PR, Maxon ME, Johnson AD. Nuclear fusion occurs during mating in *Candida albicans* and is dependent on the *KAR3* gene. Mol Microbiol. 2005; 55:1046–1059. [PubMed: 15686553]
14. Bennett RJ, Uhl MA, Miller MG, Johnson AD. Identification and characterization of a *Candida albicans* mating pheromone. Mol Cell Biol. 2003; 23:8189–8201. [PubMed: 14585977]
15. Magee BB, Legrand M, Alarco AM, Raymond M, Magee PT. Many of the genes required for mating in *Saccharomyces cerevisiae* are also required for mating in *Candida albicans*. Mol Microbiol. 2002; 46:1345–1351. [PubMed: 12453220]
16. Lockhart SR, Zhao R, Daniels KJ, Soll DR. Alpha-pheromone-induced "shmooing" and gene regulation require white-opaque switching during *Candida albicans* mating. Eukaryot Cell. 2003; 2:847–855. [PubMed: 14555467]
17. Panwar SL, Legrand M, Dignard D, Whiteway M, Magee PT. *MFalpha1*, the gene encoding the alpha mating pheromone of *Candida albicans*. Eukaryot Cell. 2003; 2:1350–1360. [PubMed: 14665468]
18. Tsong AE, Miller MG, Raisner RM, Johnson AD. Evolution of a combinatorial transcriptional circuit: a case study in yeasts. Cell. 2003; 115:389–399. [PubMed: 14622594]
19. Tsong AE, Tuch BB, Li H, Johnson AD. Evolution of alternative transcriptional circuits with identical logic. Nature. 2006; 443:415–420. [PubMed: 17006507]

**20. Baker CR, Booth LN, Sorrells TR, Johnson AD. Protein modularity, cooperative binding, and hybrid regulatory states underlie transcriptional network diversification. Cell. 2012; 151:80–95. This paper demonstrated how regulatory transcription circuits could contain hybrid states that can be resolved by different routes. [PubMed: 23021217]

21. Slutsky B, Staebell M, Anderson J, Risen L, Pfaller M, Soll DR. "White-opaque transition": a second high-frequency switching system in *Candida albicans*. J Bacteriol. 1987; 169:189–197. [PubMed: 3539914]

**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]

23. Huang G, Wang H, Chou S, Nie X, Chen J, Liu H. Bistable expression of *WOR1*, a master regulator of white-opaque switching in *Candida albicans*. Proc Natl Acad Sci U S A. 2006; 103:12813–12818. [PubMed: 16905649]

24. Srikantha T, Borneman AR, Daniels KJ, Pujol C, Wu W, Seringhaus MR, Gerstein M, Yi S, Snyder M, Soll DR. *TOS9* regulates white-opaque switching in *Candida albicans*. Eukaryot Cell. 2006; 5:1674–1687. [PubMed: 16950924]

25. Zordan RE, Galgoczy DJ, Johnson AD. Epigenetic properties of white-opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop. Proc Natl Acad Sci U S A. 2006; 103:12807–12812. [PubMed: 16899543]

*26. Hernday AD, Lohse MB, Fordyce PM, Nobile CJ, DeRisi JL, Johnson AD. Structure of the transcriptional network controlling white-opaque switching in *Candida albicans*. Mol Microbiol. 2013; 90:22–35. A detailed analysis of the transcriptional networks regulating white-opaque switching. [PubMed: 23855748]

27. Zordan RE, Miller MG, Galgoczy DJ, Tuch BB, Johnson AD. Interlocking transcriptional feedback loops control white-opaque switching in *Candida albicans*. PLoS Biol. 2007; 5:e256. [PubMed: 17880264]

*28. Porman AM, Alby K, Hirakawa MP, Bennett RJ. Discovery of a phenotypic switch regulating sexual mating in the opportunistic fungal pathogen *Candida tropicalis*. Proc Natl Acad Sci U S A. 2011; 108:21158–21163. Demonstration that mating in *C. tropicalis* is regulated by a white-opaque switch similar to that in *C. albicans*. [PubMed: 22158989]

29. Pujol C, Daniels KJ, Lockhart SR, Srikantha T, Radke JB, Geiger J, Soll DR. The closely related species *Candida albicans* and *Candida dubliniensis* can mate. Eukaryot Cell. 2004; 3:1015–1027. [PubMed: 15302834]

30. Xie J, Du H, Guan G, Tong Y, Kourkoumpetis TK, Zhang L, Bai FY, Huang G. N-acetylglucosamine induces white-to-opaque switching and mating in *Candida tropicalis*, providing new insights into adaptation and fungal sexual evolution. Eukaryot Cell. 2012; 11:773–782. [PubMed: 22544905]

31. Rikkerink EH, Magee BB, Magee PT. Opaque-white phenotype transition: a programmed morphological transition in *Candida albicans*. J Bacteriol. 1988; 170:895–899. [PubMed: 2828333]

32. Lachke SA, Lockhart SR, Daniels KJ, Soll DR. Skin facilitates *Candida albicans* mating. Infect Immun. 2003; 71:4970–4976. [PubMed: 12933839]

33. Soll DR. Why does *Candida albicans* switch? FEMS Yeast Res. 2009; 9:973–989. [PubMed: 19744246]

*34. Tao L, Cao C, Liang W, Guan G, Zhang Q, Nobile CJ, Huang G. White cells facilitate opposite- and same-sex mating of opaque cells in *Candida albicans*. PLoS Genet. 2014; 10:e1004737. This work showed how 'sterile' white cells can promote mating between competent opaque cells. [PubMed: 25329547]

35. Dumitru R, Navarathna DH, Semighini CP, Elowsky CG, Dumitru RV, Dignard D, Whiteway M, Atkin AL, Nickerson KW. *In vivo* and *in vitro* anaerobic mating in *Candida albicans*. Eukaryot Cell. 2007; 6:465–472. [PubMed: 17259544]

36. Huang G, Srikantha T, Sahni N, Yi S, Soll DR. CO_2 regulates white-to-opaque switching in *Candida albicans*. Curr Biol. 2009; 19:330–334. [PubMed: 19200725]

37. Huang G, Yi S, Sahni N, Daniels KJ, Srikantha T, Soll DR. N-acetylglucosamine induces white to opaque switching, a mating prerequisite in *Candida albicans*. PLoS Pathog. 2010; 6:e1000806. [PubMed: 20300604]
38. Alby K, Bennett RJ. Stress-induced phenotypic switching in *Candida albicans*. Mol Biol Cell. 2009; 20:3178–3191. [PubMed: 19458191]
39. Xie J, Tao L, Nobile CJ, Tong Y, Guan G, Sun Y, Cao C, Hernday AD, Johnson AD, Zhang L, et al. White-opaque switching in natural *MTL*a/alpha isolates of *Candida albicans*: evolutionary implications for roles in host adaptation, pathogenesis, and sex. PLoS Biol. 2013; 11:e1001525. [PubMed: 23555196]
40. Ramirez-Zavala B, Reuss O, Park YN, Ohlsen K, Morschhauser J. Environmental induction of white-opaque switching in *Candida albicans*. PLoS Pathog. 2008; 4:e1000089. [PubMed: 18551173]
41. Butler G, Rasmussen MD, Lin MF, Santos MA, Sakthikumar S, Munro CA, Rheinbay E, Grabherr M, Forche A, Reedy JL, et al. Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. Nature. 2009; 459:657–662. [PubMed: 19465905]
42. Tzung KW, Williams RM, Scherer S, Federspiel N, Jones T, Hansen N, Bivolarevic V, Huizar L, Komp C, Surzycki R, et al. Genomic evidence for a complete sexual cycle in *Candida albicans*. Proc Natl Acad Sci U S A. 2001; 98:3249–3253. [PubMed: 11248064]
43. Diener AC, Fink GR. *DLH1* is a functional *Candida albicans* homologue of the meiosis-specific gene *DMC1*. Genetics. 1996; 143:769–776. [PubMed: 8725225]
*44. Bennett RJ, Johnson AD. Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. Embo J. 2003; 22:2505–2515. First demonstration that *C. albicans* completes its sexual cycle by concerted chromosome loss. [PubMed: 12743044]
45. Hickman MA, Paulson C, Dudley AM, Berman J. Parasexual ploidy reduction drives population heterogeneity through random and transient aneuploidy in *Candida albicans*. Genetics. 2015
46. Forche A, Alby K, Schaefer D, Johnson AD, Berman J, Bennett RJ. The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. PLoS Biol. 2008; 6:e110. [PubMed: 18462019]
47. Berman J, Hadany L. Does stress induce (para)sex? Implications for *Candida albicans* evolution. Trends Genet. 2012; 28:197–203. [PubMed: 22364928]
48. Bennett RJ, Forche A, Berman J. Rapid mechanisms for generating genome diversity: whole ploidy shifts, aneuploidy, and loss of heterozygosity. Cold Spring Harbor Perspect Med. 2014; 4(10)
49. Seervai RN, Jones SK Jr, Hirakawa MP, Porman AM, Bennett RJ. Parasexuality and ploidy change in *Candida tropicalis*. Eukaryot Cell. 2013; 12:1629–1640. [PubMed: 24123269]
50. Selmecki A, Bergmann S, Berman J. Comparative genome hybridization reveals widespread aneuploidy in *Candida albicans* laboratory strains. Mol Microbiol. 2005; 55:1553–1565. [PubMed: 15720560]
51. Kwon-Chung KJ, Chang YC. Aneuploidy and drug resistance in pathogenic fungi. PLoS Pathog. 2012; 8:e1003022. [PubMed: 23166494]
52. Ni M, Feretzaki M, Li W, Floyd-Averette A, Mieczkowski P, Dietrich FS, Heitman J. Unisexual and heterosexual meiotic reproduction generate aneuploidy and phenotypic diversity de novo in the yeast *Cryptococcus neoformans*. PLoS Biol. 2013; 11:e1001653. [PubMed: 24058295]
53. Hickman MA, Zeng G, Forche A, Hirakawa MP, Abbey D, Harrison BD, Wang YM, Su CH, Bennett RJ, Wang Y, et al. The ‘obligate diploid’ *Candida albicans* forms mating-competent haploids. Nature. 2013; 494:55–59. [PubMed: 23364695]
54. Nielsen K, Heitman J. Sex and virulence of human pathogenic fungi. Adv Genet. 2007; 57:143–173. [PubMed: 17352904]
55. Reedy JL, Floyd AM, Heitman J. Mechanistic plasticity of sexual reproduction and meiosis in the *Candida* pathogenic species complex. Curr Biol. 2009; 19:891–899. [PubMed: 19446455]
56. Sherwood RK, Scaduto CM, Torres SE, Bennett RJ. Convergent evolution of a fused sexual cycle promotes the haploid lifestyle. Nature. 2014; 506:387–390. [PubMed: 24390351]
57. Haber JE. Mating-type genes and *MAT* switching in *Saccharomyces cerevisiae*. Genetics. 2012; 191:33–64. [PubMed: 22555442]

58. Klar AJ. Lessons learned from studies of fission yeast mating-type switching and silencing. Annu Rev Genet. 2007; 41:213–236. [PubMed: 17614787]

**59. Hanson SJ, Byrne KP, Wolfe KH. Mating-type switching by chromosomal inversion in methylotrophic yeasts suggests an origin for the three-locus *Saccharomyces cerevisiae* system. Proc Natl Acad Sci U S A. 2014; 111:E4851–4858. Studies show that a two-locus system regulates mating-type switching in methylotrophic yeasts, and that *Candida* clade yeast probably lost this ability after diverging from other Saccharomycotina species. [PubMed: 25349420]

60. Alby K, Schaefer D, Bennett RJ. Homothallic and heterothallic mating in the opportunistic pathogen *Candida albicans*. Nature. 2009; 460:890–893. [PubMed: 19675652]

61. Alby K, Bennett RJ. Interspecies pheromone signaling promotes biofilm formation and same-sex mating in *Candida albicans*. Proc Natl Acad Sci U S A. 2011; 108:2510–2515. [PubMed: 21262815]

62. Lin X, Hull CM, Heitman J. Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. Nature. 2005; 434:1017–1021. [PubMed: 15846346]

63. Lin X, Litvintseva AP, Nielsen K, Patel S, Floyd A, Mitchell TG, Heitman J. alpha AD alpha hybrids of *Cryptococcus neoformans*: evidence of same-sex mating in nature and hybrid fitness. PLoS Genet. 2007; 3:1975–1990. [PubMed: 17953489]

64. Finkel JS, Mitchell AP. Genetic control of *Candida albicans* biofilm development. Nat Rev Microbiol. 2011; 9:109–118. [PubMed: 21189476]

65. Fox EP, Nobile CJ. A sticky situation: untangling the transcriptional network controlling biofilm development in *Candida albicans*. Transcription. 2012; 3:315–322. [PubMed: 23117819]

66. Baillie GS, Douglas LJ. Role of dimorphism in the development of *Candida albicans* biofilms. J Med Microbiol. 1999; 48:671–679. [PubMed: 10403418]

67. Ramage G, VandeWalle K, Lopez-Ribot JL, Wickes BL. The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in *Candida albicans*. FEMS Microbiol Lett. 2002; 214:95–100. [PubMed: 12204378]

*68. Daniels KJ, Srikantha T, Lockhart SR, Pujol C, Soll DR. Opaque cells signal white cells to form biofilms in *Candida albicans*. Embo J. 2006; 25:2240–2252. First demonstration that white cells of *C. albicans* respond to pheromone resulting in adherence and biofilm formation. [PubMed: 16628217]

69. Park YN, Daniels KJ, Pujol C, Srikantha T, Soll DR. *Candida albicans* forms a specialized “sexual” as well as “pathogenic” biofilm. Eukaryot Cell. 2013; 12:1120–1131. [PubMed: 23771904]

70. Daniels KJ, Park YN, Srikantha T, Pujol C, Soll DR. Impact of environmental conditions on the form and function of *Candida albicans* biofilms. Eukaryot Cell. 2013; 12:1389–1402. [PubMed: 23954841]

71. Lin CH, Kabrawala S, Fox EP, Nobile CJ, Johnson AD, Bennett RJ. Genetic control of conventional and pheromone-stimulated biofilm formation in *Candida albicans*. PLoS Pathog. 2013; 9:e1003305. [PubMed: 23637598]

72. Sahni N, Yi S, Daniels KJ, Huang G, Srikantha T, Soll DR. Tec1 mediates the pheromone response of the white phenotype of *Candida albicans*: insights into the evolution of new signal transduction pathways. PLoS Biol. 2010; 8:e1000363. [PubMed: 20454615]

73. Ramirez-Zavala B, Weyler M, Gildor T, Schmauch C, Kornitzer D, Arkowitz R, Morschhauser J. Activation of the Cph1-dependent MAP kinase signaling pathway induces white-opaque switching in *Candida albicans*. PLoS Pathog. 2013; 9:e1003696. [PubMed: 24130492]

74. Lockhart SR, Pujol C, Daniels KJ, Miller MG, Johnson AD, Pfaller MA, Soll DR. In *Candida albicans*, white-opaque switchers are homozygous for mating type. Genetics. 2002; 162:737–745. [PubMed: 12399384]

75. Odds FC, Bougnoux ME, Shaw DJ, Bain JM, Davidson AD, Diogo D, Jacobsen MD, Lecomte M, Li SY, Tavanti A, et al. Molecular phylogenetics of *Candida albicans*. Eukaryot Cell. 2007; 6:1041–1052. [PubMed: 17416899]

76. Booth LN, Tuch BB, Johnson AD. Intercalation of a new tier of transcription regulation into an ancient circuit. Nature. 2010; 468:959–963. [PubMed: 21164485]

77. Bougnoux ME, Pujol C, Diogo D, Bouchier C, Soll DR, d'Enfert C. Mating is rare within as well as between clades of the human pathogen *Candida albicans*. Fungal Genet Biol. 2008; 45:221–231. [PubMed: 18063395]

Highlight Points

- *C. albicans* was long considered to be an obligate asexual species
- An unusual parasexual cycle has now been uncovered in *C. albicans*
- Mating competency is dependent on cells undergoing a white-to-opaque switch
- Both heterothallic and homothallic modes of mating exist
- In place of meiosis cells undergo concerted chromosome loss

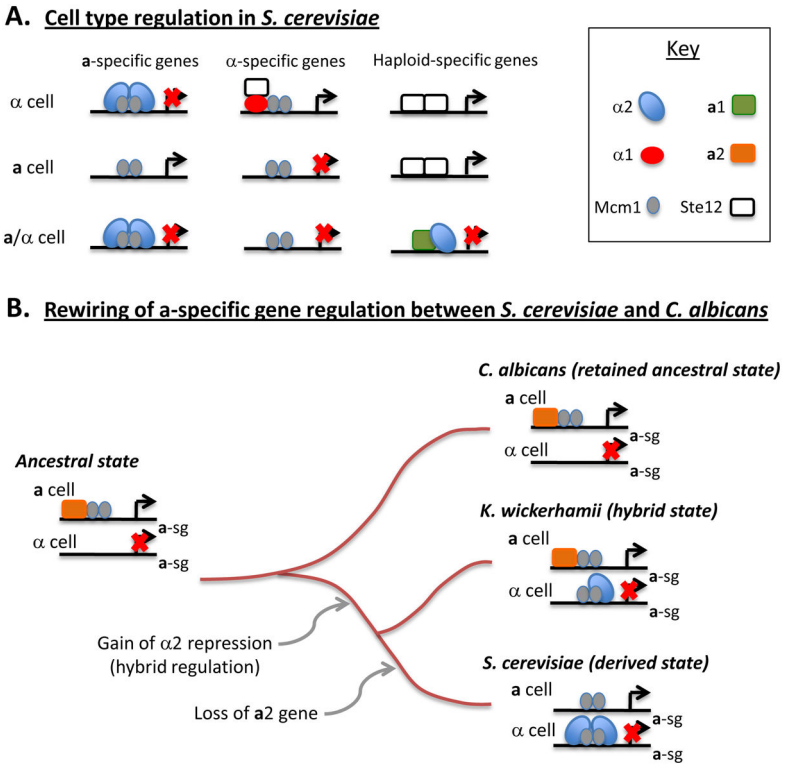


Figure 1. Cell type regulation in *S. cerevisiae* and *C. albicans*

(A) Schematics show transcription factors regulating **a**-specific genes, α-specific genes, and haploid-specific genes in *S. cerevisiae*. The transcription factors **a**1, α1, α2, Mcm1, and Ste12 act coordinately to regulate cell type specificity in *S. cerevisiae*.

(B) Rewiring of the regulation of **a**-specific genes (**a**-sg) between hemiascomycetes. The ancestral mode of regulation involves the **a**2 transcription factor acting in concert with Mcm1 to regulate **a**-specific genes. This mode of regulation has been retained in *C. albicans*. In some yeast lineages, expression of the **a**-specific genes also came under negative control of the α2 transcription factor. In *S. cerevisiae*, the ancestral form of regulation by **a**2 was lost, whereas in *K. wickerhamii* the hybrid form of regulation (involving both **a**2 and α2) has been retained.

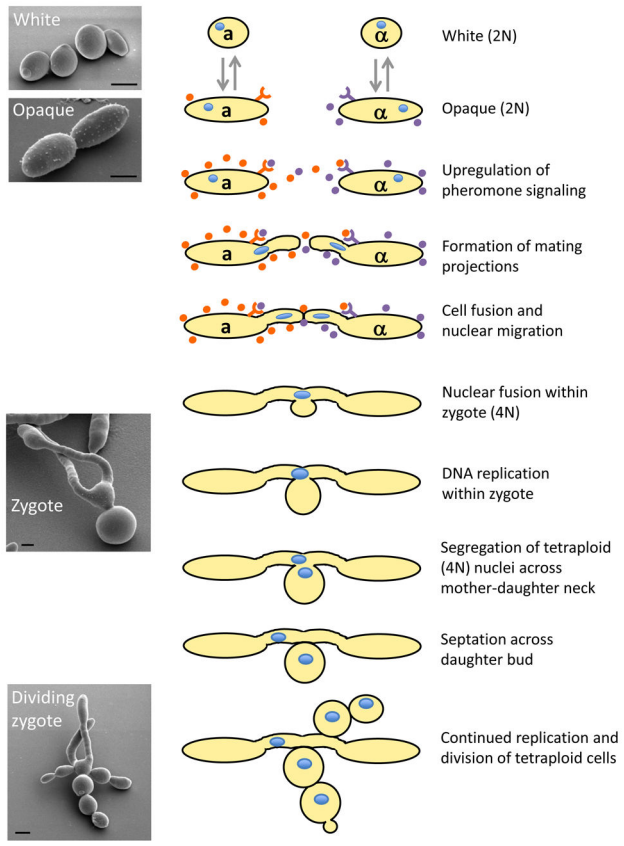


Figure 2. Mating in *C. albicans*

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).

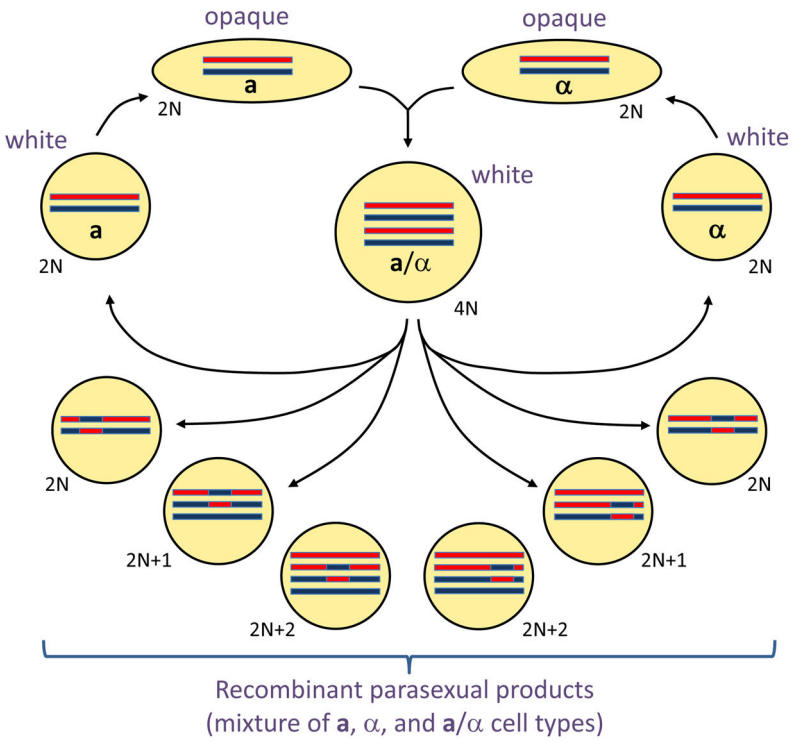


Figure 3. Genetic rearrangements during the parasexual cycle in *C. albicans*

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.