EUKARYOTIC CELL, June 2003, p. 390–397 Vol. 2, No. 3
1535-9778/03/$08.00+0 DOI: 10.1128/EC.2.3.390–397.2003

Relationship between Switching and Mating in *Candida albicans*

David R. Soll,* Shawn R. Lockhart, and Rui Zhao

Department of Biological Sciences, The University of Iowa, Iowa City, Iowa 52242

Candida albicans represents the most pervasive fungal pathogen colonizing humans (5, 36). Its success stems in part from its capacity to live as a benign commensal in a majority of healthy individuals in one or more of a variety of body locations, most notably the oral cavity, genitalia, and gastrointestinal tract. As an opportunistic pathogen, it lies in wait for a change in some aspect of the host physiology or microflora that normally suppresses growth and invasiveness. In response to this change, it increases in number and invades tissue. Its versatility as a pathogen is reflected in the variety of tissues it can colonize, the variety of disease states it is responsible for, and the ever increasing number of individuals, usually immunocompromised, who die from bloodstream and disseminated infections.

Until the mid-1980s, it was believed that even though its success reflected phenotypic plasticity, the only developmental program *C. albicans* possessed for generating multiple phenotypes was the bud-hypha transition. Although a variety of different observations supported the conclusion that the hyphal phenotype facilitated tissue invasion (13, 28, 43), there was still the nagging feeling that the bud-hypha transition was not complex enough to account for the remarkable versatility of this pathogen. Therefore, it should have come as no great surprise when Slutsky et al. (41) and Pomes et al. (37) reported in 1985 that different strains of *C. albicans* switched reversibly and at high frequency between a limited number of different colony morphologies. In the case of the common laboratory strain 3153A, switching occurred among at least seven colony phenotypes, each dictated by differences in the temporal dynamics and spatial distributions of budding cells, pseudohyphae, and hyphae in the colony dome (41, 44). As more strains of *C. albicans* were analyzed for switching, it became apparent that the majority switched reversibly and at high frequency between a variety of phenotypes but that the repertoires of switch phenotypes were different in different strains. Several reports also demonstrated reversible, high-frequency switching in *Candida tropicalis* (46), *Candida parapsilosis* (9), *Candida glabrata* (24, 26), and even *Cryptococcus neoformans* (10, 11).

One switching system in *C. albicans* consisted of a reversible transition between two phases: a white hemispherical colony morphology, referred to as the "white phase," and a grey flat colony morphology, referred to as the "opaque phase" (42). This switching system, referred to as "the white-opaque transition," was initially identified in strain WO-1, isolated in 1986 from the bloodstream of a bone marrow transplant patient at The University of Iowa Hospitals and Clinics (42). The patient died from this infection. Strains exhibiting a similar phase transition were identified in 1987 in a survey of switching in roughly 125 independent *C. albicans* isolates (D. R. Soll, unpublished data). It was estimated at that time that approximately 7% of *C. albicans* strains underwent the white-opaque transition. Because the white-opaque transition could be discriminated on a variety of agars, involved only two alternative phenotypes, and had dramatic pleiotropic effects on cellular phenotype (2, 42), it was selected as an experimental model for delving into the molecular mechanisms regulating switching in *C. albicans* and related species. However, in considering the basic biology of the white-opaque transition, there was always the reservation that it occurred in only a minority of natural strains and that it differed mechanistically from the more common switching systems characterized in strain 3153A. There was also the more extreme reservation by some that it simply represented a unique, unrepresentative switching system resulting from a chromosomal rearrangement that had been identified in strain WO-1 (6, 7). As we shall see, the reservation that white-opaque switching was restricted to one or a few strains proved to be unfounded.

Cellular and molecular biology of white-opaque switching. Conventional microscopy immediately revealed that opaque-phase cells were morphologically unique (1, 2, 40, 42). While white-phase cells of strain WO-1 were round to ovoid and budded like most other strains of *C. albicans*, opaque-phase cells were twice as large as white-phase cells, asymmetric, elongated, and bean shaped. Transmission electron microscopy revealed that opaque-phase cells contained a large vacuole that in turn contained vesicles, and unique cell wall pimples with channels traversing them (1, 2). An opaque-phase-specific 14.5-kDa antigen was demonstrated to be associated with these pimples (1).

Since it was obvious from early experiments on cellular phenotype that the white-opaque transition had to involve differential gene expression, strategies to identify phase-specific genes were developed in the early 1990s. Differential hybridization screens of opaque-phase cDNA libraries identified the first phase-specific gene, *PEP1* (*SAP1*), which was verified by Northern blot analysis to be expressed exclusively in the opaque phase (34). This was followed by the identification of a number of additional opaque-phase-specific genes (*OP4*, *SAP3*, *CDR3*, *EFG1*-2.2 kb, and *HOS3*-2.3 kb) (3, 14, 35, 50, 52, 53) and white-phase-specific genes (*WH11*, *EFG1*-3.2 kb, and *HOS3*-2.5 kb) (48, 50, 51, 52). The list of phase-specific genes continued to grow through the 1990s and has recently been greatly expanded by use of expression array technology (27).

Functional analyses of the promoters of phase-regulated genes supported the idea that switching involved complex regulatory mechanisms. For instance, the promoters of the white-phase-specific genes *WH11* and *EFG1*-3.2 kb were demon-

* Corresponding author. Mailing address: Department of Biological Sciences, The University of Iowa, Iowa City, IA 52242. Phone: (319) 335-1117. Fax: (319) 335-2772. E-mail: david-soll@uiowa.edu.

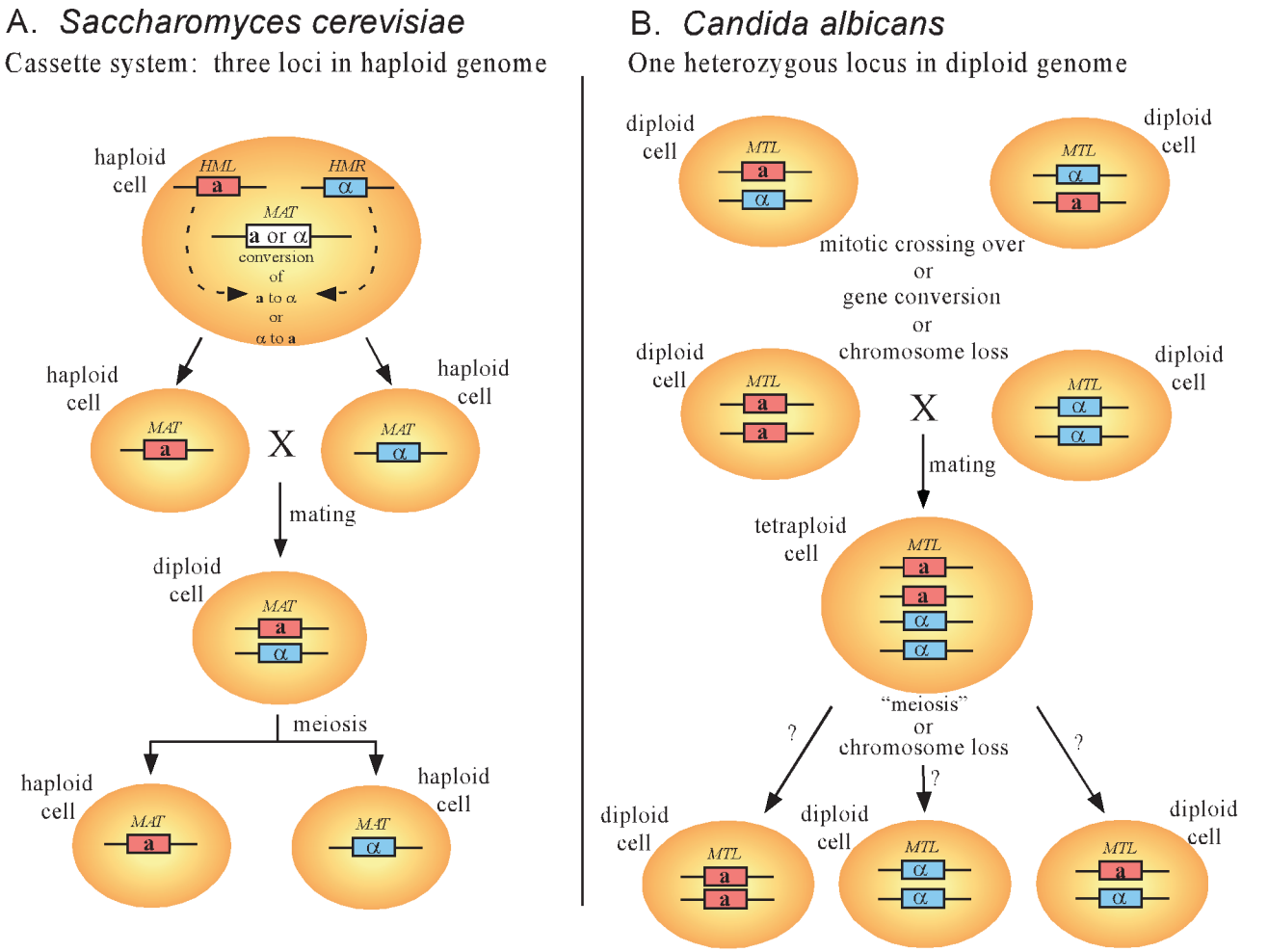


FIG. 1. The configuration of mating type loci and the mechanisms for generating cells of the opposite mating type are different for *S. cerevisiae* and *C. albicans*. Note that while *S. cerevisiae* contains a cassette system that includes two silent loci and one expressed locus, *C. albicans* is normally heterozygous for mating type at one locus. While *S. cerevisiae* changes mating type at the expression locus with no loss of the alternate mating type information, *C. albicans* loses the information of one of the two mating types when it expresses a mating type. *HML*, homothallic mating locus left; *HMR*, homothetic mating locus right; *MAT*, mating type locus; *MTL*, mating type-like locus.

strated to contain unrelated white-phase activation sequences, indicating that the regulation of these coordinately expressed genes involved *trans*-acting factors containing different DNA-binding proteins (25, 49, 50). In the case of opaque-phase-specific gene promoters, while some contained a MADS box binding site sequence at their activation sites (e.g., *OP4* and *CDR3*), others did not (e.g., *SAP1*) (31; S. Lockhart and D. R. Soll, unpublished data). Hence, just as in the case of white-phase-specific gene expression, opaque-phase-specific gene expression involves multiple *trans*-acting factors containing different DNA-binding proteins. It was, therefore, enigmatic that so complex a switching system involving genes present in all strains of *C. albicans* was expressed in only a small minority of strains.

Discovery of *MTL* loci in *C. albicans*. The idea that switching in *C. albicans* may have something to do with mating was frequently entertained, in part because switching represented two heritable but metastable states (44). Indeed, the mating type cassette system in *Saccharomyces cerevisiae* was frequently considered a candidate mechanism for switching. However, the idea was just as frequently dismissed because of the absence of mating type-like (*MTL*) genes. Things changed, however, when Hull and Johnson (15) reported the discovery of *MTL***a** and *MTL*α alleles on homologous chromosomes at the same locus in the common laboratory strain of *C. albicans*, SC5314.

In *S. cerevisiae*, there are three loci containing mating type genes (Fig. 1A) (12). Two of these loci are silent (*HML* and *HMR*). One contains the *MAT***a** genes (*MAT***a***1* and *MAT***a***2*), and the other contains the *MAT*α genes (*MAT*α*1* and *MAT*α*2*). The third locus (*MAT*) contains either the *MAT***a** or the *MAT*α genes and is expressed. The mating type of a haploid cell is defined by the genotype (**a** or α) of the *MAT* locus. In *S. cerevisiae*, the *MAT* locus switches reversibly from **a** to α or from α to **a** by site-specific recombination with a copy of the silent *HML* or *HMR* gene (Fig. 1A). Mating occurs only between an **a** and an α cell and results in the diploid heterozygote **a**/α. In *MAT*-heterozygous diploids, expression of both *MAT***a***1* and *MAT*α*2* in the same cell suppresses mating and facilitates

meiosis, under proper conditions, through the binding of a Mat**a**1p-Matα2p repressor complex (19).

Hull and Johnson (15) demonstrated that in contrast to *S. cerevisiae*, *C. albicans* strain SC5314, which is diploid, possesses only one *MTL* locus on chromosome 5. The locus is heterozygous, containing on one homolog the *MTL***a** gene *MTL***a***1*, which is homologous to *S. cerevisiae MAT***a***1*, and on the other homolog *MTL*α*1* and *MTL*α*2*, which are homologous to *S. cerevisiae MAT*α*1* and *MAT*α*2* (Fig. 1B). Both the *MTL***a** and *MTL*α loci contain three additional genes not found in the *MAT***a** and *MAT*α loci of *S. cerevisiae*. The *MTL***a** locus of *C. albicans* is missing a homolog of the *S. cerevisiae* gene *MAT***a***2*. Hence, *C. albicans* does not possess an *S. cerevisiae*-like cassette system for mating type switching. Rather, it carries opposing *MTL***a** and *MTL*α alleles at the same locus on the chromosome 5 homologs. This discovery implied that to become a functional homozygote, *C. albicans* would have to become genetically *MTL* homozygous (Fig. 1B).

First indications of mating type-dependent fusion. Hypothesizing that mating may take place between homozygous *MTL***a** and homozygous *MTL*α cells in nature, as in the case of *S. cerevisiae*, Hull et al. (17) generated hemizygous *MTL***a** (**a**/−) and hemizygous *MTL*α (α/−) strains from the laboratory strain SC5314 and infected mice with mixtures of the two. Alternative adenine and uridine auxotrophy was introduced into the two strains, so that the *MTL***a** and *MTL*α strains were *ade2 URA3* or *ADE2 ura3*, respectively. Fusants could hence be selected through complementation by plating mixed populations on a medium lacking adenine and uridine. The same in vivo experiment was performed with *MTL* heterozygotes alone, which were not expected to fuse, if their hypotheses were correct. Yeast cells from the kidneys of infected mice were then plated on selection medium and rich medium. While 44 of 10^3 total CFU retrieved from the kidneys of mice infected with mixtures of **a** and α cells proved to be *ADE2 URA3* (4.4×10^{-2}), none of 10^3 total CFU retrieved from mice infected with mixtures of *ade2 URA3* **a**/α cells and *ADE2 ura3* **a**/α cells proved to be *ADE2 URA3* ($<10^{-3}$). Hence, mixtures of homozygous **a** and homozygous α cells fused, but mixtures of heterozygous **a**/α cells did not, or did so at undetectable rates. Selected *ADE2 URA3* cells contained more DNA than parental diploids and also contained both *MTL***a** and *MTL*α genes, demonstrating fusion. Images of 4′,6′-diamidino-2-phenylindole (DAPI)-stained cells presented in a website supplement to the report of Hull et al. (17) in *Science* (www.sciencemag.org/future/data/1049869.html) further suggested that the fusants were mononuclear. These data were consistent with a mating system in which homozygous **a**/**a** and homozygous α/α strains fused to form tetraploids (Fig. 1B). However, meiosis and recombination were not demonstrated.

In a report published in parallel with that of Hull et al. (17), Magee and Magee (32) also presented evidence that fusion between hemizygous *MTL***a** and *MTL*α strains occurred in vitro. Taking advantage of the observation by Janbon et al. (18) that growth on sorbose leads to loss of one of the two chromosome 5 homologs that harbor the *MTL* loci, they generated hemizygous *MTL***a** and *MTL*α strains from auxotrophic strains and performed crosses on selective agar. Fusants were identified by complementation. Prototrophs arose in **a** × α crosses but not in **a**/α × **a**/α crosses. Again, it was reported that the fusants contained approximately twice the DNA content of either parent strain, but no evidence was presented in that study that cells were mononucleate, and again, neither meiosis nor recombination was demonstrated. Both the in vivo and in vitro demonstrations of mating type-based fusion indicated for the first time that, as in *S. cerevisiae*, heterozygosity at the mating type locus in *C. albicans* suppressed mating and homozygosity facilitated it. In both the in vivo and in vitro studies, fusion occurred between auxotrophic hemizygotes, was selected for, and appeared to be an infrequent event. In neither study was it demonstrated how resulting tetraploid cells returned to the diploid state. The obvious alternatives were meiosis and random chromosome loss.

Switching and mating: discovery of a dependent relationship. In examining the colony morphologies of the hemizygous **a** and α strains, Miller and Johnson (33) made the remarkable discovery of opaque-phase sectors. This discovery was indeed unexpected, since the parental *MTL*-heterozygous strain SC5314, which they used to generate the alternative hemizygotes, had previously been demonstrated to undergo 3153A-like switching, not the white-opaque transition (51). Miller and Johnson (33) demonstrated that cells of both the hemizygous **a** and α derivatives of SC5314 switched between white and opaque colony-forming phenotypes and that cells from the white and opaque phases shared the morphological features of white- and opaque-phase cells, respectively, of strain WO-1. In addition, they demonstrated that the four WO-1 phase-specific genes *EFG1*, *WH11*, *OP4*, and *SAP1* were similarly phase regulated during white-opaque switching in hemizygous **a** and α strains. These observations led Miller and Johnson (33) to conclude that in heterozygotes, switching, like mating, was suppressed. The ramifications of the observations of Miller and Johnson (33) were obvious. Since it appeared that a majority of naturally occurring *C. albicans* strains were **a**/α, it was quite possible that the white-opaque transition was suppressed in the majority of *C. albicans* strains and that any *C. albicans* strain could undergo the white-opaque transition if it became homozygous at the *MTL* locus.

Even more surprising and exciting was Miller and Johnson's (33) discovery that mating between opaque-phase hemizygous **a** and α cells was many orders of magnitude more efficient than mating between white-phase hemizygous **a** and α cells. The frequency of mating between opaque-phase **a** and opaque-phase α cells was 10^5 to 10^6 times higher than that between white-phase **a** and white-phase α cells and 10^3 to 10^4 times higher than that between white-phase and opaque-phase cells of opposite mating types. These results suggested that the opaque-phase phenotype represented a mating-competent form of *C. albicans*.

To test the generality of the relationship between *MTL* homozygosity and white-opaque switching among natural homozygous strains, Lockhart et al. (29) identified six new white-opaque switchers among a large collection of clinical isolates and tested them as well as strain WO-1 (a total of seven white-opaque switchers) for allelism at the *MTL* locus. They found that all seven white-opaque-switching strains were homozygous at the *MTL* locus; three were **a**/**a** and four were α/α. They next assessed the *MTL* zygosity of 220 clinical *C. albicans* isolates. These isolates were distributed among four of the five major *C. albicans* clades, which were recently identified in

cluster analyses with the complex DNA fingerprinting probe Ca3 (4, 38, 39). Of this broad collection, 213 (97%) were *MTL* heterozygous and 7 (3%) were *MTL* homozygous. Of the seven homozygous strains, five switched unambiguously between the white and opaque phases, while the remaining two switched with associated color changes but without the distinct cellular phenotype of the opaque phase. These results indicated that the great majority of homozygous strains in nature, if not all, undergo the white-opaque transition. Of 20 *MTL*-heterozygous strains randomly selected from the general collection, 18 did not undergo the white-opaque transition, while 2 did. However, further examination revealed that the latter two strains spontaneously generated *MTL*-homozygous progeny at a high frequency, and these homozygous offspring in turn underwent the white-opaque transition. The results of Lockhart et al. (29), therefore, generalized the finding of Miller and Johnson (33) that strains heterozygous at the *MTL* locus do not undergo white-opaque switching while the great majority of strains homozygous at the *MTL* locus do.

To test the generality of the relationship between switching and mating in natural homozygous strains, Lockhart et al. (30) performed crosses between natural *MTL*α- and *MTL***a**-homozygous strains expressing the white or opaque phenotype. In this analysis, they directly assessed fusion at the cellular level without selection (see the description of the cell biology of mating in the next section). They demonstrated that in 12 crosses between different opaque *MTL***a** homozygous strains and opaque *MTL*α-homozygous strains, fusion occurred. No fusions were observed in three crosses between different opaque *MTL*α-homozygous strains, in five crosses between different opaque *MTL***a**-homozygous strains, or in four crosses between different *MTL***a**-homozygous and different *MTL*α-homozygous strains when one strain was white and the other was opaque. By vitally staining opaque homozygous *MTL***a** and opaque homozygous *MTL*α cells with fluorescein isothiocyanate-concanavalin A (green) and rhodamine-concanavalin A (red), respectively, Lockhart et al. (30) also demonstrated that fusions included exclusively one red and one green cell, never a green-green or red-red combination. These results confirmed the generality of the observations by Miller and Johnson (33) on engineered hemizygous **a** and α strains from a single strain. The combined observations demonstrate that *MTL* homozygosity alone does not confer the ability to mate on *C. albicans*, as it does on *S. cerevisiae* (Fig. 2A). Rather, in *C. albicans* both homozygous *MTL***a** and homozygous *MTL*α cells must first switch to opaque in order to mate (Fig. 2B). These results indicate that the low levels of fusion observed by Hull et al. (17) in crosses between white cells of opposite mating types were not the result of white-white fusion but rather resulted from fusions between opaque-phase cells generated at low levels in each white-phase population through switching.

Visualizing the mating process. Mating in *S. cerevisiae* involves a carefully orchestrated developmental program that includes a specific sequence of morphological steps (8). In response to a pheromone released from the opposite mating type, a haploid *S. cerevisiae* cell bulges to form a "shmoo" shape and then extends a wide, unconstricted projection ("conjugation tube"). When tubes from opposite mating types meet, they fuse at their apices. Parent cell nuclei, which are arrested in G_1, migrate to the ends of the tubes, and after tube fusion, they associate and fuse, forming a diploid nucleus. A daughter cell then forms from the conjugation bridge of the zygote. The nucleus, positioned at the junction of bridge and daughter cell, divides, and one of the two diploid nuclei enters the daughter cell. The daughter cell then divides as a diploid, returning to a haploid state when transferred to starvation medium through the process of sporulation, which includes meiosis.

Employing continuous videomicroscopy, computer-assisted three-dimensional reconstruction of living cells, and fluorescence microscopy, Lockhart et al. (30) have demonstrated that the morphological stages in the *C. albicans* mating process parallel those in the *S. cerevisiae* mating process. In mixed **a**/**a** and α/α cultures, cells form unconstricted projections (conjugation tubes) that continue to elongate (Fig. 3). If tubes of the opposite mating types do not undergo fusion, they continue to grow, reaching lengths up to the equivalent of 5 cell diameters. These conjugation tubes do not contain septa, and in the absence of fusion, they form buds at their apices, in essence reverting to the budding growth form. In the process of reversion, the nucleus then migrates from the mother cell to the junction of conjugation tube and daughter bud, where it divides, and the daughter cell and mother cell each receive a nucleus (30). The tube ends up nucleus free, and a septum forms only at the tube–daughter cell junction.

If the conjugation tubes of an **a**/**a** cell and an α/α cell attract each other through chemotropism, the tubes extend until their apices meet and then fuse (Fig. 3). The nuclei of the **a** and α cells migrate to the tube apices. Upon tube fusion (formation of a "zygote"), the nuclei end up physically associated but do not fuse (Fig. 3) under the conditions employed by Lockhart et al. (30). In each zygote, the conjugation bridge swells as a vacuole forms in it. As the vacuole enlarges, it separates the two nuclei, which return to the tube–mother cell junctions and undergo asynchronous nuclear division (Fig. 3). A daughter cell then forms from the conjugation bridge and receives one or two nuclei. The daughter cell nuclei can originate from either or both mother cells. A septum then forms exclusively at the conjugation bridge–daughter cell junction and provides a landmark for identifying the daughter cell in fixed preparations (30). The daughter cell, tube, and mother cell then form secondary buds (Fig. 3). The sequence of morphological stages and fusion events observed by Lockhart et al. (30) was not rare. Indeed, a majority of cells in mixed cultures of homozygous *MTL***a** and homozygous opaque *MTL*α cultures shmooed and formed conjugation tubes, and a significant minority (10%) underwent fusion. Except for the lack of nuclear fusion, the stages of cellular fusion and zygote development clearly resembled mating in *S. cerevisiae*.

So far, no demonstrated meiosis. Both Hull et al. (17) and Magee and Magee (32) demonstrated mixing of markers upon mating type-based fusion of **a** and α cells. They also demonstrated associated increases in cellular DNA content. As noted, Hull et al. (17) also provided evidence in a website supplement to their publication that fusion progeny were mononucleate. These observations indicated that karyogamy had occurred. Lockhart et al. (30), however, did not observe nuclear fusion (karyogamy) in the conjugation bridges of zygotes. Their nuclear staining experiments suggested that after cellular fusion, the nuclei from the original **a**/**a** and α/α cells did not undergo karyogamy. Rather, the nuclei translocated back to the tube-

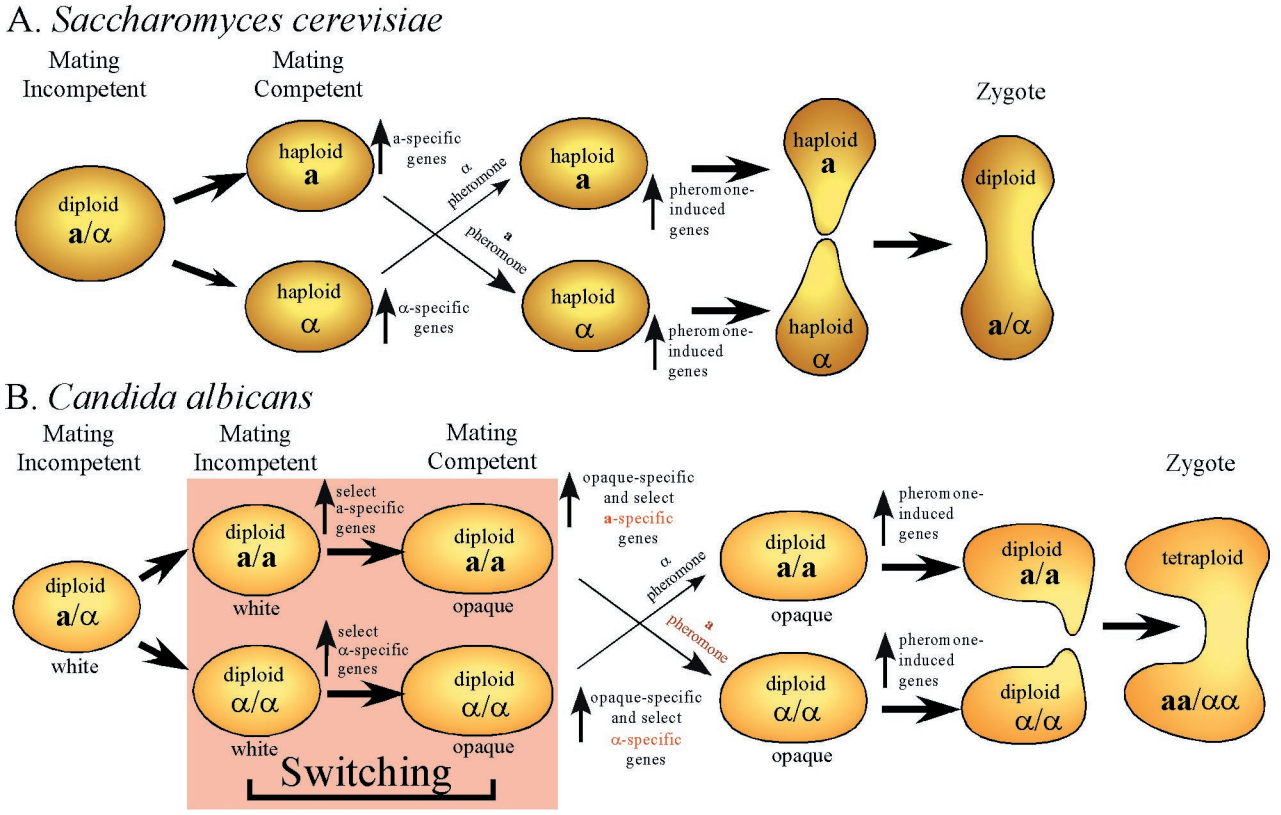
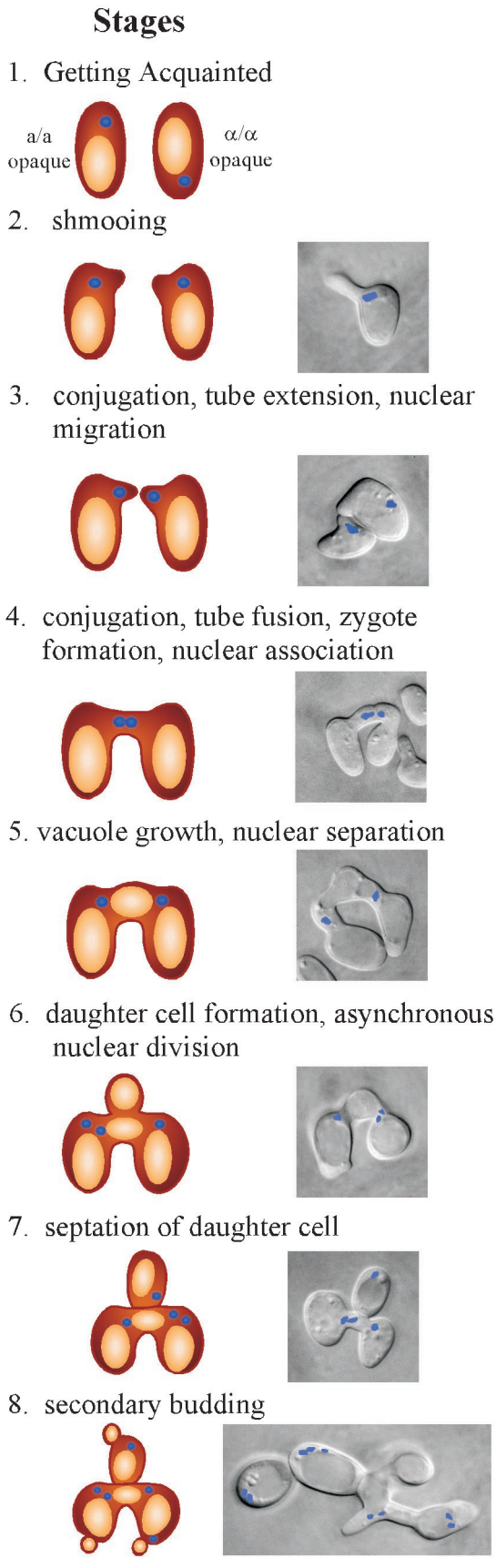


FIG. 2. *C. albicans* has inserted an extra developmental step, the switch from white to opaque, into the mating process. In *S. cerevisiae*, **a** and α cells are immediately mating competent, and all **a**-specific and α-specific genes are upregulated. In *C. albicans*, a homozygous **a** or α cell is not mating competent unless it switches to the opaque phenotype. In *C. albicans*, upregulation of **a**-specific and α-specific gene expression is divided between the transition to a homozygous state and the transition from white to opaque.

cell junctions through expansion of the conjugation bridge vacuole. Lockhart et al. (30) also failed to find mixing or segregation of mating type and other genetic markers, which would have been proof of karyogamy. The apparent difference between the results of Lockhart et al. (30) and those of Hull et al. (17), Magee and Magee (32), and Miller and Johnson (33) may be due to the absence of selection, the high frequency of observed fusions, and possibly the natural origin of true homozygous rather than hemizygous strains in the experiments performed by Lockhart et al. (30). While the studies by Johnson and colleagues (17, 33) and by Magee and Magee (32) involved crosses between *MTL*-hemizygous auxotrophs and the selection of rare prototrophs, the studies by Lockhart et al. (30) involved natural *MTL*-homozygous strains and the microscopic identification of fusions. The proportion of cells observed by Lockhart et al. (30) that underwent fusion was far higher than the proportions observed by Hull et al. (17), Magee and Magee (32), and Miller and Johnson (33), leaving open the likely possibility that karyogamy may have occurred in a minority of fusants in the Lockhart study but may have been missed both cytologically and in random genetic analysis of only a limited number of daughter cells. Lockhart et al. (30) did find that a minority of daughter cells formed by zygotes were binucleate and gave rise to binucleate **a**/α as well as mononucleate **a**/**a** and α/α cells. However, there was no mixing or segregation of markers on different chromosomes in their limited analysis, suggesting that binucleate daughter cells did not readily undergo karyogamy. In more-recent experiments using different incubation conditions, Lockhart and colleagues (S. Lockhart, R. Zhao, K. Daniels, and D. R. Soll, unpublished data) found evidence for rare but relatively stable tetraploid fusants.

Gene regulation during mating. While *C. albicans* generates homozygous **a**/**a** and homozygous α/α cells by a mechanism different from the cassette replacement mechanisms of *S. cerevisiae* (Fig. 1), the *MTL* genes are homologous to *MAT* genes of the *S. cerevisiae* mating system (15). Because of this homology, one can hypothesize the regulatory roles of the *MTL* genes from those of their *MAT* homologs. In **a**/α diploid cells of *S. cerevisiae*, the gene products of *MAT***a** and *MAT*α are both expressed (19). Mat**a**1p and Matα2p form a heterodimer which represses the transcription of haploid-specific genes, including *HO*, which encodes the nuclease for mating type switching; *RME1*, a repressor of early meiosis; and several genes encoding components of the mating-specific mitogen-activated protein kinase pathway. In **a**/α diploid cells, **a**-specific genes are repressed by the Matα2p–Mcm1p repressor complex (20, 21). *MAT*α*1*, which encodes an activator of α-specific genes, is also repressed by the Mat**a**1p–Matα2p repressor complex. Hence, no **a**-specific or α-specific genes are expressed in a diploid **a**/α cell. In haploid *MAT***a** cells, **a**-specific genes are expressed due to the absence of the Matα2p–Mcm1p repressor complex, and



α-specific genes are not expressed due to the absence of the Matα1p–Mcm1p activator complex. In haploid *MAT*α cells, α-specific genes are expressed through activation by the Matα1p–Mcm1p complex, and **a**-specific genes are repressed by the Matα2p–Mcm1p repressor complex. When **a** or α haploid *S. cerevisiae* cells are exposed to the pheromone of the opposite mating type, the mating-specific mitogen-activated protein kinase pathway is activated through the respective pheromone receptors, resulting in the activation of a number of additional mating-type-specific genes, including those involved in shmooing and fusion (47).

Therefore, in *S. cerevisiae*, **a** cells express **a**-specific genes and α cells express α-specific genes (Fig. 2A). In *S. cerevisiae*, **a** and α cells are immediately mating competent. When treated with pheromones, both **a** and α cells express additional genes involved in fusion and zygote development (Fig. 2A). In *C. albicans*, *MTL* **a**/α heterozygotes are unable to switch, suggesting that an Mtl**a**1p–Mtlα2p complex, homologous to the Mat**a**1p–Mat1α2p complex, may be involved in the suppression of switching. Because *C. albicans* has added an additional step to the acquisition of mating, the possibility arises that some genes turned on in **a** and α cells of *S. cerevisiae* (Fig. 2A) may not be turned on in white **a**/**a** or in white α/α cells of *C. albicans*. Instead, these genes may be turned on after the transition from white to opaque (Fig. 2B) and hence may be regulated by an opaque-specific rather than a homozygous-specific mechanism. Indeed, Zhao and colleagues (R. Zhao, W. Wu, S. Lockhart, and D. R. Soll, unpublished data) recently demonstrated that although *MTLα1* is expressed in both homozygous α/α white-phase and homozygous α/α opaque-phase cells, the α pheromone gene *MF*α is expressed in homozygous α/α opaque-phase but not in homozygous α/α white-phase cells. Neither is expressed in heterozygous **a**/**a** cells. Hence, *MTLα1* must be activated when a white **a**/α cell becomes a white α/α cell, but *MF*α is not activated. *MF*α is activated upon a subsequent switch to the opaque phase. Therefore, it is likely that genes coordinately activated when *S. cerevisiae* becomes haploid will separate into two groups in *C. albicans*: those similarly activated when white cells become homozygous, and those activated by the transition to the opaque phase (Fig. 2B).

The functional reason for the insertion of an additional complex developmental program (Fig. 2B) into the mating process of *C. albicans* is not immediately obvious, given that it is not an apparent component of mating in other fungi (16). Presumably, there is an advantage specific for *C. albicans* that may relate to its commensalism and pathogenesis. The unique phenotypic characteristics of opaque-phase cells may shed light on this enigma and are therefore worth reviewing. First, opaque-phase cells form a cell wall that is morphologically distinct from that of white-phase cells. The opaque-phase cell wall possesses unique pimples with channels from the plasma membrane to the wall surface (1, 2). Until now, there were no good suggestions for the role pimples played in the basic biol-

FIG. 3. Cell biology of mating in *C. albicans*. Models are drawn of the stages in the left-hand vertical column, and combined-phase images of the cell bodies and zygotes and fluorescent images of the nuclei are presented in the right-hand vertical column.

ogy of *C. albicans*. Given the role of secretion and adhesion in yeast mating, we can now suggest that pimples may play a role in chemotropism and/or fusion.

Second, opaque-phase cells differentially express and secrete two aspartyl proteinases (14, 34, 53). Although these proteinases have been assumed to be involved in tissue penetration and have been shown to facilitate opaque-phase-specific cavitation on newborn-mouse skin (23), we must now consider their possible involvement in the mating process.

Third, and perhaps most intriguing, is the relationship between the white-opaque transition and temperature. Opaque-phase cells are stable at 25°C but not at 37°C, which is body temperature (42). Transferring opaque-phase cells from 25 to 37°C induces conversion en masse to the white phase (35, 40, 42, 45, 48). The phenotypic transition, assessed as the point of phenotypic commitment to the white phase, occurs semisynchronously at the second cell doubling at 37°C (45, 48). Since mating requires expression of the opaque phenotype, it may be limited to locations outside the human body, such as the surface of skin or environmental reservoirs, where the temperature is below body temperature. It could be that there is a disadvantage to mating within the human body, or that mating per se and pathogenesis within the human body are incompatible.

Fourth, and related to the preceding characteristic, opaque-phase cells are far more virulent than white-phase cells in a cutaneous mouse model (23). Is it possible that mating is restricted to the opaque phenotype in order to target the process to a location outside the human body, namely, skin? Is it possible that this restriction removes the process from environments that harbor the human immune system, or targets the process to an environment more prone to multiple strain colonization?

One may also ask why homozygotes do not simply express the opaque phenotype constitutively. Why have they developed a switching system fine-tuned by the deacetylases Hda1p and Rpd3, which maintain frequencies of switching between the two phases of around 10^{-3} (22, 52)? Obviously there is some advantage for homozygotes to express the white phenotype as well as the opaque phase. That advantage, again, is not immediately obvious.

There is little doubt that these newly discovered dependent relationships between *MTL* homozygosis, white-opaque switching, and mating in *C. albicans* have challenged us with a number of truly fascinating and unexpected enigmas and questions related to the basic biology and pathogenesis of *C. albicans*. There is also little doubt, given the differences between *S. cerevisiae* and *C. albicans*, that regulatory mechanisms unique to *C. albicans* mating will be discovered. The rapid rate of discovery experienced in the past few years should, therefore, be matched, if not outdistanced, by the discoveries that will be made in the next few years. Who would have thought only a few years ago that *C. albicans* has sex, and that it must switch to do it?

ACKNOWLEDGMENTS

We are indebted to T. Srikantha and C. Pujol for valuable discussions and to K. Daniels for help with the figures.

The research from the Soll laboratory on switching and mating reported here was supported by NIH grants AI2392 and DE14219.

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