Mating in *Candida albicans* and the Search for a Sexual Cycle

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Abstract

Candida albicans is a normal part of the human microflora, but it is also an opportunistic fungal pathogen that causes both mucosal infections and life-threatening systemic infections. Until recently, *C. albicans* was thought to be asexual, existing only as an obligate diploid. However, a mating locus was identified that was homologous to those in sexually reproducing fungi, and mating of *C. albicans* strains was subsequently demonstrated in the laboratory. In this review, we compare and contrast the mating process in *C. albicans* with that of other fungi, particularly *Saccharomyces cerevisiae*, whose mating has been most intensively studied. Several features of the mating pathway appear unique to *C. albicans*, including aspects of gene regulation and cell biology, as well as the involvement of "white-opaque" switching, an alteration between two quasi-stable inheritable states. These specializations of the mating process may have evolved to promote the survival of *C. albicans* in the hostile environment of a mammalian host.

Contents

INTRODUCTION

The diploid fungus *Candida albicans* is a normal part of the human gastrointestinal microflora and can be detected in at least 70% of the healthy population (58). However, *C. albicans* is an opportunistic pathogen and, given the right conditions, has the ability to cause local mucosal infections and even, more rarely, systemic infections in which the fungus can spread to all major organs of the body. Systemic infections are life threatening and are most common in individuals with an immune system that has been severely depressed, such as HIV-compromised individuals or patients treated with immunosuppressive drugs following organ transplantation.

C. albicans has several attributes that enable it to rapidly adapt to changing environmental signals, and these attributes aid it in colonizing the host. The best studied of these is the ability of *Candida* to switch between growing as unicellular yeast and growing as multicellular filaments (the yeast-hyphal transition) (7, 39, 48, 68, 76). In addition, *C. albicans* strains show different forms of "phenotypic switching," in which cells change their morphology and physiology by reversible switching between alternative variant phenotypes (61–63). What distinguishes phenotypic switching from other forms of regulation (such as the yeast-hyphal transition) is the inheritance of a phenotypic state for many generations after the initial switching event. Both the yeast-hyphal transition and phenotypic switching affect the virulence of *C. albicans* in models of systemic disease. *C. albicans* also has evolved multiple regulatory pathways that recognize a range of external cues, including pH, temperature, and the presence of serum. It therefore appears that *C. albicans* can rapidly adapt to different host microenvironments with both heritable and nonheritable mechanisms, and these can help promote tissue invasion and evasion of the host immune system.

Since its "modern" classification around 100 years ago, *C. albicans* has been described as an asexual fungus. In the 1970s and 1980s, natural isolates of *C. albicans* were shown to be diploid and there were no observations suggesting a sexual reproductive cycle (50, 56, 75). This was surprising, however, given the close relationship of *C. albicans* to sexually reproducing yeast species such as *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Furthermore, by the mid-1990s several *S. cerevisiae* genes known to function in mating and

meiosis were found to have close orthologs in *C. albicans*. Some of the *C. albicans* orthologs could even substitute for their counterparts when expressed in *S. cerevisiae* (14, 36, 74). In recent years, this list has grown much longer owing to the sequencing of the *C. albicans* genome.

Population studies of *C. albicans* have also been carried out to determine the extent of genetic exchange between strains in the natural population. These studies demonstrated that most *C. albicans* isolates propagated clonally, but they also provided evidence for limited genetic exchange (i.e., recombination) in the population (21, 71). These lines of evidence neither confirmed nor eliminated a role for mating and recombination in the life of *C. albicans*.

Since the discovery of *C. albicans* mating in 2000, studies carried out in several laboratories have shown that *C. albicans* contains an elaborate mating program that has many similarities but also several major differences when compared with those of other fungi. Aspects of mating and its regulation specific to *C. albicans* may have evolved to limit mating to specific locations in the mammalian body, or to allow mating to take place under less-than-optimal conditions. This review summarizes current knowledge about mating in *C. albicans* and uses the work in *S. cerevisiae* as a convenient reference point. We also discuss the recent description of a parasexual cycle of *C. albicans* through which genetic exchange can occur in the absence of meiosis. As meiosis has not been demonstrated in *C. albicans*, this parasexual cycle may be used in *C. albicans* as an alternative to a true sexual cycle.

DISCOVERY OF MATING IN *C. ALBICANS*

Identification of the *MTL* Locus in *C. albicans*

In the model yeast *S. cerevisiae*, sexual mating is controlled by genes encoded at a single genetic locus—the *MAT* locus. The *S. cerevisiae* *MAT* locus consists of two alleles: *MAT***a** and *MAT*α (see **Figure 1**). Cells expressing the genes at *MAT***a** mate as **a**-type cells, cells expressing genes at *MAT*α mate as α-type cells, and cells expressing the genes at both loci are unable to mate but are competent to go through meiosis. *MAT***a** codes for a single protein, **a**1, a homeodomain protein, and *MAT*α codes for a homeodomain protein (α2) and an alpha-domain protein (α1). [In this review the gene encoding each transcriptional regulator is called by its full name (e.g., *MAT**a**1*), and the gene product is abbreviated (e.g., **a**1)]. The three transcriptional regulators, **a**1, α1, and α2, control mating type via a simple, combinatorial circuit. In haploid α cells, α1 turns on α-specific genes while α2 represses **a**-specific genes, directing the cell to mate as an α cell. In haploid **a** cells, the **a**1 protein has no known function, but **a**-specific genes are expressed constitutively and α-specific genes are not (because α1 is absent), directing the cell to mate as an **a**-cell. Cells that have mated express both **a**1 and α2 proteins, and these form a heterodimer that represses many of the mating genes, thereby blocking mating by the **a**/α cell but permitting meiosis under favorable environmental conditions (23, 30, 38, 64).

Although mating had yet to be observed, the discovery in 1999 of an *MTL* locus in *C. albicans* revealed that the key master regulators of mating in *S. cerevisiae* were conserved in *C. albicans* (25). The genes encoding these proteins were arranged in a similar fashion along the chromosome (see **Figure 1**) and even contained introns in conserved positions. A fourth transcriptional regulator, *MTL**a**2*, was subsequently discovered to reside at the *MTL***a** locus in *C. albicans*, although an ortholog of this gene is missing at the *S. cerevisiae* *MAT* locus (72). This gene encodes a putative DNA binding protein homologous to the high mobility group box proteins found in the mating loci of several fungi, including *Cryphonectria parasitica*, *Potentilla anserina*, *K. lactis*, and *Neurospora crassa*. The identification of an "**a**" and an "α" version of the *C. albicans* *MTL* locus revealed

***MAT*:** mating-type

***MTL*:** mating-type like

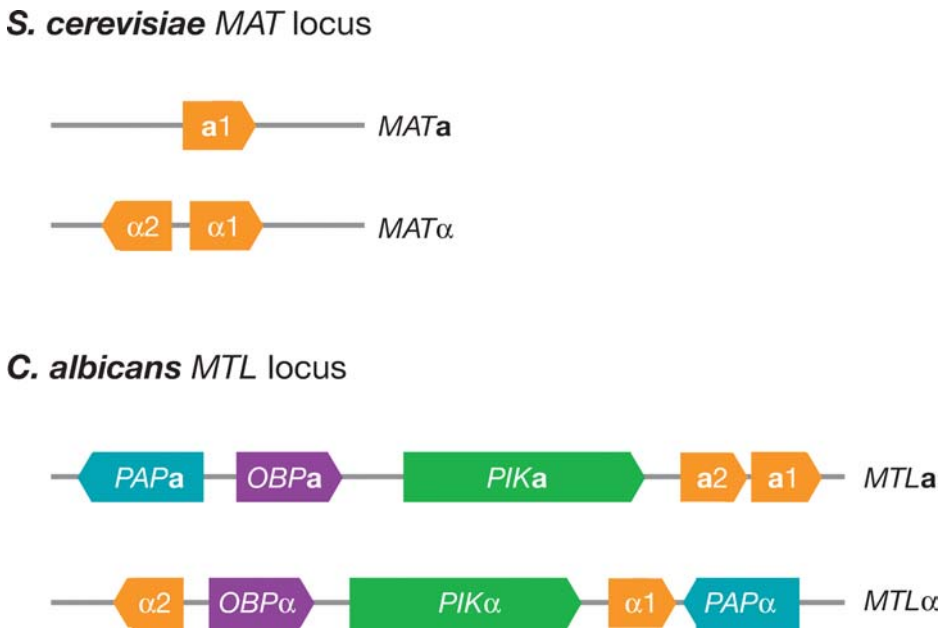

Figure 1

The *S. cerevisiae* and *C. albicans* mating-type loci. The *S. cerevisiae* locus has two alleles, *MAT***a** and *MAT*α, which together encode three transcriptional regulators: **a**1, α1, and α2. The *C. albicans* mating locus also contains two alleles, *MTL***a** and *MTL*α. *MTL***a** encodes the **a**1 and **a**2 transcriptional regulators, and *MTL*α encodes the α1 and α2 transcriptional regulators. The *C. albicans* mating locus also contains three additional pairs of genes, *PAP*, *PIK*, and *OBP*, which encode a poly(A) polymerase protein, a phosphoinositol kinase protein, and an oxysterol binding protein, respectively.

that the standard laboratory strain of *C. albicans* (SC5314) corresponded to an **a**/α cell.

The overall size of the *MTL* locus (9 kb) in *C. albicans* was much larger than that of the *MAT* locus (0.7 kb) in *S. cerevisiae*, principally due to the presence of three additional open reading frames at each loci. These additional open reading frames were arranged in pairs, one member of which was present in *MTL***a** and one member in *MTL*α (see **Figure 1**). These genes encoded poly(A) polymerases, orthologs of the *S. cerevisiae* phosphatidylinositol kinase Pik1, and orthologs of the human oxysterol binding proteins. These genes, called *PAP***a**, *PAP*α, *PIK***a**, *PIK*α, *OBP***a**, and *OBP*α, are not found at the mating locus of most other fungi. Each pair of alleles shares approximately 60% identity with each other, in contrast with the much greater similarity (often > 99%) of the two alleles of the typical *C. albicans* gene. The sequence divergence between alleles of *PAP*, *PIK*, and *OBP* raises the possibility that one allele may carry out a function distinct from the other. The *C. albicans MTL* locus may represent a position in the *C. albicans* genome where allele diversification can be maintained. A final important observation was that *MTL***a** and *MTL*α sequences were each present as single copies in the *C. albicans* strain studied (SC5314) (25), ruling out the possibility of silent mating-type "cassettes," as found in *S. cerevisiae*, *K. lactis*, *Schizosaccharomyces pombe*, and, more recently, *Candida glabrata* (6, 66).

Mating of *C. albicans* Strains

C. albicans was shown to mate in two separate reports published in 2000, albeit using somewhat different approaches (26, 45).

In both cases, a normally heterozygous (i.e., **a**/α) strain of *C. albicans* (e.g., SC5314) was manipulated in the laboratory to create **a** and α derivatives, which were then observed mating.

In the Johnson laboratory, **a**-type mating strains were constructed by deleting either the entire *MTL*α locus or the *MTL*α*1* and *MTL*α*2* transcriptional regulators. Similarly, α-type mating strains were constructed either by deletion of the entire *MTL***a** locus or by deletion of the *MTLa1* and *MTLa2* genes (26). [*MTLa2* was fortuitously inactivated in the original experiments—it had not yet been identified but the *MTLa1* deletion also inactivated *MTLa2*]. These strains are described as hemizygous *MTL* strains in this review (see **Figure 2**).

A different approach used by the Magee laboratory (45) took advantage of the fact that the *MTL* of *C. albicans* resides on chromosome 5. Prior studies by Janbon et al. (27) had made the surprising discovery that one homolog of chromosome 5 was often lost during growth of *C. albicans* strains on certain selective media. In particular, while the standard laboratory strain of *C. albicans* was unable to grow on sorbose medium, variants arose that could assimilate sorbose, and these had typically lost one homolog of chromosome 5 (27). It is thought that a negative regulator of the sorbose-utilization gene (*SOU1*) resides on

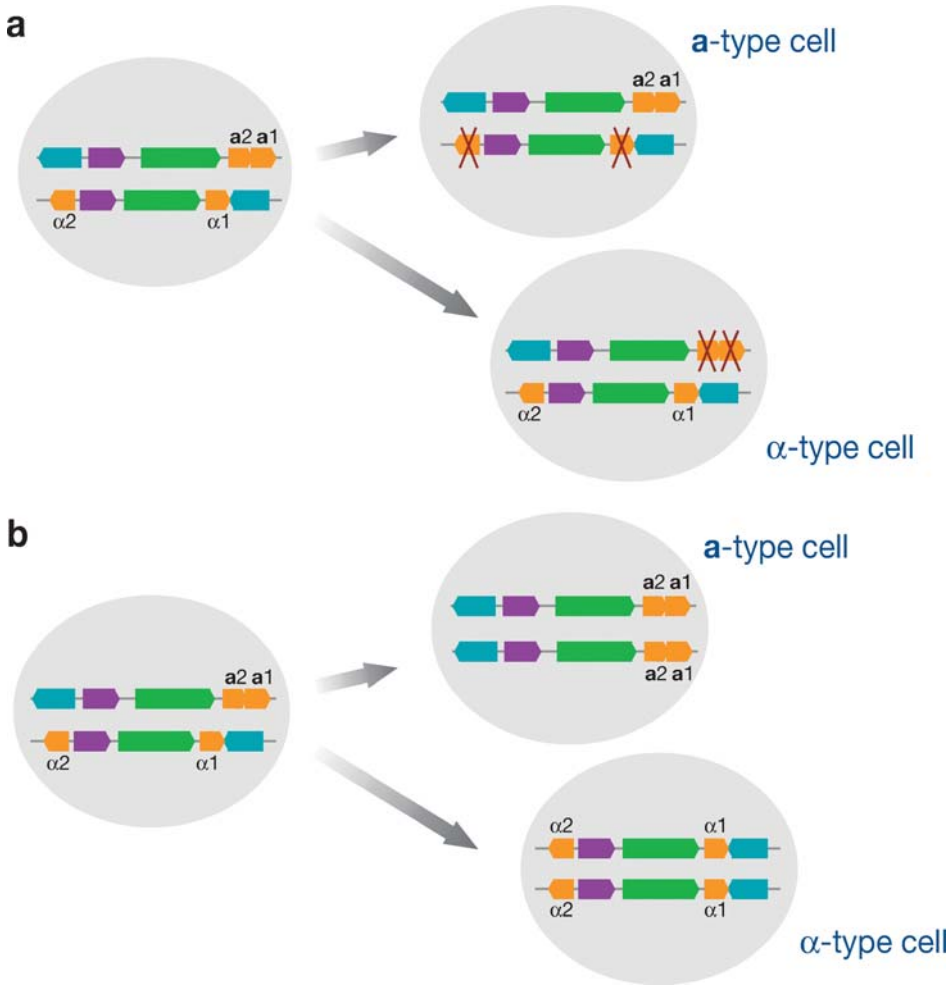

Figure 2

C. albicans mating strains have been constructed in two ways. (*a*) Deletion of the regulatory genes at the *MTL* generates hemizygous **a**-type and α-type mating strains. (*b*) Loss of one homolog of chromosome 5, which carries the *MTL* allele, followed by reduplication of the remaining homolog results in homozygosis of the *MTL* locus. In addition, naturally occurring **a**-type and α-type mating strains have been identified in clinical isolates (37, 42).

chromosome 5 and that a reduction in expression of this regulator (e.g., by loss of one copy on chromosome 5) allows growth on sorbose medium. Removal of sorbose selection led to duplication of the remaining homolog of chromosome 5, restoring the normal diploid complement of chromosomes (27, 28). Using this technique of sorbose selection, Magee & Magee (45) converted the **a**/α parental versions of several strains (including SC5314) into *MTL***a/a** and *MTL*α/α homozygous derivatives (see **Figure 2**).

To demonstrate mating between the newly constructed **a** and α strains, both laboratories selected prototrophic conjugants resulting from crosses between strains carrying complementary auxotrophic markers. Mating between the homozygous strains occurs on both laboratory media and in a mammalian host, in this case after injection of **a** and α strains into the tail vein of a mouse and recovery of mating products from the kidney. The mating products were mononuclear and tetraploid in DNA content. The efficiency of *C. albicans* mating, however, both in vitro and in vivo was relatively low, in some cases as low as one successful mating event per ten million potential mating partners.

WHITE-OPAQUE SWITCHING

Regulation of Mating in *C. albicans* by White-Opaque Switching: A Master Switch

In *S. cerevisiae*, **a** and α cells are fully competent to mate. This is not the case in *C. albicans*, in which **a** and α cells must undergo a phenotypic switch from the predominant white form to the rarer and less stable opaque form to become mating competent. Discovered in 1987 by Soll and colleagues (60), white-opaque switching refers to the ability of some, but not all, *C. albicans* strains to undergo a reversible switch between the white and opaque phases; after switching has occurred, the phase of the parental cell is then passed on for many generations to its descendants. White-phase cells appear round and form dome-shaped, white colonies on solid agar, and opaque-phase cells appear more elongated and form flatter, darker colonies on solid agar (60) (see **Figure 3**). The rate of switching between white and opaque phases was described by Rikkerink et al. (56a). Switching in the white-to-opaque direction occurred at a frequency of 10^{-4} to 10^{-5} per cell generation, and switching from the opaque to white phase occurred at the slightly higher frequency of 5×10^{-4} per cell generation (56a). Switching between white and opaque phases can be observed in single colonies showing both white and opaque sectors (**Figure 3**). The role of white-opaque switching was initially studied in the context of pathogenesis: Soll's laboratory showed that opaque-phase cells were more virulent in cutaneous models of infection and that white-phase cells were more virulent in systemic infection (32, 73).

Miller & Johnson (47) subsequently described two key connections between white-opaque switching and mating: (*a*) The ability of a *C. albicans* strain to undergo white-opaque switching was regulated by the *MTL* locus, and (*b*) opaque-phase **a** and α cells mated approximately 10^6 times more efficiently than these same cells in the white phase. These findings explained why only a subset of clinical isolates of *C. albicans* underwent white-opaque switching; **a** and α cells, but not **a**/α cells, were competent for switching. It also explained the low level of mating previously reported, as the prior studies used *C. albicans* white cells.

Examination of a number of clinical *C. albicans* isolates has revealed that between 3% and 7% can undergo white-opaque switching; these are naturally occurring homozygous **a/a** or α/α strains. The remainder of the clinical isolates were heterozygous (**a**/α) at the *MTL* and did not undergo white-opaque switching (37, 42). Some strains were heterozygous at the *MTL* but generated homozygous progeny with high frequency, and these progeny could then undergo white-opaque switching (42). The existence of natural homozygotes of *C. albicans* suggests that mating may not be

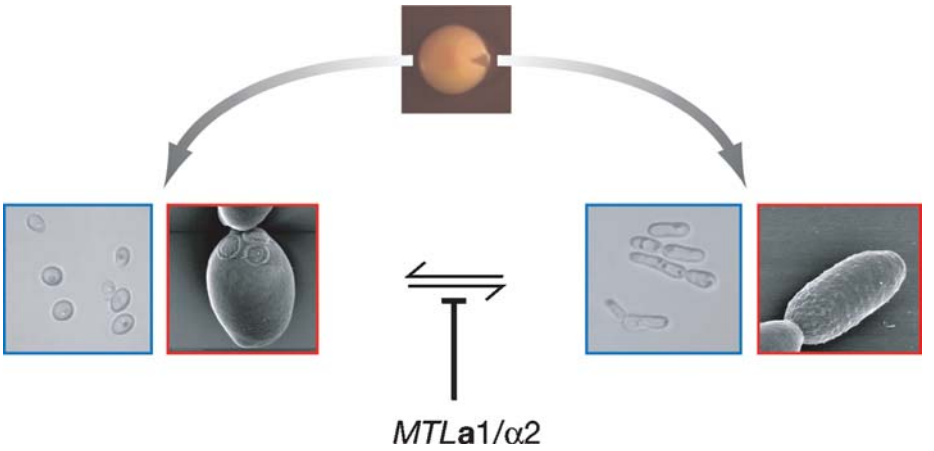

Figure 3

White-opaque switching in *C. albicans*. The top part of the figure shows a sectored colony on an agar plate, in which most of the cells are in the white phase and a sector contains cells in the opaque phase. The lower panels show scanning electron micrographs at ×1000 magnification and bright field images of white and opaque cells. Electron micrographs were prepared by fixing samples with 2% glutaraldehyde and post-fixed with 1% osmium tetroxide. They were then dehydrated with ethanol, and dried and sputter-coated with 15 nm of gold palladium. Switching to the opaque phase is blocked by the **a**1 and α2 proteins, ensuring that **a**/α strains are not permissive for white-opaque switching, whereas **a** and α strains are. Electron micrographs are provided courtesy of Mathew Miller, University of California, San Francisco.

limited to the laboratory but may also occur in the wild. This view is supported by the observation that opaque cells can undergo mating at frequencies (40%) approaching those of other fungi (47).

Regulation of white-opaque switching occurs through repression exacted by **a**1 and α2, which likely function as heterodimers to repress transcription. In *S. cerevisiae*, the **a**1-α2 heterodimer acts to block mating of diploid **a**/α cells by repression of **a**- and α-specific genes. In *C. albicans*, the **a**1-α2 heterodimer acts indirectly to block mating by preventing switching of white-phase cells to opaque-phase cells (see **Figure 3**; additional transcriptional regulation of mating is discussed below). Thus, following mating of opaque-phase **a** and α cells, the conjugant **a**/α cell switches back to the white phase owing to control of switching by the **a**1-α2 heterodimer (47). Control of switching by the *MTL* makes intuitive sense, as it ensures that only those cells competent for mating, i.e., **a** and α cells, are permitted to switch to the opaque phase.

Opaque Cells: Primed to Mate

Mating of *C. albicans* strains likely occurs only between opaque cells, and the low level of mating observed between white cells of the opposite mating type is likely due to spontaneously arising opaque cells. Opaque cells are stable only at lower temperatures in the laboratory (room temperature rather than 30° or 37°C). This could explain why the initial mating experiments, performed using white cells, found that room temperature led to a higher degree of mating than did higher temperatures (45).

Transcriptional profiling of white and opaque cells has revealed that approximately 400 genes are differentially regulated between the two phases, with approximately 230 genes upregulated in the opaque phase and approximately 170 genes upregulated in the

MAPK: mitogen-activated protein kinase

white phase (35, 72). A few of the opaque-phase-specific genes play a role in mating in *C. albicans*. These include the *STE4* and *CEK2* genes, which encode two components of the mating MAPK cascade involved in the response to mating pheromones (described below). In addition, the *MF*α gene, which encodes the α-mating pheromone, is preferentially expressed in opaque α cells (35). It therefore appears that opaque cells, but not white cells, express genes necessary for efficient mating.

Opaque cells also differ from white cells morphologically. In addition to their more elongated shape, opaque cells exhibit "pimples" on the cell surface, as seen by scanning electron microscopy (1) (see **Figure 3**). These have been proposed to represent regions where the plasma membrane protrudes, possibly facilitating cell fusion (47). Although opaque cells appear to be primed for mating in *C. albicans*, most of the 400 genes differentially regulated by white-opaque switching show no obvious link with mating (35, 72). Approximately one third of this group of genes has been implicated in metabolism, with opaque cells biased toward an oxidative metabolism and white cells biased toward a fermentative metabolism. Other regulated genes are involved in diverse processes such as adhesion, stress response, drug resistance, and tissue invasion. The main purpose of white-opaque switching may be to allow *C. albicans* to occupy different host niches, and this possibility, and its relationship with mating, is discussed below.

The Role of White-Opaque Switching

Why has *C. albicans* incorporated white-opaque switching into its mating behavior? One possibility is that white-opaque switching has evolved to allow *Candida* to direct mating to a specific environmental niche in the host. Perhaps certain steps in the mating process, such as pheromone signaling and membrane fusion, are incompatible with *C. albicans*' survival in certain hostile environments of a mammalian host (20). As discussed above, opaque-phase cells are less robust than white-phase cells in systemic infection of a mammalian host, and they are also unstable at 37°C, switching back en masse to the white phase (60). These findings suggested that the role of opaques might be to limit mating to environments outside the human body. As opaque cells are adept at colonizing the skin (32, 73), mating on the skin of newborn mice (surface temperature 31.5°C) was examined and found to be highly efficient, with up to 40% of opaque **a** and α cells fusing during a 24-h period (34). Synthetic surfaces, such as glass, plastic, and silicone elastomer, were much less conducive to mating, with little, if any, cell fusion observed. These results suggest that skin cells may be generating metabolic signals that aid in the mating process in *C. albicans*. The skin surface may also be an ideal place for mating to occur because multiple strains might be expected to come into contact with one another at this location.

In opposition to the idea that mating occurs only outside of the body is the observation that opaque cells have been isolated from inside mammalian hosts (32, 33). In addition, white **a** and α cells injected systemically into a mouse host underwent mating at an appreciable level, suggesting that switching to the opaque phase followed by mating must have occurred with reasonable efficiency within the host (26). As discussed below, signals from the host can impact the expression of *MTL* genes, and it may be that signals also exist to stabilize the opaque phase in specific locations within the host.

MATING PATHWAYS

Transcriptional Circuit Controlling Mating in *C. albicans*

As described above, the transcriptional regulatory proteins encoded at the *S. cerevisiae MAT* locus specify mating behavior by

activating and repressing appropriate sets of genes. In the absence of *MAT* entirely, cells mate as an **a**-type cell, as **a**-specific genes are constitutively expressed. Thus, the **a**-type cell is the default cell type. An α-type cell requires expression of the *MATα1* gene, whose product turns on α-specific genes, and the *MATα2* gene, whose product represses **a**-specific genes. Mating is blocked in the **a**/α cell type, since expression of many genes required for the mating of both **a**- and α-type cells (the haploid specific genes) is repressed by the **a**1-α2 heterodimer (see **Figure 4**). The entire circuitry of *S. cerevisiae* mating-type regulation, including the target genes, has recently been described (19).

The **a**1, α1, and α2 proteins also coordinate expression of the **a**- and α-specific genes in *C. albicans* together with the additional transcriptional regulator encoded at the **a** mating-type locus, the **a**2 protein. Although there are some similarities between the transcriptional circuits that regulate mating in *S. cerevisiae* and *C. albicans*, there are also several important differences between the circuits in these fungi (72) (summarized in **Figure 4**). In brief, there are three main differences. (*a*) Individual genes (e.g., *NEJ1*, *RAM2*) are controlled by the mating-type

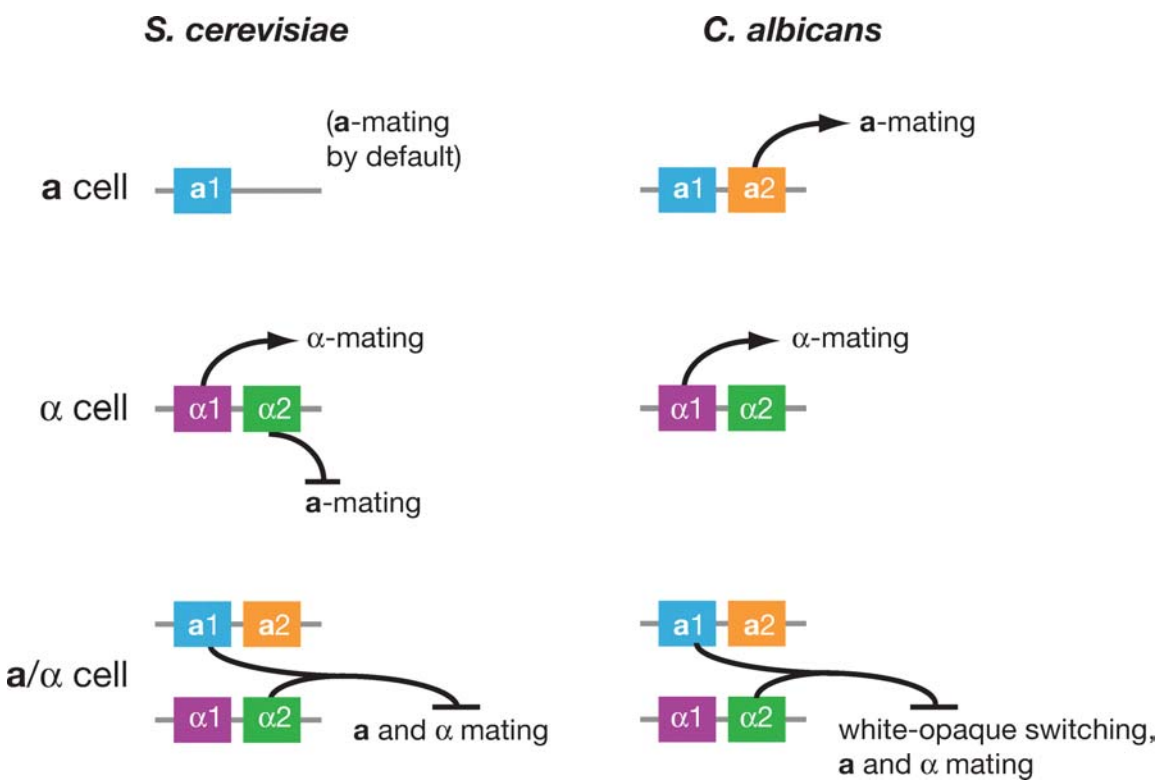

Figure 4

The transcriptional circuits regulating mating type in *S. cerevisiae* and *C. albicans*. In *S. cerevisiae*, the default pathway is the formation of an **a**-type cell, which requires no input from the *MAT* genes. In α-type cells, α1 turns on the α-specific genes and α2 represses the **a**-specific genes. The circuit is different in *C. albicans*: **a**2 activates the **a**-specific genes, and α1 activates the α-specific genes. In both organisms expression of **a**1 and α2 act together to repress mating; in *S. cerevisiae* **a**1-α2 directly represses mating genes, and in *C. albicans* **a**1-α2 acts both directly to repress mating genes and indirectly by inhibiting the white-to-opaque transition.

regulators in one organism but not in the other. These differences presumably result from sequence differences in the regulatory regions of the target genes. (*b*) The *C. albicans* mating-type locus has a positive activator of **a**-specific genes that is missing in *S. cerevisiae*. To compensate for its absence, *S. cerevisiae* has an extra circuit branch; in addition to working with **a**1 to turn off the haploid-specific genes (as it does in both *S. cerevisiae* and *C. albicans*), α2 also represses the **a**-specific genes in *S. cerevisiae*. This circuit branch is absent in *C. albicans*. (*c*) White-opaque switching, which involves the regulation of ~400 genes, is present in *C. albicans*, in which it is controlled by the mating-type locus, but is absent in *S. cerevisiae*.

Of all these evolutionary changes, perhaps the second is the easiest to attempt to reconstruct. By examining the mating-type locus in many fungi, it was proposed that the *S. cerevisiae* branch of the fungal lineage lost the **a**2 protein and that the redeployment of α2 to turn off the **a**-specific genes occurred relatively recently in evolution (6, 72) (**Figure 5**). According to this idea, the role of α2 as half of the **a**1-α2 heterodimer is ancient and present in many fungi, but its **a**1-independent role (repression of the **a**-specific genes) is a peculiarity of the *S. cerevisiae* lineage.

Regulation of the Mating Circuit by Host Factors: A Role for Hemoglobin?

In most fungi, cell type is determined simply by the identity of the mating-type loci present in that cell. In *C. albicans*, however,

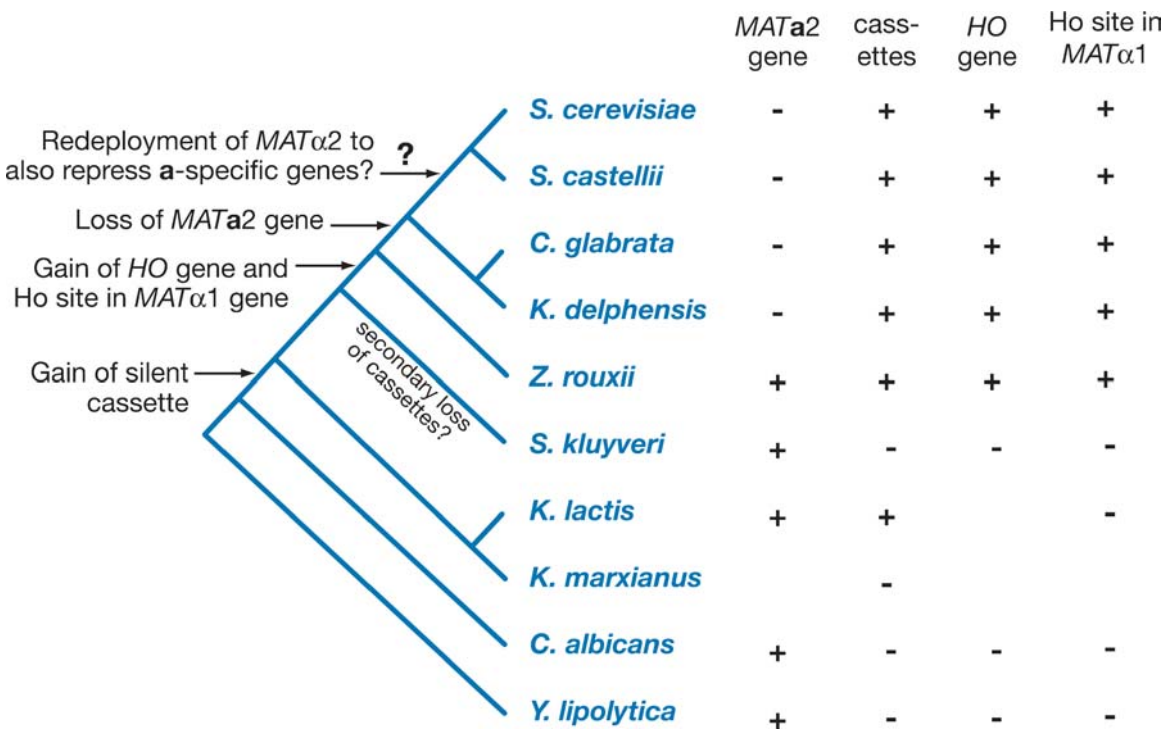

Figure 5

Proposed evolution of the mating-type locus. As depicted, a direct ancestor of *S. cerevisiae* first gained silent mating cassettes, then subsequently gained both the Ho endonuclease and the *HO* gene, and finally lost the ancestral *MAT**a**2* gene. *C. albicans* appears to contain the ancestral mating-type locus, with an intact *MAT**a**2* gene, and no silent mating cassettes or *HO* gene. Figure modified from Butler et al. (6).

environmental factors may alter cell type determination. In *C. albicans*, expression of the *MTL**a**1*, *MTLα1*, and *MTLα2* genes (the *MTL**a**2* gene was not examined in this study) is influenced by the levels of the hemoglobin response gene (*HBR1*). A heterozygous *hbr1*$^{-}$*/HBR1*$^{+}$ strain showed a marked decrease in expression of *MTLα1* and *MTLα2* and slightly enhanced *MTL**a**1* expression, whereas overexpression of *HBR1* resulted in increased expression of both *MTLα* genes (52) (see **Figure 6**). The heterozygote *hbr1*$^{-}$*/HBR1*$^{+}$ strain also exhibited white-opaque switching, even in the presence of the *MTL**a**1* and *MTLα2* genes, which normally act to prevent switching. Presumably, the reduced level of expression of *MTLα2* in this mutant is sufficient to relieve the block on white-opaque switching. Furthermore, an **a**/α *hbr1*$^{-}$*/HBR1*$^{+}$ strain was shown to mate as an **a**-type cell, in spite of a full complement of *MTL* genes (52).

HBR1 was originally identified (and named) because of its elevated expression in cells that have been exposed to hemoglobin (53). *C. albicans* cells show a pleiotropic response to hemoglobin, including the induction of receptors for fibronectin, laminin, and fibrinogen, suggesting that hemoglobin might be an important signal for *C. albicans*' physiology in the host. The in vivo significance of the control of the mating-type regulators by *HBR1* is not yet clear, as wild-type (*HBR1*$^{+}$*/HBR1*$^{+}$) **a**/α strains of *C. albicans* have yet been shown to undergo white-opaque switching in either the presence or absence of hemoglobin. Of interest, however, is the fact that *HBR1*$^{+}$*/HBR1*$^{+}$ cells require

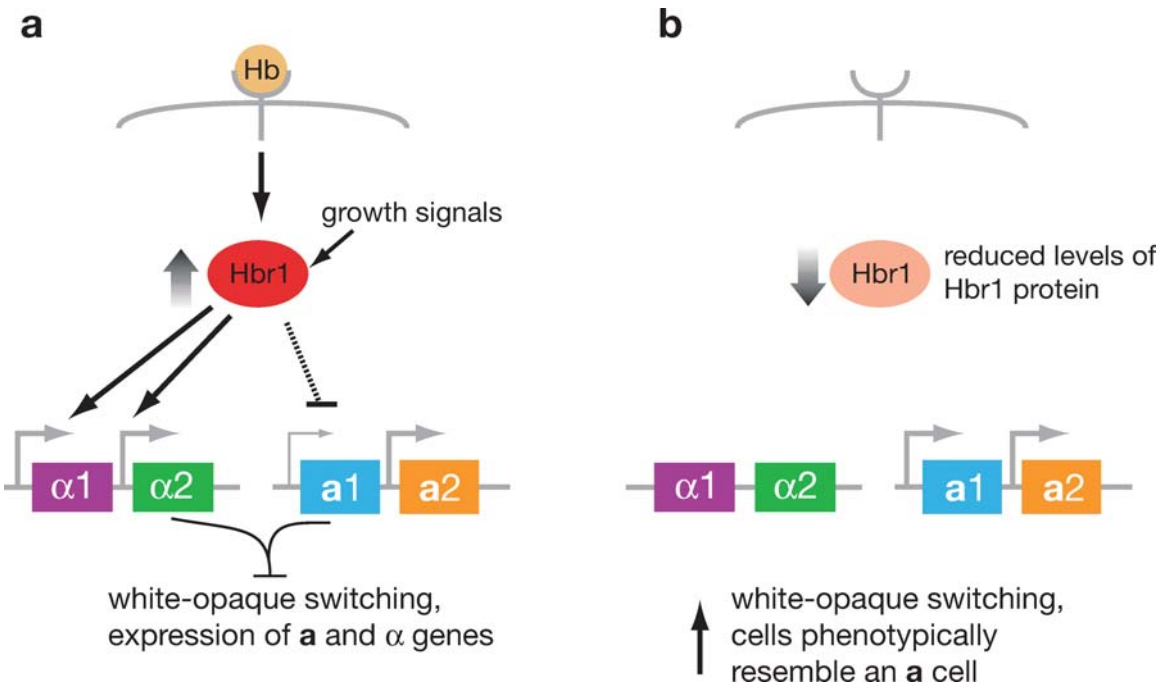

Figure 6

Regulation of *MTL* expression by the Hbr1 protein. (*a*) Hbr1 expression is increased in the presence of hemoglobin (Hb), which in turn can stimulate expression of the *MTLα1* and *MTLα2* genes and can repress the *MTL**a**1* gene. Under these conditions, white-opaque switching is blocked. (*b*) If the level of Hbr1 falls below a threshold level (thus far only achieved by deletion of one copy), then expression of *MTLα1* and *MTLα2* genes is turned off and the cell behaves phenotypically as an **a**-type mater.

hemoglobin to maintain expression of both Hbr1 and the *MTL*α genes once they reach the stationary phase (52). Thus, the link between hemoglobin and white-opaque switching may be physiologically relevant under specific conditions.

A Conserved Mating Signaling Cascade: Identification of a Mating Pheromone

Initial microscopy experiments of mixtures of opaque **a** and α cells suggested that, as in other fungi, mating cells of *C. albicans* communicated with each other through pheromone signaling (41, 47). Mating mixes showed cells forming long polarized projections, similar in principle but not appearance to mating projections of the "shmoo" forms that *S. cerevisiae* cells assume when exposed to pheromone (for review of *S. cerevisiae* mating, see Reference 65). In *S. cerevisiae*, **a** cells secrete **a**-pheromone that is recognized by α cells by a specialized receptor encoded by *STE3*. Conversely, α cells secrete α-pheromone that is sensed by **a** cells expressing the *STE2* gene. Both pheromones and pheromone receptors are cell type specific, ensuring signaling specificity. Pheromone signaling is essentially the first step in mating: **a** and α cells within signaling range rearrange their cytoskeleton to grow toward one another and also undergo numerous changes in gene expression to prime themselves for subsequent mating events.

Several laboratories reported the identification of a *C. albicans* mating pheromone produced only by α cells (4, 35, 43, 49, 51). Similar to α-pheromone secreted by *S. cerevisiae*, the mature pheromone requires processing of the precursor by the serine proteinase Kex2 (see **Figure 7**). Indeed, the processing sites are more highly conserved than other features of the protein, and they provided a major clue in the identification of the *MF*α gene. Deletion of either the *MF*α gene or the *KEX2* gene blocked mating of α cells but had no effect on the mating of **a** cells (4, 51). Conversely, deletion of the *C. albicans STE2* ortholog (the α-factor receptor) in **a** cells blocked mating but had no effect on mating when deleted from α cells (4). Although the overall structure of the *MF*α gene was similar to that from *S. cerevisiae*, the mature α-factor peptide showed no sequence conservation at the amino acid level (see **Figure 7**). Processing of the precursor protein by Kex2 is predicted to generate two peptides, 13 and 14 amino acids long, and both peptides are active, inducing the formation of mating projections in opaque **a** cells (51). In contrast to opaque cells, white-phase **a** cells showed no morphological response to α-pheromone (4, 43, 51).

Closer comparison of the 13- and 14-amino-acid peptides (here called MF13 and MF14) suggests that they may differ in their activity, stability, or both. For example, fewer cells underwent morphological changes in response to MF14 than to MF13, and the addition of MF13, but not MF14, to the medium of mating cells complemented a *kex2*Δ strain (51). Using a "halo" assay, Panwar et al. (51) also found evidence that MF13 was better at inducing cell cycle arrest than MF14 . In this assay, the peptides were spotted onto a lawn of opaque **a** cells and a halo around the peptides suggested that cells growing in this region underwent cell cycle arrest.

Addition of the MF13 peptide to opaque **a** cells induced expression of 65 genes by more than threefold (4). In *S. cerevisiae*, approximately 150 genes are induced more than twofold in response to α-pheromone (57). Remarkably, the two sets of genes overlap by only 25%, indicating a major evolutionary divergence of the pheromone response between these two yeasts. Genes induced by α-pheromone in both *C. albicans* and *S. cerevisiae* included several components of the conserved mating MAPK cascade. One third of the pheromone-induced genes in *C. albicans* have unknown function and most of these are not present in the *S. cerevisiae* genome. Perhaps the most interesting class of *C. albicans* genes induced by α-factor encode cell surface and secreted factors previously

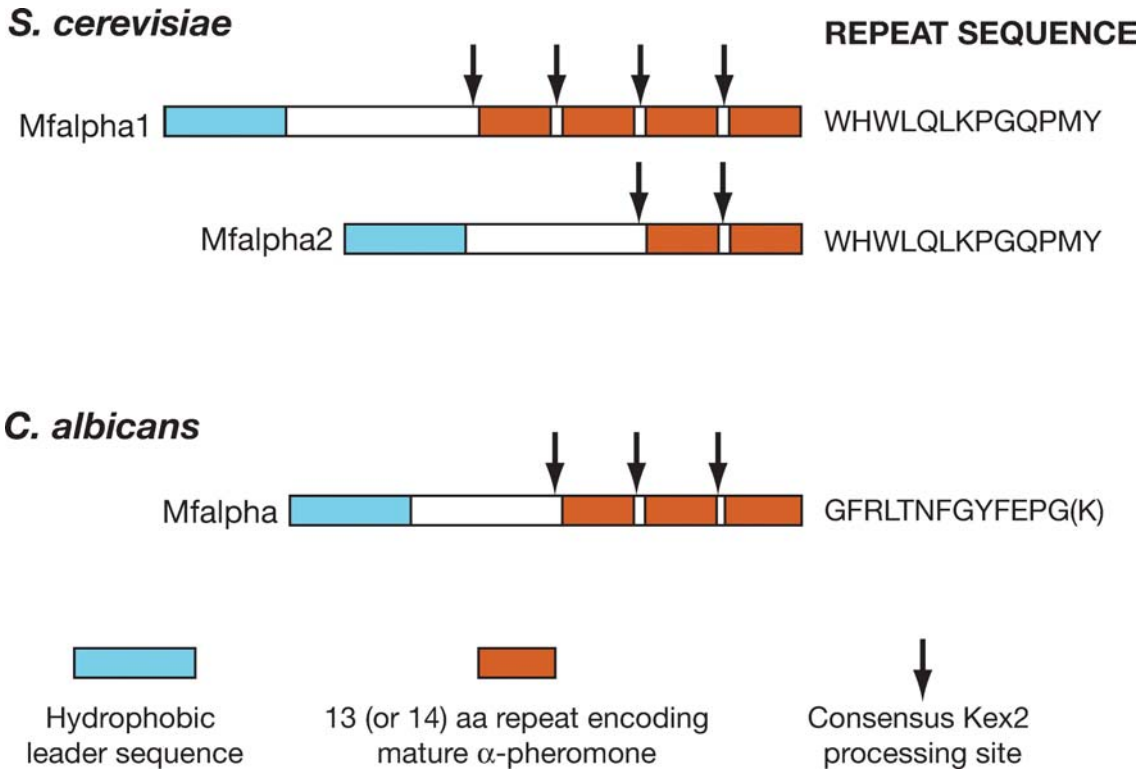

Figure 7

Identification of a mating pheromone in *C. albicans*. Two genes, *MFα1* and *MFα2*, encode the alpha pheromone in *S. cerevisiae*. Processing of the precursor protein by Kex2 protease releases the mature, 13-amino-acid pheromone. In *C. albicans*, α-pheromone is encoded by the single gene *MFα*, which contains three repeats of the mature pheromone sequence. Kex2 processing of the MFα protein produces two 13-amino-acid peptides and one 14-amino-acid peptide with an additional lysine residue at its C terminus.

shown to be required for full virulence of *C. albicans* strains in systemic infection. These included the *HWP1* gene (4, 13), which encodes a glycosylphosphatidylinositol-modified cell wall protein thought to mediate covalent attachment of *Candida* cells to host epithelial cells (67, 70). Localization studies show that Hwp1 is localized to the cell wall of mating projections following induction by α-pheromone (13). Genes encoding the Rbt1 protein (a putative cell wall protein of unknown function), the Rbt4 protein (a secreted protein related to plant pathogenesis proteins), and several secreted aspartyl proteases (Sap4–6) are also upregulated in response to pheromone and are required for full virulence in the mouse-tail-vein model (5, 10, 17, 67). These results indicate an intriguing link between virulence and mating in *C. albicans* and raise the possibility that components of cell-cell communication originally involved in mating were adapted during evolution for use by *C. albicans* in host-pathogen interactions (4).

Several issues in the *C. albicans* response to mating pheromones remain. For example, although white-phase **a** cells show no morphological response to α-pheromone, they express the pheromone receptor and upregulate a subset of pheromone-responsive genes upon pheromone exposure (43, 72). It remains to be seen at what step white-phase cells are blocked

in making a full physiological response to α-pheromone and what the purpose of this limited response might be. The gene encoding **a**-pheromone in *C. albicans* has yet to be identified, although several observations point to its presence. For example, the receptor for **a**-pheromone, encoded by the *STE3* gene, has been identified by homology with the gene from *S. cerevisiae*. Also, the *HST6* gene, an ortholog of the *STE6* gene required for export of **a**-pheromone in *S. cerevisiae*, is also required for mating of *C. albicans* **a** cells (44). Finally, opaque α cells, when mixed with opaque **a** cells, undergo morphological changes indicating they have been exposed to **a**-factor (47). Thus, although the evidence in favor of the existence of **a**-factor is overwhelming, it has not yet been identified and characterized.

A Conserved Mating Signaling Cascade: The MAPK Pathway

In *S. cerevisiae*, a conserved MAPK pathway transduces the pheromone signal from outside of the cell into a transcriptional response in the nucleus (16, 38, 59) (**Figure 8**). In *S. cerevisiae*, pheromone binding to its receptor leads to activation of a heterotrimeric G protein and dissociation of the Gα subunit (Gpa1), allowing the G$\beta\gamma$ subunits (Ste4 and Ste18) to initiate the MAPK cascade. The cascade involves activation of Ste20, which in turn leads to successive phosphorylation of Ste11, Ste7, and finally of the MAPK itself, Fus3. In the absence of Fus3, the MAPK Kss1 can substitute for its function, although its role is normally limited to the signaling involved in filamentation. Fus3 activates the sequence-specific DNA binding protein Ste12, which, together with Mcm1, is responsible for activating the pheromone-responsive genes in *S. cerevisiae*. Orthologs of each of these signaling components have been identified in *C. albicans*, and a number of them are required for mating, including *CST20* (*STE20*), *HST7* (*STE7*), *CEK1* (*KSS1*), *CEK2* (*FUS3*), and *CPH1* (*STE12*) (*S. cerevisiae* orthologs are given in parentheses) (8, 44) (see **Figure 8**). One possible difference between *C. albicans* and *S. cerevisiae* may be the absence of the scaffold protein Ste5 in *C. albicans*. Ste5 acts to tether the components of the MAPK cascade to one another and is one mechanism that prevents crosstalk between signaling in the mating pathway and other pathways that utilize the same core components. However, scaffold proteins are much less conserved than other proteins of the MAPK cascade, so the apparent absence of Ste5 from *C. albicans* may simply be due to an inability to recognize it.

As in other fungi, the central components of the MAPK pathway in *C. albicans* are used in both mating and filamentation cascades. For example, filamentation defects in *C. albicans* strains deleted for *CST20*, *HST7*, *CEK1*, and *CPH1* have been reported (12, 31, 36, 40). Several other pathways regulating filamentous growth in *C. albicans* have been identified, and these act independently of the MAPK pathway. For this reason, inactivation of the MAPK cascade in *C. albicans* has a strong effect on mating but a more modest effect on filamentous growth.

Although of minor importance in *S. cerevisiae*, nutritional cues play a major role in regulating mating in *C. albicans*, as they do in *S. pombe*, *Cryptococcus neoformans*, and *Ustilago maydis* (38). In these latter fungi, nutrient starvation is signaled via the cAMP pathway, and this acts in concert with the MAPK pathway to initiate mating. Although the mechanism is not known, media composition has a large influence on mating efficiency in *C. albicans*. For example, media containing glucose are less inducive to mating than media containing mannitol (4, 9). Optimal mating has been reported on Lee's medium and spider medium, two conditions that also activate filamentation in *C. albicans* (4, 8, 41), further evidence of the close relationship between the mating and filamentation pathways. Much work remains in understanding how *C. albicans* mating is regulated. Whether the host makes substantial contributions to this regulation is also an important and largely unanswered question.

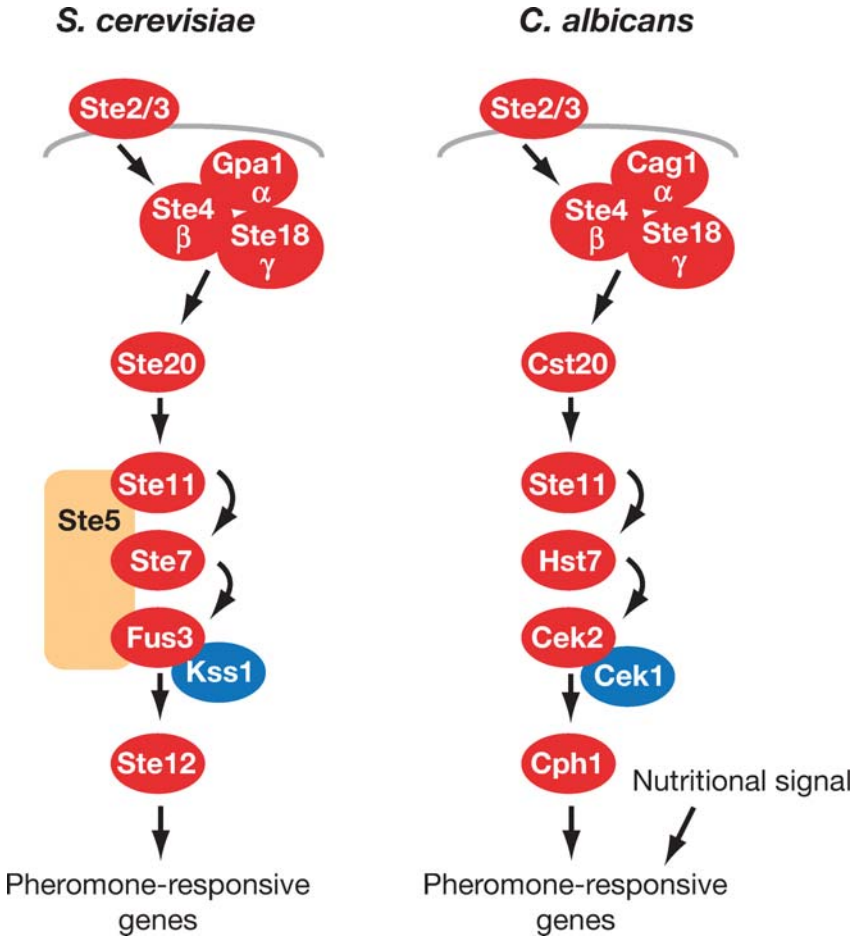

Figure 8

A conserved MAPK cascade transduces the pheromone signal to the nucleus. *S. cerevisiae* and *C. albicans* share most of the components of the G-protein-coupled MAPK pathway. Pheromone binding to its receptor (Ste2 or Ste3) leads to activation of a trimeric G protein and subsequent activation of the MAPK cascade. Fus3 (*S. cerevisiae*) and Cek2 (*C. albicans*) are the major kinases activated by the cascade, although Kss1 and Cek1 can replace them. The scaffold protein Ste5 has not been identified in *C. albicans*. In addition, the response to pheromone in *C. albicans* is regulated by a nutritional signaling pathway apparently absent in *S. cerevisiae*.

CELL BIOLOGY OF MATING

As observed under the microscope, the process of *C. albicans* mating largely mirrors the mating process in *S. cerevisiae*, although there are important differences (41) (see **Figure 9**). Mixtures of diploid opaque **a** and α cells exhibit mating projections that, unlike the shmoo forms in *S. cerevisiae*, grow up to many times the length of the cell. Projections from **a** and α cells are observed to grow toward one another, presumably because they follow mating pheromone gradients, and they eventually undergo cell fusion. The nuclei of the two mating partners then migrate toward one another into the conjugation bridge. In the study by Lockhart et al. (41) the nuclei came into close proximity but did not undergo karyogamy. Instead, the nuclei were forced apart by

Karyogamy: the coming together and fusing of cell nuclei

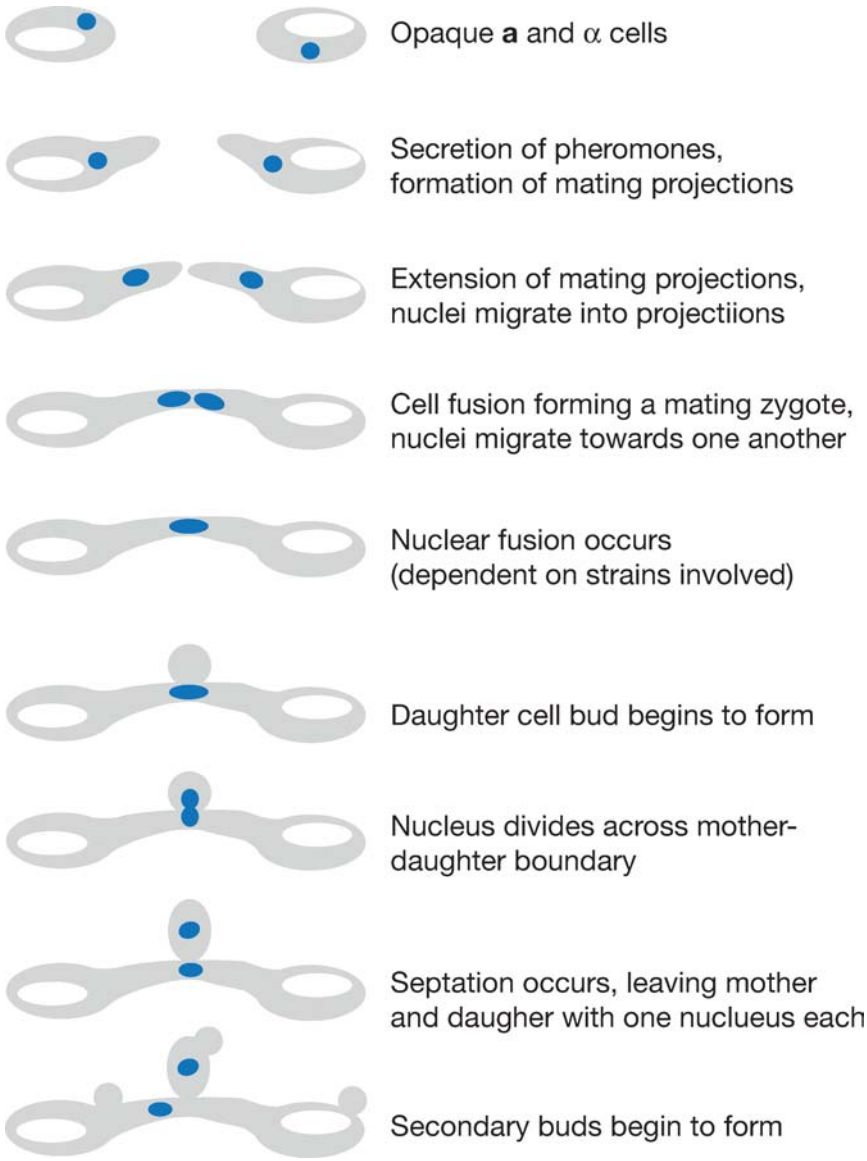

Figure 9

Steps in the mating of *C. albicans*. Nuclei are depicted in blue and vacuoles in white. The parental mating cells are diploid, and the mating products are mononuclear and tetraploid. The vacuole is not depicted in the daughter cell.

the formation of a vacuole in the conjugation bridge, and one or both of the parental nuclei entered the emerging daughter bud.

More recent studies have shown that karyogamy does occur at high efficiency during mating in a number of strains including clinical isolates and derivatives of the standard laboratory strain SC5314 (3). However, a few clinical isolates of *C. albicans* carry out nuclear fusion inefficiently, including the isolates used in the original study by Lockhart et al. (41) Crosses between clinical isolates from different clades exhibited karyogamy, indicating that there is no genetic barrier to nuclear fusion and genetic exchange between different clades of *C. albicans*. Finally, the efficiency of fusion seems influenced by the media composition, suggesting that nuclear fusion in *C. albicans* may be regulated by external signals as well as by genetic background (3).

COMPLETING THE MATING CYCLE

Chromosome Loss in *C. albicans* Tetraploids

Mating of diploid *C. albicans* cells generates tetraploid cells that must undergo a reductional DNA division back to the diploid state to complete a sexual mating cycle. In most fungi this process occurs by meiosis, in which two consecutive rounds of DNA segregation take place following one round of DNA replication. However, in other fungi a reduction in ploidy can occur through chromosome loss during rounds of mitotic division, thereby forming a parasexual cycle (11, 15, 22). While no meiotic cycle in *C. albicans* has been identified (see below), a parasexual cycle involving efficient and cooperative chromosome loss has been described (2). Growth of tetraploid mating products of *C. albicans* at 37°C either on *S. cerevisiae* pre-sporulation (pre-spo) medium or on sorbose medium resulted in tetraploid strains losing chromosomes with high efficiency and apparently at random. Chromosome loss appeared concerted, as loss of one or more chromosomes seemed to predispose the cell to lose additional chromosomes. Tetraploid cells that had undergone chromosome loss demonstrated a range of ploidy, although in the study by Bennett & Johnson (2), approximately one third of the cells were diploid in DNA content, as determined by fluorescence-activated cell sorting and by analysis of genetic markers. Previous experiments had also showed instability of *C. albicans* tetraploids; in these cases the tetraploids were formed by fusion of diploid spheroplasts or protoplasts (24, 54). Subsequent treatment with heat shock or drug selection induced mitotic instability and chromosome loss, producing a number of diploid progeny.

The establishment of a simple parasexual cycle (see **Figure 10**) permits several basic genetic manipulations to be performed in *C. albicans*, including (*a*) linking a phenotype with a genotype, (*b*) showing genetic linkage, and (*c*) constructing new strains (2). In natural environments a parasexual cycle could also be used by *C. albicans* to generate phenotypic diversity. This could occur by mating between different strains or even by self-mating of identical, or closely related, strains (29). In the latter case, allelic differences between genes in the diploid genome of *C. albicans* could encode for proteins with subtly different properties. Shuffling of these alleles through a parasexual cycle could generate strains with different phenotypes, providing *C. albicans* with a genetic advantage during an infection. Many pathogens have mechanisms that introduce bursts of genetic variation during infection, and *C. albicans* could in principle use mating and the parasexual cycle for this purpose (29), although this has not been demonstrated experimentally.

The Search for Meiosis

Although the parasexual cycle of **Figure 10** provides many of the advantages of a true sexual cycle, it is possible that *C. albicans* can also undergo meiosis. Examination of the genome of *C. albicans* has identified orthologs of several genes involved in meiosis in other fungi, including *DMC1* (*DLH1*), *SPO11*, and *HOP1* (for a list of orthologs of meiosis-specific genes, see Reference 74). On the other hand, several important meiosis genes appear to be missing in the genome of *C. albicans*, suggesting that if meiosis occurs in *C. albicans*, its structure and regulation may significantly differ from that of other fungi (74).

RECENT DISCOVERY OF MATING IN OTHER *CANDIDA* SPECIES

C. albicans has a close relative, *Candida dubliniensis*, that shares many of the characteristics of *C. albicans*, including the bud-hypha transition, formation of chlamydospores, and phenotypic switching. In fact, these two fungi were recognized as distinct species only in

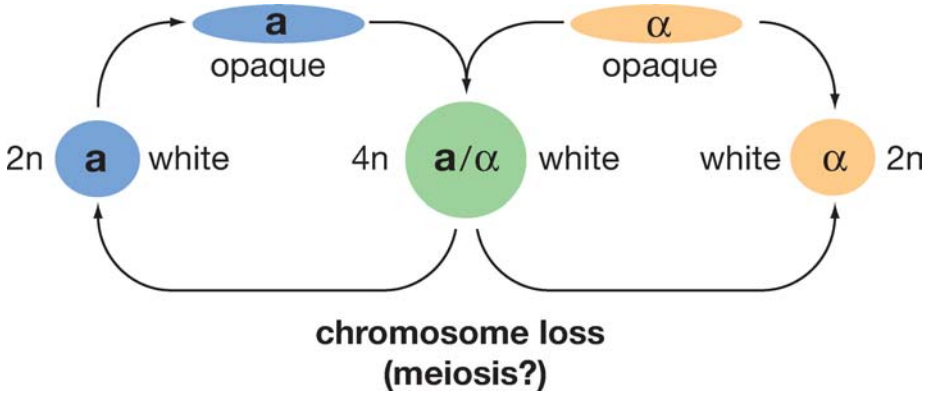

Figure 10

A parasexual cycle for *C. albicans*. Diploid **a** and α cells must first switch from the white phase to the opaque phase for mating. Mating of opaque cells generates a mononuclear, tetraploid **a**/α cell. A reduction in ploidy can be achieved by chromosome loss, regenerating **a** and α mating-competent progeny as part of a parasexual cycle. Note that a/α progeny (nonmating) are also formed via chromosome loss from the tetraploid (not shown). A meiotic program has not been identified in *C. albicans*.

1995 (69). Like *C. albicans*, *C. dubliniensis* **a** and α strains undergo the white-to-opaque transition, and opaque cells are competent for mating (55). However, the proportion of natural homozygous **a** and α strains in the *C. dubliniensis* population (33%) was significantly higher than that for *C. albicans* (3% to 7%). The close relationship between these *Candida* species was further demonstrated by the fact that mating (and karyogamy) could occur between them. The cell biology of interspecies mating resembled that described for *C. albicans* intraspecies mating (55). *C. dubliniensis* strains were also tested for mating on mouse skin, but unlike *C. albicans*, these strains showed no morphological changes and mating was not observed. In contrast, interspecies mating between *C. albicans* and *C. dubliniensis* occurred efficiently on mouse skin (55). Given the sequence divergence between the genomes of these two species, it seems either that interspecies mating does not occur in the wild or that it fails to generate progeny that can compete with the parental species for growth.

After *C. albicans*, *Candida glabrata* is the most common *Candida* species identified in humans (18). Like *C. albicans*, *C. glabrata* is a commensal in healthy individuals, but it can also cause mucosal and systemic infections. Despite the similarities in infection profile and name between *C. albicans* and *C. glabrata*, the latter is much more closely related to *S. cerevisiae* (see **Figure 5**). In keeping with this, it appears that *C. glabrata* has two silent mating cassettes and an active *MAT* locus and undergoes mating-type interconversion via an Ho-type endonuclease (66, 77). Mating has not been formally demonstrated in *C. glabrata*; however, its discovery seems likely in the near future.

FUTURE PERSPECTIVES

Since the discovery of mating in *C. albicans* five years ago, a great deal has been uncovered about this process. While some aspects of mating are similar to those of other fungi, others, most notably the incorporation of white-opaque switching, are limited to *C. albicans* or closely related species. Several possibilities exist that explain why mating in *C. albicans* is closely associated with white-opaque switching. First, it is possible

that white-opaque switching is used to direct mating to a specific host niche or microenvironment (e.g., the skin). The process of mating may make cells particularly susceptible to the host immune system, so *C. albicans* protects itself by spending most of its life in the white (nonmating) form. Alternatively, it is possible that the mating pathway has been retained in *C. albicans* not as a mechanism to generate recombinant progeny, but rather as a specialized program for regulating the white-to-opaque transition (46). This model is consistent with the population genetics, which suggests that recombination occurs rarely in natural isolates, with most strains propagating clonally. Finally, it is possible that white-opaque switching in *C. albicans* plays an as yet undiscovered role in pathogenesis, as the expression of nearly 400 genes differs between cells in the white and opaque phases.

Additional questions to be addressed concerning mating in *C. albicans* include: What is the molecular mechanism of white-opaque switching? What is the full range of physiological differences between white and opaque cells? Where are opaques formed in the wild, and where does mating occur? How (and why) is cell fusion and nuclear fusion regulated nutritionally? What is the role of the other genes (*PIK*, *PAP*, and *OBP*) at the mating-type locus? Does *C. albicans* undergo meiosis? Is chromosome loss used as an alternative to meiosis for completing a mating cycle? How is mating related to filamentation and virulence? Given the importance of *C. albicans* as a human pathogen, and the dual role that white-opaque switching plays in virulence and mating, these answers cannot help but to illuminate the long coevolution of this pathogen and its mammalian host.

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