Evidence for Mating of the "Asexual" Yeast *Candida albicans* in a Mammalian Host

Christina M. Hull,* Ryan M. Raisner,* Alexander D. Johnson†

Since its classification nearly 80 years ago, the human pathogen *Candida albicans* has been designated as an asexual yeast. In this report, we describe the construction of *C. albicans* strains that were subtly altered at the mating-type–like (*MTL*) locus, a cluster of genes that resembles the mating-type loci of other fungi. These derivatives were capable of mating after inoculation into a mammalian host. *C. albicans* is a diploid organism, but most of the mating products isolated from a mouse host were tetrasomic for the two chromosomes that could be rigorously monitored and, overall, exhibited substantially higher than 2n DNA content. These observations demonstrated that *C. albicans* can recombine sexually.

The yeast *Candida albicans* is found as a commensal organism in the digestive tract of mammals. It is also the most common human fungal pathogen, causing both mucosal and systemic infections, particularly in immunocompromised people (*1*). *C. albicans*, a diploid yeast, has been classified as asexual because no direct observation of mating or meiosis has been reported. Population studies of *C. albicans* indicate that some low level genetic exchange may occur (*2*), but there is no conclusive evidence for recent sexual recombination (*3*–*5*). The apparent absence of sexual reproduction in *C. albicans* is especially intriguing because its relatives in the budding yeast family (e.g., *Saccharomyces cerevisiae* and *Kluyveromyces lactis*) have retained sexual cycles. In fact, sexual reproduction is common throughout the fungal kingdom, ranging from yeasts to mushrooms.

Although mating and meiosis have not been observed for *C. albicans*, homologs of genes that function in both mating [e.g., *GPA1* (*6*), *STE20* (*7*), and *STE6* (*8*)] and meiosis [e.g., *DMC1* (*9*)] in *S. cerevisiae* have been identified in *C. albicans*. These genes are intact and code for well-conserved proteins, and several have been shown to complement *S. cerevisiae* mutants. The full range of the functions of these gene products in *C. albicans* has not been determined, but their presence suggests that *C. albicans* has a cryptic sexual cycle or that *C. albicans* lacks a sexual cycle and these conserved gene products have been coopted for different purposes.

Sexual reproduction in fungi is typically controlled by genes that reside in a specified genetic locus called a mating-type, or *MAT*, locus. Two features distinguish this locus from other portions of the genome. First, in diploid cells, the *MAT* locus is usually heterozygous; it codes for different (but usually related) genes on each of the two homologous chromosomes. Second, the genes encoded by the *MAT* locus of different fungi generally fall into three specific categories: DNA binding proteins that regulate the expression of sexual cycle genes, structural genes that code for mating pheromones, and structural genes that code for mating pheromone receptors (*10*–*13*).

The genes in the *MAT* locus of the sexually reproducing yeast *S. cerevisiae* have been well characterized (*11*, *14*). They encode three transcriptional regulatory proteins that, together with other proteins encoded elsewhere in the genome, control the transcription of many target genes. The patterns of expression of these target genes give rise to three distinct types of cells (**a**, α, and **a**/α) that are responsible for the *S. cerevisiae* sexual cycle. The two types of mating cells (**a** and α) are typically haploid and carry different genetic information at the *MAT* locus: **a** cells carry *MATa*, and α cells carry *MAT*α. *MATa* codes for a single regulatory protein (the homeodomain protein a1), and *MAT*α codes for two proteins (the homeodomain protein α2 and the alpha domain protein α1). Mating between an **a** cell and an α cell forms the **a**/α cell, which is usually diploid, carries both *MATa* and *MAT*α, and is capable of undergoing meiosis and spore formation.

We recently described a mating-type–like (*MTL*) locus in *C. albicans* (Fig. 1) that resembles the mating-type (*MAT*) locus of the sexually reproducing yeast *S. cerevisiae* in two important respects: First, it encodes proteins similar to the three transcriptional regulators encoded by *MAT* (a1, α1, and α2), and second, it is heterozygous in the diploid laboratory strain of *C. albicans* that we analyzed (SC5314), with an a1-like gene carried on one chromosome and α1- and α2-like genes carried on the other. Other similarities in the arrangement of the genes, the positions of introns, and the function of the a1 protein (it is a transcriptional repressor in both organisms) further support a close connection between the *MTL* locus of *C. albicans* and the *MAT* locus of *S. cerevisiae* (*15*).

Like its counterpart in *S. cerevisiae*, the *MTL* locus could regulate a sexual cycle in *C. albicans*. If the analogy between the *MTL* locus in *C. albicans* and the *MAT* locus of *S. cerevisiae* holds, then the SC5314 laboratory strain of *C. albicans* would be an **a**/α strain and would not be expected to mate. To test the hypothesis that *C. albicans* has the inherent capacity to mate, we genetically altered an SC5314 derivative (CAI4) to create two types of "**a**" strains and two types of "α" strains. For the **a** strains, either the entire *MTL*α locus was deleted from CAI4 (to give an *MTLa/mtl*αΔ strain) or only the α*1* and α*2* genes were deleted (to give an *MTLa/mtl*α*1mtl*α*2* strain). Likewise, α strains

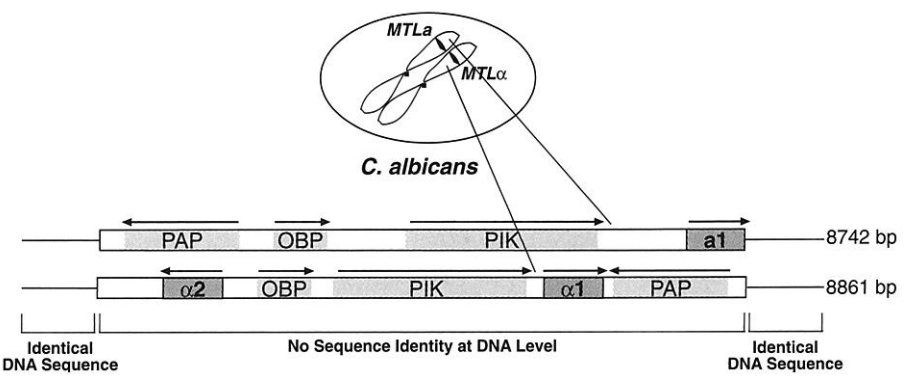

Fig. 1. Features of the *C. albicans* mating-type–like (*MTL*) locus (*15*). The *C. albicans MTL* locus contains open reading frames for nine proteins from four families of proteins: three gene regulatory proteins (**a**1, α1, and α2), two phosphatidylinositol kinases (PIK), two oxysterol-binding proteinlike proteins (OBP), and two poly(A) polymerases (PAP). The region of DNA sequence that differs between the *MTLa* and *MTL*α segments is 8742 base pairs (bp) for *MTLa* and 8861 bp for *MTL*α.

Department of Microbiology and Immunology and Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: ajohnson@socrates.ucsf.edu

were constructed either by deleting the entire *MTLa* locus (*mtlaΔ/MTLα*) or by deleting only the *a1* gene (*mtla1/MTLα*) (*15*, *16*). In addition, *ade2/ade2* (Ade^-Ura^+) and *ura3/ura3* (Ade^+Ura^-) derivatives of these strains were constructed to allow successful mating events to be detected by selecting for cells with the ability to grow on media lacking adenine and uracil (Ade^+Ura^+ prototrophs) (*16*).

To test for mating, we mixed various strains as outlined in Fig. 2A. In addition to mixtures that contained putative mating pairs (an **a** strain and an α strain), we also included pairs that would not be expected to mate, for example, an *MTLa/MTLα ura3/ura3* and an *MTLa/MTLα ade2/ade2* (mix 1, Fig. 2A). Because mammals are natural hosts for *C. albicans*, we introduced the combinations of strains into mice through tail vein injection (*17*). Pilot experiments had revealed that, after injection, the *ade2/ade2* strains persisted in mice at substantially lower levels than did the *ura3/ura3* strains (*18*). To compensate for this difference, we injected three times as many *ade2/ade2* cells as *ura3/ura3* cells for each test mating. Twenty-four hours after injection, the mice were euthanized, the kidneys were removed and homogenized, and the mixture was plated on various media. For all the test matings, about 10^3 *C. albicans* colonies per kidney formed on rich medium, conditions under which all the starting strains readily grow. On medium lacking adenine and uracil, we recovered 44 colonies from the **a** + α mix (*MTLa/mtlα1mtlα2* + *mtla1/MTLα*) (mix 2 of Fig. 2A), one colony from the mixture containing the **a** and α locus deletion strains (*mtlaΔ* + *mtlαΔ*) (mix 3 of Fig. 2A), and no colonies from the control mix (mix 1 of Fig. 2A). Additional test matings revealed similar results (*19*). Other experiments showed that prototrophs did not appear when comparable titers of the starting strains were simply mixed together and incubated on various types of laboratory media and then plated to selective medium (*20*).

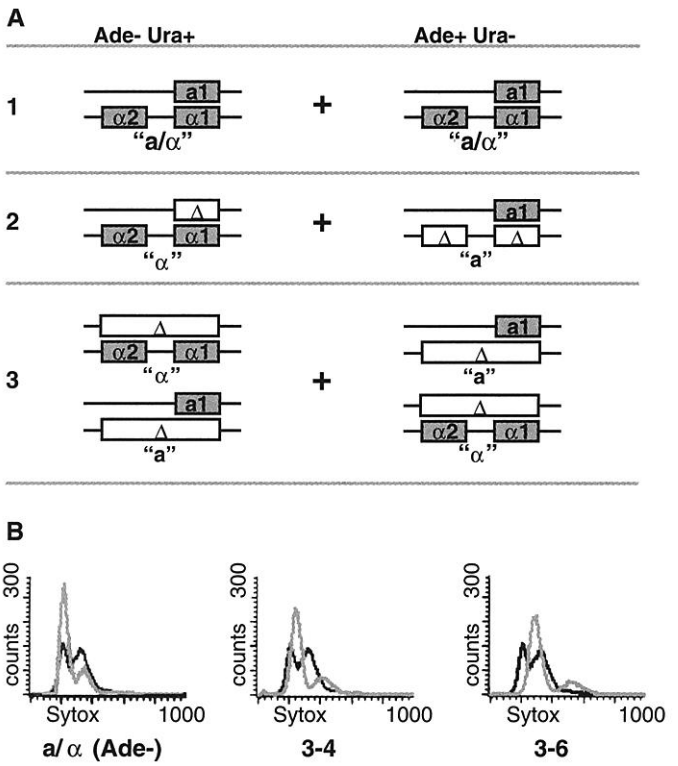

Fig. 2. (**A**) *MTL* configurations of strains used in test mating experiments. Strains containing different deletions at the *MTL* locus were mixed together and tested for mating in a mouse tail vein model. The *MTLa* and *MTLα* configurations for the different mutants are represented schematically for each of three different injection mixes labeled 1 though 3. Each *MTL* configuration has been labeled "**a**" or "α" to indicate the analogous behavior of a similarly modified diploid in *S. cerevisiae*. Mix 1, intact *MTL* Ade^- + intact *MTL* Ura^-; mix 2, *mtla1* deletion Ade^- + *mtlα1mtlα2* deletion Ura^-; mix 3, *mtlaΔ* Ade^-, *mtlαΔ* Ade^- + *mtlαΔ* Ura^-, *mtlaΔ* Ade^- (*19*). (**B**) FACS analysis of prototrophs. Strains were analyzed by fluorescence-activated cell scan for DNA fluorescence. The *x* axis of each graph (Sytox) represents a logarithmic scale of fluorescence, and the *y* axis (counts) represents a linear scale of cell number. In each case, the control strain (CAI4) is in black, and the test strain is in gray. The first graph [a/α (Ade^-)] shows an overlay of an Ade^- *MTLa/MTLα* strain over the Ura^- *MTLa/MTLα* strain (CAI4). The *mtla1* and *mtlα1α2* starting strains gave similar profiles (*18*). The graphs labeled 3-4 and 3-6 show profiles for two of eighteen prototrophs tested and represent the majority of the FACS profiles, showing an increase in fluorescence that is consistent with an increase in DNA content (*21*).

After the Ade^+Ura^+ phenotype of the prototrophs was confirmed by purifying for single colonies on selective medium, they were subjected to several tests. In a representative sample, treatment with serum elicited rapid germ tube formation, indicating that the prototrophs were indeed *C. albicans* (*18*). Fluorescence-activated cell sorting (FACS) analysis suggested that cells from most of the colonies tested (12 of 15) had substantially increased DNA content compared with that of the starting strains (Fig. 2B) (*21*). DAPI (4′,6′-diamidino-2-phenylindole) staining showed the presence of a single nucleus in the cells of all of the prototrophs (*21*).

We next determined whether the prototrophs carried genetic markers derived from both parents. For the *MTLa/mtlα1mtlα2* × *mtla1/MTLα* cross (mix 2 of Fig. 2A), all four alleles of *MTL* were distinguishable, two from each starting strain (*22*). Southern analysis (*23*) of 16 of the prototrophs recovered from this experiment revealed that 12 strains carried all four alleles of *MTL* [*MTLa/mtla1/MTLα/mtlα1mtlα2* (Fig. 3, examples shown in lanes 10 through 16)] and four strains carried three alleles of *MTL* (Fig. 3, example shown in lane 9). These four strains represent three of the four possible *MTL* locus combinations (*MTLa/mtla/MTLα*, *MTLa/mtla/mtlα*, and *MTLa/MTLα/mtlα*). The starting strains each carried only one version of *MTLa* and one version of *MTLα* (Fig. 3, lanes 7 and 8); hence, all the prototrophs contained genetic information from both of the parent strains, and we will hereafter refer to them as conjugants. "Wild" strains of *Candida* could not have confounded this analysis because two of

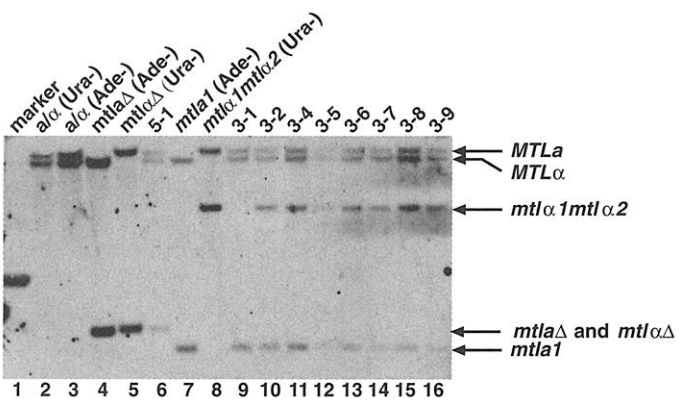

Fig. 3. Southern blot confirming the presence of three or more alleles of *MTL* in the conjugant strains. *C. albicans* genomic DNA was isolated from conjugants and subjected to restriction enzyme digestion before electrophoresis. Lane 1, marker lane; lane 2, *MTLa/MTLα ura3/ura3* auxotroph; lane 3, *MTLa/MTLα ade2/ade2* auxotroph; lane 4, *mtlaΔ/MTLα ade2/ade2* auxotroph; lane 5, *MTLa/mtlαΔ ura3/ura3 2*; lane 6, prototrophic conjugant from mating test 3 of Fig. 2A; lane 7, *mtla1/MTLα ade2/ade2* auxotroph; lane 8, *MTLa/mtlα1mtlα2 ura3/ura3* auxotroph; lane 9, prototrophic conjugant from mating test mix 2 of Fig. 2A showing the presence of three configurations of *MTL*; lanes 10 to 16, prototrophic conjugants from mating test 2 of Fig. 2A showing the presence of four configurations of *MTL*. See (*25*) for details.

the *MTL* alleles in this experiment (one from each starting strain) were created for use in this experiment and do not exist outside the laboratory.

The strain recovered from the *MTL* locus deletion experiment (mix 3 of Fig. 2A) was also analyzed and found to carry *MTLa*, *MTL*α, and at least one *mtl* deletion. (DNA restriction fragments for the *mtla* deletion and the *mtl*α deletion are identical in size and could not be distinguished in this experiment.) Again, because none of the starting strains in this test mix carried both *MTLa* and *MTL*α (Fig. 3, lanes 4 and 5). We can conclude that the conjugant carried genetic information from each parent. Moreover, although there was a mixture of several different strains injected in this experiment (mix 3 of Fig. 2A), the conjugant must have formed from one **a** parent and one α parent.

The same type of marker analysis was performed on the conjugants for the *ADE2* locus, which is located on a different chromosome from that of *MTL* (*24*). Of the four *ADE2* alleles that entered the host, three can be unambiguously identified: The two *ade2* disruptions can be distinguished from each other and from the intact *ADE2* alleles, but the two intact *ADE2* alleles are indistinguishable (*22*). Southern analysis indicated that all of the conjugant strains carried all three of the distinguishable alleles of *ADE2* (examples shown in Fig. 4); moreover, the normalized ratio of the signal of the intact *ADE2* gene to that of either disruptant was about 2:1 in at least four of the conjugants (3-4, 3-6, 3-1, and 3-3) (*18*), consistent with the idea that these conjugants carried two copies of the intact *ADE2* gene and one copy each of the two disrupted alleles. Because all the strains in this experiment have the same type of disruption at the *ura3* locus, the analysis of this locus is not informative; the Ura^+ parental strains have *URA3* integrated at the *ade2* locus.

In summary, all the prototrophs we recovered from the "**a**" × "α" crosses contain genetic markers from both parental strains. For the *MTLa/mtl*α*1mtl*α*2* × *mtla1/MTL*α cross (mix 2, Fig. 2A), 12 of 16 prototrophs tested contain all four alleles of the *MTL* locus, two derived from each parent. The simplest interpretation of this result is that the two diploid parental strains mated to form a tetraploid cell. The four prototrophs recovered from this cross that lacked one *MTL* allele probably arose through chromosome loss or homozygosis of the *MTL* locus after mating, perhaps during the passaging of strains on laboratory media required for their analysis. Although not as definitive as the analysis of the *MTL* locus, the analysis of the *ADE2* locus is consistent with the idea that the prototrophs arose from the formation of a tetraploid strain from the two diploid parents. To date, mating has been detected in six independent crosses; in all cases, mating was observed only between an "**a**" and an "α" strain. Control crosses ("**a**" × "**a**," "α" × "α," and "**a**/α" × various strains) did not produce Ade^+Ura^+ prototrophs in the same experiments.

Although we cannot rigorously conclude that successful conjugation occurs only when an "**a**" strain is crossed with an "α" strain, this is the only combination we have observed to date, and the results correlate well with this idea. This strong correlation supports the idea that the observed conjugation arose from bona fide mating and not from nonspecific cell and nuclear fusion events, the latter would be expected to occur between any two strains irrespective of the *MTL* configuration. *C. albicans* appears inherently able to mate and raises the question of why this appears to happen so rarely in nature. Assuming that the *MTLa/MTL*α configuration of *MTL* (as found in SC5314) is the prevalent wild form in *C. albicans*, it is possible that mating requires "**a**" and "α" strains to arise by homozygosis of the *MTL* locus or by chromosome loss. These derivatives may be lost quickly from natural populations, and without laboratory intervention, the appropriate pairs of **a** and α cells may arise in the same host only rarely.

These data raise the possibility that *C. albicans* has a complete sexual cycle, perhaps one in which diploids can mate to form tetraploids that can undergo meiosis to produce diploids. Experiments are under way to test for meiosis in tetraploid strains of *C. albicans*. It is also possible that after mating, chromosomes could be lost from tetraploids, gradually reducing them to the diploid state. The recovery of conjugants that appear trisomic for the *MTL*-containing chromosome locus provides some support for this notion. Finally, the fact that mating was observed in mice but not on a variety of laboratory media suggests the possibility that a signal or condition conducive to mating is provided by the mammalian host.

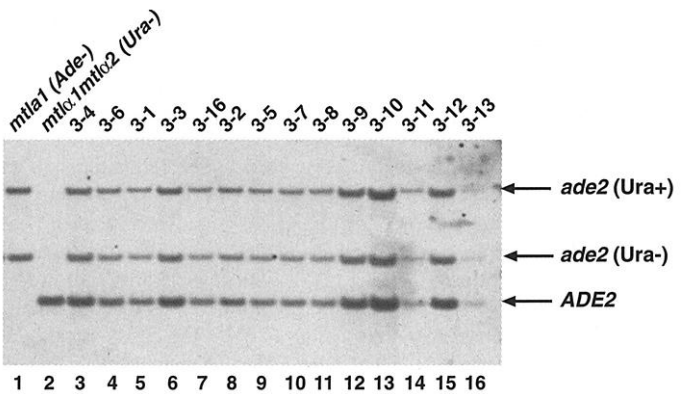

Fig. 4. Southern blot confirming the presence of at least three alleles of *ADE2* in the conjugant strains. *C. albicans* genomic DNA was isolated from conjugants and subjected to restriction enzyme digestion before electrophoresis. Lane 1, *mtla1/MTL*α *ade2/ade2* auxotroph; lane 2, *mtla*Δ*/MTL*α *ura3/ura3* auxotroph; lanes 3 to 16, prototrophic conjugants from mating test mix 2 of Fig. 2A showing the presence of at least three configurations of *ADE2*. See (*25*) for details.

References and Notes

1. F. C. Odds, *Candida and Candidosis* (Bailliere Tindall, London, ed. 2, 1988).
2. Y. Graser *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12473 (1996).
3. M. Tibayrenc, *Trends Microbiol.* **5**, 253 (1997).
4. R. Vilgalys, Y. Graser, G. Schonion, W. Presber, T. Mitchell, *Trends Microbiol.* **5**, 254 (1997).
5. J. W. Taylor, D. M. Geiser, A. Hurt, V. Koufopanou, *Clin. Microbiol. Rev.* **12**, 126 (1999).
6. C. Sadhu, D. Hoekstra, M. J. McEachern, S. I. Reed, J. B. Hicks, *Mol. Cell. Biol.* **12**, 1977 (1992).
7. E. Leberer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13217 (1996).
8. M. Raymond *et al.*, *Mol. Microbiol.* **27**, 587 (1998).
9. A. C. Diener and G. R. Fink, *Genetics* **143**, 769 (1996).
10. L. A. Casselton and N. S. Olesnicky, *Microbiol. Mol. Biol. Rev.* **62**, 55 (1998).
11. I. Herskowitz, J. Rine, J. Strathern, in *Molecular and Cellular Biology of the Yeast Saccharomyces*, E. W. Jones, J. R. Pringle, J. R. Broach, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992), vol. 2, pp. 583–656.
12. J. W. Kronstad and C. Staben, *Annu. Rev. Genet.* **31**, 245 (1997).
13. B. G. Turgeon, *Annu. Rev. Phytopathol.* **36**, 115 (1998).
14. A. D. Johnson, *Curr. Opin. Genet. Dev.* **5**, 552 (1995).
15. C. M. Hull and A. D. Johnson, *Science* **285**, 1271 (1999).
16. The construction of disruption plasmids for *MTL*, *MTL*α*1*, and *ADE2* is described on *Science* Online (*25*). Ura^-Ade^+ strains were created through counter selection on 5-fluoroorotic acid to eliminate the *URA3* marker gene from the penultimate disruption strain. Ade^-Ura^+ strains were created by deleting both copies of *ADE2* in Ura^- strains and allowing the cells to retain *URA3* after the second round of transformation. Because *C. albicans* does not contain silent cassettes of *MTL* (*15*), the various *MTL* deletions would be expected to be sufficient to create strains analogous to **a** and α.
17. At least two female Balb-C mice for each test mating combination were injected with 2.5×10^7 yeast cells into the lateral tail vain. Cells for injection were grown to saturation at 30°C and then back-diluted and grown for 4 hours to mid logarithmic phase. The cells were pelleted, resuspended in 0.9% saline, and counted by hemacytometer. Mice were euthanized after 24 hours, and the kidneys were removed, homogenized, and plated in fractions onto standard synthetic medium plus dextrose (SD)-Ade-Ura plates and incubated at 30°C. A fraction of each sample was also plated to medium composed of yeast extract, peptone, plus dextrose (YPD) for total colony counts of recovered cells.
18. C. M. Hull, R. M. Raisner, A. D. Johnson, unpublished data.
19. Data from additional test matings can be found on *Science* Online (*25*).
20. Supplemental information on the in vitro conditions tested is available on *Science* Online (*25*).
21. Supplemental data and protocol information on the

FACS and DAPI analyses are available on *Science* Online (*25*).
22. Additional *MTL* and *ADE2* Southern blot information is available on *Science* Online (*25*).
23. Supplemental information on the isolation of genomic DNA and Southern blot protocol and probes is available on *Science* Online (*25*).
24. Chromosome information was obtained from alces.med.umn.edu/Candida.html. Specific assignments were made by the Stanford *Candida albicans* sequencing project. Sequence data for *C. albicans* were obtained from the Stanford DNA Sequencing and Technology Center Web site at www-sequence.stanford.edu/group/candida.
25. Supplemental data are available at www.sciencemag.org/feature/data/1049869.shl.
26. The authors acknowledge V. Nguyen and additional members of the J. Li laboratory (University of California, San Francisco) for assistance with the FACS analysis, A. Uhl, I. Herskowitz, D. Ganem, and R. Brazas for helpful comments, and D. Inglis and additional members of the Johnson Laboratory for continuing support and assistance. Sequencing of *C. albicans* by the Stanford DNA Sequencing and Technology Center was accomplished with the support of the NIDR and the Burroughs Wellcome Fund. This work was supported by NIH grant GM37049 and a Burroughs Wellcome Merit Award to A.D.J.

25 February 2000; accepted 25 May 2000

Induction of Mating in *Candida albicans* by Construction of *MTL*a and *MTL*α Strains

B. B. Magee and P. T. Magee*

Although the diploid fungus *Candida albicans*, a human pathogen, has been thought to have no sexual cycle, it normally possesses mating-type–like orthologs (*MTL*) of both of the *Saccharomyces cerevisiae* mating-type genes (*MAT*) a and α. When strains containing only *MTL*a or *MTL*α were constructed by the loss of one homolog of chromosome 5, the site of the *MTL* loci, *MTL*a and *MTL*α strains mated, but like mating types did not. Evidence for mating included formation of stable prototrophs from strains with complementing auxotrophic markers; these contained both *MTL* alleles and molecular markers from both parents and were tetraploid in DNA content and mononucleate.

C. albicans has become the third or fourth most common nosocomial isolate in many hospitals, and systemic infection has a mortality rate of 30 to 70% (*1*). This yeast is almost always diploid as isolated (*2*) and until now was thought to lack a sexual phase (*3*).

Several hypotheses have been put forward for the way in which *C. albicans* generates genetic variability, including phenotypic transition, or switching, extensively studied in the Soll laboratory (*4*), and chromosomal rearrangements (*5*, *6*). Both are associated with changes in the phenotype, but would be expected to yield less variability than sexual recombination.

Evidence for asexuality in *C. albicans* consists of the failure of many laboratories to find either mating or sporulation, the frequent isolation of strains with extensive chromosomal translocations that would lead to aneuploid progeny after meiosis (*7*, *8*), and evidence for linkage disequilibrium, typical of clonal inheritance (*9*).

However, with the sequencing of the *C. albicans* genome, evidence has emerged suggesting that sexual recombination has occurred relatively recently in this fungus. This evidence includes the discovery of orthologs of most, if not all, of the genes involved in mating and sporulation in *Saccharomyces cerevisiae*, including genes for pheromone receptors and heterotrimeric GTP-binding proteins, and specifically the demonstration, with information from the Stanford Genome Center *Candida albicans* Sequencing Project (*10*), that at least one strain of *C. albicans* is heterozygous for orthologs of the two *S. cerevisiae* mating types *MAT*a and *MAT*α. These loci are called mating-type–like (*MTL*) **a** and α in *C. albicans* (*11*). There is also evidence for a lack of linkage of some molecular chromosomal markers (*12*).

Taking advantage of the location of the *MTL* locus on a chromosome, 5, that can be reduced to monosomy by changing the simple growth conditions (*13*), we constructed several sets of *C. albicans* strains that lack either the **a** or the α *MTL* allele; these strains mated in **a**-α combinations to yield tetraploid recombinants.

C. albicans is normally unable to grow on sorbose (Sou^-). The Sou^+ variants, which appear on the background of nongrowing cells plated on sorbose as a sole source of carbon, are monosomic for chromosome 5; furthermore, either homolog can be lost under these conditions (*13*). Using this technique, we isolated Sou^+ derivatives of strains CAI-4, RM1000, BWP17, B23, and B26 lacking either *MTL*a or *MTL*α as shown by Southern blots (*14*) (Fig. 1A, lanes 3, 5, 7, 9, 10, 12, and 13). Table 1 summarizes the properties of the strains used in the experiments described below.

If strains containing only one allele of the *MTL* locus are mating-competent, selectable prototrophic conjugants should arise from complementation of the various auxotrophic markers in the two sets of strains. The hemizygotic strains (or homozygotic; under nonselective conditions the strains often duplicate the remaining homolog of chromosome 5 and become homozygous for the entire chromosome) listed in Table 1 were grown on YEPD (yeast extract–peptone–dextrose) (*15*) plates until they were in stationary phase. They were cross-streaked on YEPD, YPB (YEPD plus phloxine B), and PD (potato dextrose agar) plates, incubated for 36 hours, and replica-plated onto minimal medium. In each case, several prototrophic colonies grew from one of the cross-streaked areas where potential complementation products would be formed by an **a** × α mating. Prototrophs formed by mating of the *MTL*a × *MTL*α strains should contain both *MTL* alleles. The cells were restreaked on minimal medium. Polymerase chain reaction (PCR) revealed that the prototrophs possessed both *MTL* alleles (*16*) and that, although they were able to grow on medium lacking uridine, they contained the λ_{imm434} fragment which replaces the *URA3* locus in CAI4 (*17*, *18*). Thus, the prototrophic cells contained genomic material from both parents (Fig. 1A, lanes 14 and 15).

More efficient mating was found to occur when the parents were allowed to remain in contact on nonselective medium for 3 to 12 days before replication onto minimal (selective) medium (*19*). Under this regimen, profuse growth occurred on the selective medium, in crosses between *MTL*a- *MTL*α strains but not between those of like mating type. More prototrophs were formed when mating plates were incubated at room temperature than at 30° or 37°C (Fig. 2A). Mating at any temperature is a slow process, with the yield of recombinants rising from a few after 4 days on nonselective medium to many at 8 days (Fig. 2B).

To verify that the recombinants were genetically prototrophic and not the result of cross-feeding or necrophagy of dead cells at the cross-streak intersection, we restreaked them onto minimal medium. The resulting large colonies were chosen to prepare DNA. The small colonies were revertants of the MG30 derivatives, and one revertant (X28)

Department of Genetics, Cell Biology and Development, University of Minnesota, 1445 Gortner Avenue, St. Paul, MN 55108, USA.

*To whom correspondence should be addressed. E-mail: ptm@biosci.cbs.umn.edu