membranes. The presence of a putative synaptotagmin isoform (70 kD) in CHO cells was detected with a polyclonal antiserum to the C2B domain of synaptotagmin (Fig. 4A). GST-C2B blocked this immunoreactivity (Fig. 4B). CHO cells were bound to poly-D-lysine–coated beads, ruptured by sonication, and washed to generate bead-attached plasma membrane sheets with their cytosolic face exposed to the medium (*23*) (Fig. 4C). Plasma membrane–coated beads were then incubated with rat brain cytosol in the presence or absence of the YQRL or AQRL peptides. Recruitment of AP-2 was stimulated by the YQRL peptide, but not by its AQRL mutant (Fig. 4D) (*24*). The YQRL peptide also increased the amount of the putative synaptotagmin isoform recovered in anti–AP-2 immunoprecipitates from CHO cell extracts (Fig. 4E). These data show that in non-neuronal cells, YXX∅-based endocytic motifs cooperate with a docking site, which most likely includes synaptotagmin, in recruiting AP-2 to the membrane.

Binding of YXX∅-based endocytic motifs to AP-2 may increase AP-2's affinity for synaptotagmin and thereby facilitate coated pit formation. A similar mechanism for coupling coat nucleation and cargo protein selection has been reported for the assembly of COPII-coated buds at the endoplasmic reticulum (*25*). Thus, the common picture that emerges from these studies is that cargo proteins participate in the nucleation of coat assembly and facilitate their own sorting into transport vesicles.

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27. AP-2 and AP-1 adaptor proteins were prepared by separation of the clathrin coat proteins (3 mg/ml) on a Sepharose CL4B gel filtration column in 0.5 M tris-HCl pH 7.4. The adaptor (AP-2 plus AP-1) and clathrin peaks were pooled and concentrated to 0.5 mg/ml and 1 mg/ml, respectively, and either used directly or frozen in liqid nitrogen and stored at–70°C.
28. LP_2 membranes (1 mg; at 2 mg/ml) were prepared from a total rat brain homogenate, mixed with 2 mg of dialyzed rat brain cytosol (20 mg/ml) (*11*), solubilized in 1% triton X-100 containing cytosolic buffer (*21*), and cleared by centrifugation. To examine the effect of nucleotides or PLD, we first incubated the LP_2-cytosol mix for 10 min at 37°C with these reagents before solubilization in 1% triton X-100 containing cytosolic buffer (*21*).

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Identification of a Mating Type–Like Locus in the Asexual Pathogenic Yeast *Candida albicans*

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Candida albicans, the most prevalent fungal pathogen in humans, is thought to lack a sexual cycle. A set of *C. albicans* genes has been identified that corresponds to the master sexual cycle regulators a1, α1, and α2 of the *Saccharomyces cerevisiae* mating-type (*MAT*) locus. The *C. albicans* genes are arranged in a way that suggests that these genes are part of a mating type–like locus that is similar to the mating-type loci of other fungi. In addition to the transcriptional regulators a1, α1, and α2, the *C. albicans* mating type–like locus contains several genes not seen in other fungal *MAT* loci, including those encoding proteins similar to poly(A) polymerases, oxysterol binding proteins, and phosphatidylinositol kinases.

The yeast *C. albicans* is the most common human fungal pathogen causing most cases of oral and vaginal thrush as well as severe mucosal and systemic infections in immunocompromised individuals (*1*). A principle difficulty in studying *C. albicans*, compared with other yeasts, is that *C. albicans* has no known sexual cycle and is therefore not amenable to conventional genetic analysis. It is a diploid organism for which no haploid state has been observed, nor has any process resembling meiosis or spore formation been detected. Sexual reproduction in fungi is typically controlled by genes that reside in a genetic locus called a mating-type, or *MAT*, locus. In the budding yeast *Saccharomyces cerevisiae*, the genes residing at the *MAT* locus can be either the **a** type or the α type. These genes, which code for transcriptional regulators, specify the three cell types involved in the *S. cerevisiae* sexual cycle (*2*, *3*).

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A cell containing only the *MATa* locus is an **a** cell; a cell containing only the *MAT*α locus is an α cell; and a cell that contains both *MATa* and *MAT*α (typically diploid and therefore heterozygous at the *MAT* locus) is an **a**/α cell. *MATa* codes for the homeodomain protein **a**1, which has no known function in **a** cells. *MAT*α codes for a homeodomain protein (α2) and an α-domain protein (α1) that cause the repression of **a**-specific genes and the activation of α-specific genes, respectively (Fig. 1A). Because the **a**- and α-specific genes encode proteins required for each of the cell types to mate, these changes in gene expression differentiate the **a** cell from the α cell. The product of a successful mating is an **a**/α diploid cell in which **a**1 and α2 are both expressed. The **a**1 and α2 proteins are the key regulators of the **a**/α cell type, and together they bind to a specific DNA sequence to repress the transcription of many target genes (including α1). This **a**1/α2 regulatory activity shuts off the ability of cells to mate and at the same time permits meiosis and sporulation in the presence of the appropriate external nutritional signals.

Shown in Fig. 1B are two genomic fragments from *C. albicans* that contain clusters of genes that bear a marked resemblance to the *MATa* and *MAT*α genes of *S. cerevisiae* (denoted mating type–like, or *MTLa* and *MTL*α, respectively). The *MTLa* gene cluster was obtained by chromosome walking with a lambda library of *C. albicans* genomic fragments. The beginning probe for the walk was based on a sequence trace from the Stanford *C. albicans* Sequencing Project that resembled a portion of the *S. cerevisiae MATa1* gene. *MTL*α was obtained by walking downstream of *MTLa* to its flanking DNA sequence and then back into and through *MTL*α (*4*, *5*). *MTLa* contains four open reading frames (ORFs) that encode a gene regulatory protein, a poly(A) polymerase, an oxysterol binding protein–like protein, and a phosphatidylinositol kinase. *MTL*α contains four genes whose products are closely related to those in *MTLa* plus an additional ORF coding for another gene-regulatory protein. The DNA sequences within *MTLa* and *MTL*α are ~48% identical overall; however, the DNA sequences flanking them are greater than 99% identical. We have defined the borders of *MTLa* and *MTL*α as the points at which their DNA sequences become greater than 99% identical.

Although the clusters of genes in the *MTL* locus are much larger than those of the *S. cerevisiae MAT* locus (9 versus 0.7 kb), three features of the *MTL* locus in *C. albicans* are markedly similar to those of the *MAT* locus in other fungi, especially *S. cerevisiae* (Fig. 1). First, three of the proteins coded for by the *C. albicans* locus have predicted amino acid sequences very similar to those of the transcriptional regulators **a**1, α1, and α2 encoded by the *S. cerevisiae MAT* locus. The *C. albicans MTLa* segment codes for a homeodomain protein similar in sequence to the *S. cerevisiae* **a**1 protein (30% identity and 56% similarity over the entire protein and 43% identity and 59% similarity in the homeodomain region). The *C. albicans MTL*α segment codes for an α-domain protein similar to the *S. cerevisiae* α1 protein and for a homeodomain protein similar to the *S. cerevisiae* α2 protein. The predicted *C. albicans* α1 protein is 26% identical (49% similar) to the *S. cerevisiae* α1, and the *C. albicans* α2 is 28% identical (58% similar) to the *S. cerevisiae* α2 protein, with particularly strong similarity seen in the homeodomain region (44% identity and 69% similarity) (Fig. 2).

A second similarity between the *C. albicans MTL* and *S. cerevisiae MAT* loci concerns the overall organization of these three genes. As is true for the *S. cerevisiae* genes, the *C. albicans MTL*α*1* and *MTL*α*2* genes are transcribed divergently from one chromosome, and the *MTLa1* gene is found on the other chromosome (Fig. 1). This feature is particularly notable because gene order and organization are not generally conserved between *C. albicans* and *S. cerevisiae* (*6*, *7*).

A third similarity between *C. albicans* and *S. cerevisiae* is the conserved positions of the introns in the *MATa1* and *MTLa1* genes. In *S. cerevisiae*, the *MATa1* gene is one of only a few genes interrupted by two introns (*8*). The *C. albicans MTLa1* gene also appears to be interrupted by two introns, one of which is in the same position (in the "recognition" helix of the homeodomain) as that in the *S. cerevisiae MATa1* (Fig. 2). The locations of the introns in the *C. albicans MTLa1* gene were initially predicted from the DNA sequence and were verified by observing the sizes of reverse transcription–polymerase chain reaction (RT-PCR) products (*9*). In *S. cerevisiae*, the *MAT*α*2* gene is free of introns, but the *C. albicans MTL*α*2* gene contains a single intron located in the same position within the recognition helix of the homeodomain as the COOH-terminal introns in the *C. albicans MTLa1* and *S. cerevisiae MATa1* genes.

Candida albicans is diploid, and several lines of evidence support the idea that *MTLa* and *MTL*α reside at the same position on homologous chromosomes. Two types of *C. albicans MTLa1* deletion mutants were constructed (a complete deletion of the *MTLa1* ORF and a deletion of only the homeodomain) by homologous replacement by disrupted genes (*10*). After a single round of transformation (*11*), the resultant strains were tested by PCR (*12*) and Southern (DNA) analysis (*9*). The mutant forms of the *MTLa1* gene were readily detected (Fig. 3), but the wild-type gene was absent. This result indicates that the *MTLa1* gene is present in only a single (haploid) copy in the *C. albicans* genome and contrasts with the case for many other genes in *C. albicans*, where two rounds of disruption have been necessary (one for each copy) to destroy a gene. The same approach was used to make a complete disruption of the *C. albicans MTL*α*2* gene. After a single round of transformation, the disrupted

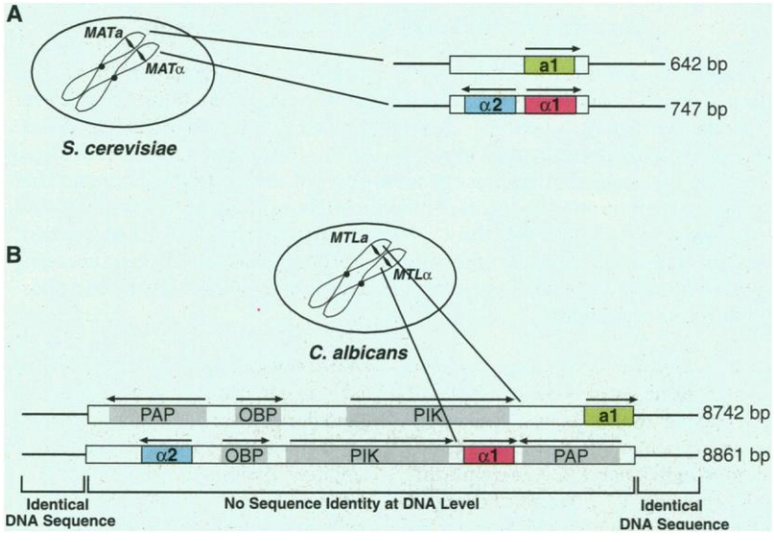

Fig. 1. Features of the *S. cerevisiae* mating-type (*MAT*) locus and the *C. albicans* mating type–like (*MTL*) locus. (**A**) The *S. cerevisiae MAT* locus contains ORFs for three gene-regulatory proteins, **a**1, α1, and α2, that are located on homologous chromosomes. The region of heterologous DNA sequence between the two chromosomes is 642 base pairs (bp) for the **a** chromosome and 747 bp for the α chromosome. (**B**) The *C. albicans MTL* locus contains ORFs for nine proteins from four families of proteins: three gene-regulatory proteins, two phosphatidylinositol kinases, two oxysterol binding protein–like proteins, and two poly(A) polymerases. The region of DNA sequence that differs between the *MTLa* and *MTL*α segments is 8742 bp for *MTLa* and 8861 bp for *MTL*α.

allele, but not the naturally occurring *MTLα2* gene, was detected by PCR (*9*). From these results, we conclude that both *MTLa1* and *MTLα2* exist in only a single copy in the *C. albicans* genome. These results also indicate the absence of silent mating-type "cassettes" in the *C. albicans* genome because PCR primers to the *MTLa1* (Fig. 3) and *MTLα2* (*9*) ORFs do not detect these genes in the disrupted strains. These data provide strong support for the idea that the *MTL* locus of *C. albicans* is heterozygous, whereas most of the *C. albicans* genome is homozygous. Because the two *MTL* loci are each embedded in nearly identical DNA sequences, the simplest interpretation is that the *MTL* loci reside on homologous chromosomes as they do in *S. cerevisiae*.

One hypothesis for the absence of a sexual cycle in *C. albicans* is that *C. albicans* was originally an **a**/α cell (to use the *S. cerevisiae* nomenclature) and through recombination lost one allele of the mating locus, becoming an **a**/**a** or α/α cell unable to undergo meiosis and return to a haploid state (*13*). The discovery of two *MTL* gene segments in *C. albicans*, one that resembles *MATa* and one that resembles *MATα*, appears to rule out this simple idea. The identification of *MTLa* and *MTLα* also suggests that the **a**/α configuration of the *C. albicans MAT*-like locus is a stable one. Because homologous recombination by way of the sequences flanking the locus could in principle result in the loss of either *MTLa* or *MTLα*, it seems likely that some sort of recombinational suppression exists. For example, one or more of the genes in each locus could be essential for cell viability, or the region could be under some type of mechanistic recombinational suppression.

In *S. cerevisiae*, the **a**1 and α2 proteins form a heterodimer that binds to specific DNA sequences (the haploid-specific gene, or hsg, operators) and represses transcription of the haploid-specific genes (*2*, *14*, *15*). To see whether *C. albicans* has an **a**1/α2 repression activity and whether it is dependent on the genes of the *MTL* locus, we used two different hsg operators: One is the consensus hsg operator from *S. cerevisiae*, and the other is an hsg-like sequence found upstream of the *C. albicans CAG1* gene, which encodes the α subunit of a trimeric GTP-binding protein. Although the function of this hsg-like sequence in *C. albicans* is not known, the sequence is recognized by *S. cerevisiae* **a**1/α2 (*16*). Five *GFP* (green fluorescent protein) reporter constructs were analyzed in *C. albicans* cells (*17*). In each construct, the *GFP* gene was placed under transcriptional control of the *C. albicans ADH1* promoter, and in addition contained (i) no insert, (ii) three *S. cerevisiae* hsg operator consensus sequences, (iii) three *S. cerevisiae* hsg operators mutated in two nucleotide positions to prevent recognition by **a**1/α2 (*18*), (iv) three copies of the hsg operator-like sequences found upstream of the *C. albicans CAG1* gene, or (v) three copies of the *C. albicans CAG1* sequence mutated in a way predicted to destroy recognition by **a**1/α2. The operators were inserted in the *ADH1* upstream region 260 base pairs upstream of the *GFP* gene.

Candida albicans cells containing the construct that lacks the operator expressed *GFP*, as evidenced by their bright green fluorescence (Fig. 4). The introduction of the *S. cerevisiae* hsg operators into the promoter markedly decreased fluorescence, whereas the mutant *S. cerevisiae* hsg operators showed no significant difference from the control construct that lacks the operators. The presence of the hsg-like sequence from *CAG1* also significantly repressed *GFP* production, and the point-mutations introduced into it relieved this repression.

To determine whether the *C. albicans MTL* gene cluster was required for the **a**1/α2-like repression activity, the *GFP* reporters were transformed into *MTLa1* deletion strains and evaluated for fluorescence. In contrast to the wild-type *C. albicans* strains, the *MTLa1* mutant strains showed the same levels of fluorescence for all of the reporter constructs, indicating that the *MTLa1* gene is required for the transcriptional repression activity (Fig. 4). Similar behavior was seen for both the complete deletion of the *MTLa1* gene and for the *MTLa1* homeodomain deletion, consistent with the DNA-binding domain of **a**1 being required for the repression activity (*9*). Northern (RNA) analysis also showed that transcription from the reporter constructs containing the functional hsg operators was derepressed in the *MTLa1* deletion mutants compared with the wild-type strain; however, in the absence of **a**1, the functional hsg operators still showed a slight amount of repression when compared with the mutated hsg operators (*9*). This repression could be due to the effect of the α2 alone or to a different *C. albicans* activity that has some overlapping function with **a**1/α2. Taken together, these results show that *C. albicans* has an **a**1/α2-like

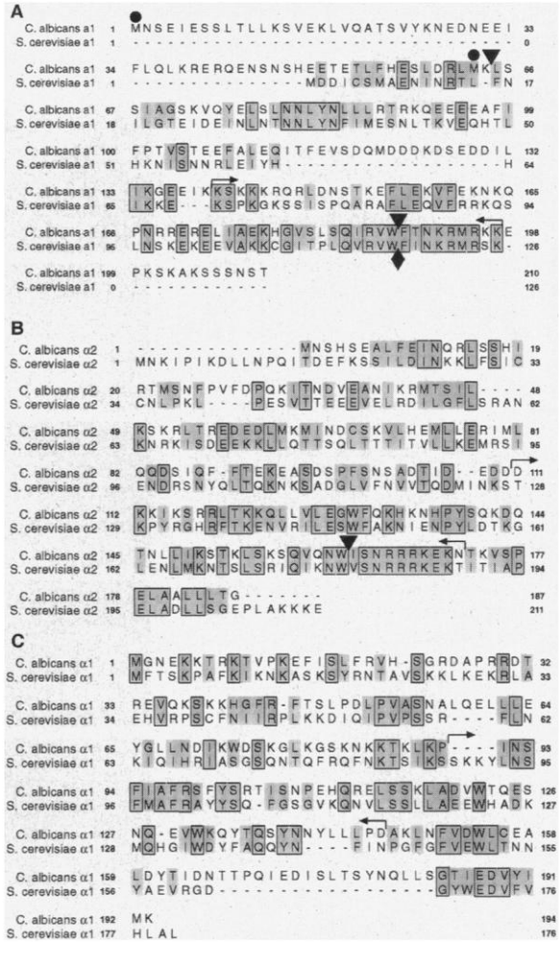

Fig. 2. Sequence comparisons between the *C. albicans* and *S. cerevisiae* **a**1, α1, and α2 proteins. Alignment was performed with the GCG8 program pileup (Genetics Computer Group, Madison, WI) and displayed with the program SeqVu 1.1 GES algorithm (Garvan Institute of Medical Research, Darlinghurst, Sydney, Australia). Boxes indicate sequence identity and shading indicates similar and identical resides. **(A)** Protein alignment based on predicted amino acid sequence for *C. albicans* **a**1. Dots indicate possible start codons for the proteins, and inverted triangles show the positions of introns in the *C. albicans* gene. The first intron is 74 bp long (*S. cerevisiae* first intron is 51 bp), and the second intron is 256 bp (*S. cerevisiae* second intron is 53 bp). The diamond shows the position of intron 2 in the *S. cerevisiae MATa1* gene. Arrows at *C. albicans* amino acid positions 140 and 197 delineate the homeodomain. **(B)** Protein alignment based on predicted amino acid sequence for *C. albicans* α2. Inverted triangle indicates position of the intron in the *C. albicans* (59 bp) gene, and arrows at *C. albicans* amino acid positions 111 and 172 delineate the homeodomain. **(C)** Protein alignment based on predicted amino acid sequence for the *C. albicans* α1 protein. Several α1-like proteins have been identified in ascomycete fungi, and a conserved region designated the α domain (*29*) has emerged. Arrows at *C. albicans* amino acid positions 90 and 146 delineate the α domain. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

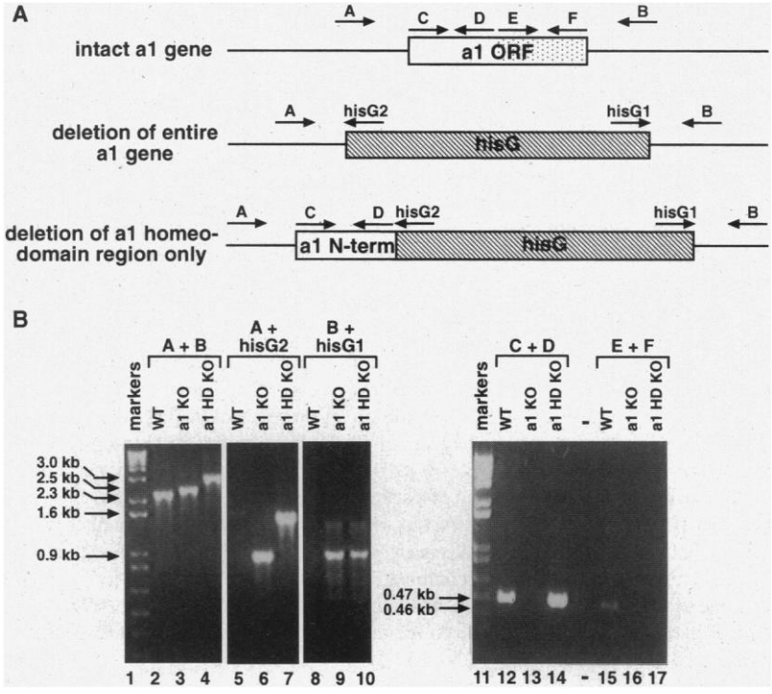

Fig. 3. Products of PCR reactions showing deletion of the *C. albicans MTLa1* gene and the absence of genes for **a**1 at other loci. (**A**) Schematic of the genomic locus. Labeled arrows indicate locations of primers used in the PCR reactions in (B). (**B**) Agarose gels showing fragments produced in PCR reactions with indicated primer sets on genomic DNA isolated from different strains. Lanes: WT, genomic DNA from wild-type cells as the template; **a**1 KO, genomic DNA from the complete *MTLa1* deletion strain as template; **a**1 HD KO, genomic DNA from the *MTLa1* homeodomain deletion strain as template. Predicted sizes of the PCR products are indicated on the left (in kilobases). All primer sets yielded the appropriately sized products or no product as predicted. Lane 1, DNA marker fragments; lanes 2 to 4, primers A and B on indicated genomic DNA; lanes 5 to 7, primers A and hisG2 on indicated genomic DNA; lanes 8 to 10, primers B and hisG1 on indicated genomic DNA; lane 11, DNA marker fragments; lanes 12 to 14, primers C and D on indicated genomic DNA; lanes 15 to 17, primers E and F on indicated genomic DNA.

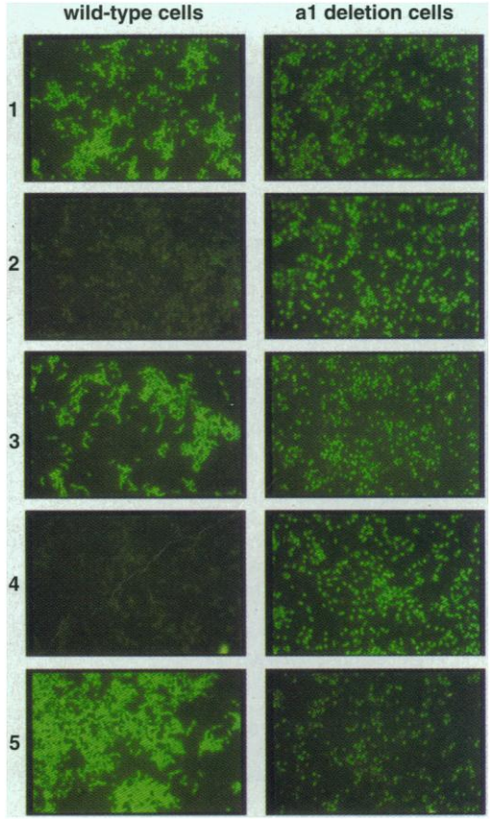

Fig. 4. Identification of an **a**1/α2 transcriptional repression activity in *C. albicans* mediated by the hsg operator and the *MTLa1* gene. Transcriptional repression activity was determined as a measure of *GFP* fluorescence in a heterologous reporter system in the presence of test DNA binding sites as inserts. In each case, the reporter consists of the *C. albicans ADH1* promoter controlling *GFP* expression. Reporter (1) no insert, (2) three *S. cerevisiae* hsg operator consensus sequences, (3) three *S. cerevisiae* hsg operators mutated at two nucleotide positions to prevent recognition by **a**1/α2 (*18*), (4) three copies of the hsg operator-like sequences located upstream of the *C. albicans CAG1* gene, and (5) three copies of the *C. albicans CAG1* sequence mutated in a way predicted to destroy recognition by **a**1/α2. Repression of the reporter is observed only when the reporter contains an intact hsg operator and when the *MTLa1* gene is present.

transcriptional repression activity and that this activity is dependent on the *C. albicans MTLa1* gene. We think it likely that *MTLa* and *MTLα* together encode the **a**1/α2 activity. In *S. cerevisiae*, **a**1 and α2 mediate transcriptional repression by bringing the corepressor Tup1 to DNA, and the **a**1/α2 repressor activity observed in *C. albicans* is at least partially dependent on the *C. albicans* Tup1 protein (*9*).

In *S. cerevisiae*, genes regulated by the products of the *MAT* locus encode proteins involved in many aspects of the sexual cycle. *C. albicans* contains close relatives of many of these genes, including those involved in *S. cerevisiae* mating [for example, *GPA1* (*16*), *STE20* (*19*), *STE6* (*20*)] and meiosis [for example, *DMC1* (*13*)]. Although some of the *C. albicans* relatives of the *S. cerevisiae* sexual cycle genes control filamentous growth, the complete functions of most of these genes have not been determined. These observations raise the question of whether any of these *C. albicans* genes are regulated by the *MTL* locus. We showed above that a DNA sequence found upstream of the *C. albicans CAG1* gene (*GPA1* homolog) can bring about *MTL*-dependent transcriptional repression when placed into a test promoter. In addition, we know that expression of the endogenous *CAG1* gene is indeed regulated by the *MTL* locus (*9*). Thus, like the analogous situation in *S. cerevisiae*, *C. albicans CAG1* is a natural target of the products of the *MTL* locus. These results have prompted us to reexamine the possibility of a sexual cycle in *C. albicans*. In preliminary experiments, mating in *C. albicans* has not been observed; however, a more extensive analysis is now underway to construct strains with the appropriate configurations of the *MTL* locus and to screen them under numerous environmental conditions, including those conducive to mating in a variety of fungi.

In addition to the three transcriptional regulators **a**1, α1, and α2, six other ORFs were identified in the *MTL* locus: three in *MTLa* and three in *MTLα* (Fig. 1). These additional ORFs

are arranged in pairs, one member of which is in *MTLa* and the other in *MTLα*. One pair of the ORFs is similar to the *S. cerevisiae PIK1* gene, a phosphatidylinositol kinase (*PIK*); the second pair of ORFs is similar to the *S. cerevisiae YKR003W*, a member of a class of genes similar to the human oxysterol binding protein (OSBP) gene, referred to here as *OBP*; and the remaining pair of ORFs is similar to *S. cerevisiae PAP1*, a poly(A) polymerase (*PAP*) (*21*). The proteins encoded by the related ORF pairs are ~60% identical to one another. This level of divergence between the members of each gene pair suggests that, although clearly related, they may have subtly different functions from each another. *MAT* loci have been characterized in several fungi, and to date, the proteins encoded by them fall into the categories of sequence-specific DNA binding proteins, pheromones, and pheromone receptors (*22–26*). A few other *MAT* genes (*Schizosaccharomyces pombe Mm*, and *Ustilago maydis LGA2* and *RGA2*) do not closely resemble any known genes, and their functions are as yet unknown. In contrast, six of the nine genes in the *C. albicans MTL* locus code for types of proteins not found in any of the previously described *MAT* loci.

The ORFs for all nine genes of the *C. albicans MTL* locus appear intact, suggesting that they code for functional proteins. We know this is the case for *C. albicans* **a**1 because it can mediate transcriptional repression from a test promoter bearing an hsg operator. The clustering of all nine genes into the *C. albicans MTL* locus suggests, by analogy with other fungi, that all of the genes may be involved in a single biological process. These genes could function to regulate a sexual cycle in *C. albicans* that has remained hidden from investigators, or they could be sexual cycle components derived from an evolutionary ancestor but now used to regulate another cellular process.

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4. Two different sets of overlapping lambda clones were identified with seven ^{32}P-labeled PCR products as probes against a lambda zap library of *C. albicans* strain SC5314 genomic fragments (gift from B. Braun). Sequences of primer pairs for generating the probe fragments were as follows: overlapping clone set 1: primer pair 1, 5′-cgggatcccagatggatgatgataaagatag-3′ and 5′-cgggatcctccagttaaattgtttcatattttg-3′; primer pair 2, 5′-gtctcgaatagaatccaagag-3′ and 5′-gtgcagactgatctgtgtc-3′; primer pair 3, 5′-taggaggtgtaacaccgtatgct-3′ and 5′-ctctgcgatcagcgaccagcaa-3′; overlapping clone set 2: primer pair 1, 5′-cgggatcccagatggatgatgataaagatag-3′ and 5′-cgggatcctccagttaaattgtttcatattttg-3′; primer pair 2, 5′-cgggatcccaagaactatttccaatccggaa-3′ and 5′-cgggatccaacatcctcaattgtacccg-3′; primer pair 3, 5′-ggagctacaagtatgactgt-3′ and 5′-gcgattctcatctgatcagcta-3′; primer pair 4, 5′-ctctgcgatcagcgaccagcaa-3′ and 5′-cgcagaaacgaagtgcgaga-3′. PCR products were labeled with ^{32}P in random prime reactions with the Megaprime DNA Labeling Kit (Amersham). Probe hybridizations, plaque lifts, phage purifications, and plasmid excisions were carried out according to B. Braun's Plaque lift/Phage screening protocol (www.sacs.ucsf.edu/home/JohnsonLab/). For each screening, ~10,000 plaques were screened and estimated to represent 2.5 to 5 genome equivalents. Genomic inserts were sequenced in full at the University of California, San Francisco, Biomolecular Resource Center.
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10. Disruption constructs for *MTLa1* and *MTLα2* were constructed with the URA blaster construct pMB-7 (*27*). The *MTLa1* deletion construct primers 5′-cagcacatgcatgcgagctcctttcagatcaagaaacagtttca-3′ and 5′-cagcacatgtcgacggtacccgtcctcagtatagatgc-3′ yielded a PCR fragment with Asp 718 and Sac I ends, and the primers 5′-cagcacatgtcgacggtgccacaataactccactg-3′ and 5′-cagcacatgcatgcgagtgcccgtggtaatacaaa-3′ yielded a PCR fragment with Sal I and Sph I ends. The fragments were cut and ligated sequentially into pMB-7. The Asp 718 to Sac I digest was performed as a partial digest on the PCR product because of a Sac I site in the fragment. The *MTLa1* homeodomain deletion construct primers 5′-cagcacatgcatgcgagctcctttcagatcaagaaacagtttca-3′ and 5′-cagcacatgtcgacggtacccgtcctcagtatagatgc-3′ yielded a PCR fragment with Asp 718 and Sac I ends, and the primers 5′-cagcacatgcatgcgagctcaaagtgtagagaaactagttc-3′ and 5′-cagcacatgtcgacggtacctaactaattatttttatttcctccccttta-3′ yielded a PCR fragment with Sal I and Sph I ends. The fragments were cut and ligated sequentially into pMB-7. The *MTLα2* deletion construct primers 5′-gaagatctgagctcagtctatcttgatttaggg-3′ and 5′-ggaagatctgtcttgttattgatgtgag-3′ yielded a PCR fragment with Bgl II and Sac I ends, and the primers 5′-aactgcagcttcgtataggtgtgcacttt-3′ and 5′-aactgcagaagcttgactctttggtcatgccttcc-3′ yielded a PCR fragment with Hind III and Pst I ends. The fragments were ligated sequentially into pMB-7.
11. Supplemental information on *C. albicans* transformations is available on *Science* Online at www.sciencemag.org/feature/data/1041394.shl.
12. Supplemental information on isolation of *C. albicans* genomic DNA and PCR conditions is available on *Science* Online at www.sciencemag.org/feature/data/1041394.shl. Primer sequences used in PCR are as follows: A, 5′-gttacaccacaatcaacacc-3′; B, 5′-ttacatgttggtgaacctaaag-3′; C, 5′-cagcacatgcatgcgagctcaaagtgtagagaaactagttc-3′; D, 5′-cagcacatgtcgacggtacctaactaattatttttatttcctccccttta-3′; E, 5′-cgggatccgaaacgacaaagactagac-3′; F, 5′-cgggatccctaggttgaatttgaacttg; hisG1, 5′-gcgcgtggcgatgcacatggtcag-3′; hisG2, 5′-gcgcggcggttgagtagctct-3′.
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17. The starting plasmid for *GFP* repression reporters was p*YGFP3* (gift from B. Cormack) (*28*). p*YGFP3* (pAJ699) was modified by the addition of the *C. albicans URA3* gene into the Sal I site of the vector with a PCR fragment with Sal I restriction sites on the ends by using primers 5′-tcgcctcgagtcgacgggcccagtactaataggaattg-3′ and 5′-tctcggagctcgagtcgacgggcccaggaccacctttgattgt-3′. The resulting fragment was digested with Sal I ligated into the Sal I site of p*YGFP3* to create pAJ717. A *URA3* 3′-untranslated region was inserted immediately downstream of the *GFP* gene. The *UTR* was generated as a PCR fragment with Pst I restriction sites on the ends with primers 5′-cgggatccgcgcgctgcagtagttagttaaatgtgaagggggag-3′ and 5′-cgggatccttaattaactgcagaggaccacctttgattgt-3′. The resulting fragment was digested with Pst I and ligated into the Pst I site of pAJ717 to create pAJ724. The *C. albicans ADH1* promoter was inserted immediately upstream of the *GFP* gene. *ADH1*–Bgl II was created in a chimeric PCR reaction to generate an *ADH1* promoter with Hind III restriction sites on the ends and a Bgl II restriction site located between the predicted upstream activating sequences and TATA box. Two starting fragments with overlapping ends were generated with primers 5′-cgggatccaagctttaacaaatgaa-3′, 5′-ataagagatctcttgcttgcatgacg-3′, and 5′-cgggatccaagcttaattgtttttgtatttg-3′, 5′-gcaagagatctctttattcagaattttcag-3′. These PCR products were mixed in a final PCR reaction to generate the full-length *ADH1*–Bgl II promoter with primers 5′-cgggatccaagctttaacaaatgaa-3′ and 5′-cgggatccaagcttaattgtttttgtatttg-3′. The resulting *ADH1*–Bgl II promoter fragment was digested with Hind III and cloned into pAJ724 to generate *CaADH1-GFP* (pAJ868). Reporter plasmids with three tandem repressor binding sites were constructed by cloning double-stranded oligonucleotides into the Bgl II site of the *CaADH1-GFP* plasmid. In each case two oligonucleotides containing three binding sites were phosphorylated and annealed to one another to generate a double-stranded duplex with single-stranded Bgl II–compatible overhangs. Single binding sites for each are highlighted in bold type. (i) pAJ888, *S. cerevisiae* haploid-specific gene site 5′-atc**tgatgtaattaattacatga**attgatgtaattaattacatgaattgatgtaattaattacatga-3′ annealed to 5′-gatct**catgtaattaattacatca**attcatgtaattaattacatcaattcatgtaattaattacatca-3′. (ii) pAJ898, *S. cerevisiae* haploid-specific gene site with point mutations that abolish **a**1/α2 binding in *S. cerevisiae* (*18*). 5′-gatc**tgctgtaattaattccatga**attgctgtaattaattccatgaattgctgtaattaattccatga-3′ annealed to 5′-gatc**tcatggaattaattacagca**attcatggaattaattacagcaattcatggaattaattacagca-3′. (iii) pAJ1081, **a**1/α2 binding sequence from the *C. albicans CAG1* promoter 5′-gatc**tgatgtgatttttaacatg**gattgatgtgatttttaacatggattgatgtgatttttaacatgg-3′ annealed to 5′-gatc**ccatgttaaaaatcacatca**atccatgttaaaaatcacatcaatccatgttaaaaatcacatca-3′. (iv) pAJ933, **a**1/α2 binding sequence from the *C. albicans CAG1* promoter with point mutations analogous to the mutations in the *S. cerevisiae* binding site that abolish **a**1/α2 binding 5′-gatc**tgctgtgatttttaccatgg**attgctgtgatttttaccatggattgctgtgatttttaccatgg-3′ annealed to 5′-gatcc**catggtaaaaatcacagca**atccatggtaaaaatcacagcaatccatggtaaaaatcacagca-3′. Reporter constructs were linearized through a Bsp EI site and integrated into the *ADH1* promoter. The pattern of fluorescence was confirmed for five transformants for each reporter.
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21. *PIKa* encodes a predicted protein that is 47% identical to Pik1, and *PIKα* encodes a predicted protein that is also 47% identical to the Pik1 protein. The predicted protein for *OBPa* is 51% identical to the predicted *YKR003W* protein, and the predicted protein for *OBPα* is 60% identical to the predicted *YKR003W* protein. The predicted protein for *PAPa* is 60% identical to Pap1, and the predicted protein for *PAPα* is 59% identical to Pap1.
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