

Genomic evidence for a complete sexual cycle in *Candida albicans*

Keh-Weei Tzung^{*†}, Roy M. Williams[‡], Stewart Scherer[§], Nancy Federspiel[¶], Ted Jones[¶], Nancy Hansen[¶], Vesna Bivolarevic[¶], Lucas Huizar[¶], Caridad Komp[¶], Ray Surzycki[¶], Raquel Tamse[¶], Ronald W. Davis^{*¶}, and Nina Agabian^{*†¶**}

^{*}Graduate Program in Oral Biology, Departments of [†]Stomatology and [¶]Microbiology and Immunology and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0422; [§]3938 Paseo Grande, Moraga, CA 94556; and [‡]Department of Biochemistry, School of Medicine, and [¶]Stanford Genome Technology Center, Stanford University, Palo Alto, CA 94304

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Candida albicans is a diploid fungus that has become a medically important opportunistic pathogen in immunocompromised individuals. We have sequenced the *C. albicans* genome to 10.4-fold coverage and performed a comparative genomic analysis between *C. albicans* and *Saccharomyces cerevisiae* with the objective of assessing whether *Candida* possesses a genetic repertoire that could support a complete sexual cycle. Analyzing over 500 genes important for sexual differentiation in *S. cerevisiae*, we find many homologues of genes that are implicated in the initiation of meiosis, chromosome recombination, and the formation of synaptonemal complexes. However, others are striking in their absence. *C. albicans* seems to have homologues of all of the elements of a functional pheromone response pathway involved in mating in *S. cerevisiae* but lacks many homologues of *S. cerevisiae* genes for meiosis. Other meiotic gene homologues in organisms ranging from filamentous fungi to *Drosophila melanogaster* and *Caenorhabditis elegans* were also found in the *C. albicans* genome, suggesting potential alternative mechanisms of genetic exchange.

Meiosis represents a specialized cell division that is essential for sexual reproduction; it generates haploid germ cells from diploid parental cells (1). Because a sexual phase for *Candida albicans* has historically not been detected, it is classified among the *Fungi imperfecti* (2). However, the identification of a mating-type-like (*MTL*) locus and genes such as *CPH1*, *CAG1*, *DLH1*, *NDT80*, and *HST6* in *C. albicans* (see *C. albicans* genome project information at <http://alces.med.umn.edu/Candida.html> and <http://www-sequence.stanford.edu/group/candida>), which participate in meiotic differentiation in *S. cerevisiae*, suggests that the classification of this diploid fungus belies the existence of a sexual cycle. Recently, in fact, genetic manipulation of the *MTL* locus resulted in the demonstration that *C. albicans* strains can mate to produce triploid or tetraploid progeny at very low frequency either in culture or in experimental animals (3, 4). It thus appears that *Candida* can undergo cell fusion, depending on mating type. However, completion of a sexual cycle, i.e., meiosis and sporulation, remains to be demonstrated.

C. albicans is an opportunistic pathogen that can cause disease in patients immunocompromised as a result of HIV infection, organ transplantation, and cancer chemotherapy (5). It is also a morphologically complex organism capable of proliferating either as a budding yeast or by the formation of pseudohyphae or filamentous hyphae. The inability to demonstrate a sexual cycle has significantly impeded conventional genetic analysis. Therefore the potential for its existence has both intrinsic and technical consequences.

Shotgun sequencing of the diploid *C. albicans* genome, undertaken by the Stanford Genome Technology Center, is complete with the sequencing of 10.4 haploid genome equivalents, which is sufficient to ensure identification of all of the genes in this organism. A web page and database have been made available over the World Wide Web ([\[www.stanford.edu/group/candida\]\(http://www.stanford.edu/group/candida\)\). Given the evolutionary proximity between *C. albicans* and *S. cerevisiae* \(6\) and the differences in their virulence and habitat, genomic comparisons between these fungi are likely to illuminate aspects of the unique cell biology of both organisms. In this report, we address the potential for meiosis and sexual recombination in *C. albicans*.](http://sequence-</p></div><div data-bbox=)

Materials and Methods

Sequencing Library Construction, Shotgun Sequencing, Assembly, and Analysis. *C. albicans* strain SC5314 was generously provided by Bristol-Myers Squibb for use without restriction. DNA from this strain was used for all M13 and plasmid sequencing library constructions. Electrocompetent *Escherichia coli* DH12S and DH10B (GIBCO/BRL Life Technologies) were used for transformation of M13 and plasmid libraries, respectively. *C. albicans* cultures were grown in yeast/peptone/dextrose broth with shaking at 30°C. Spheroplasts were made, treated with SDS/proteinase K, and the DNA purified on a sucrose gradient. The purified *C. albicans* SC5314 genomic DNA was sheared by a point-sink shearing device (7) to a fragment size of 3–6 kb for cloning into the plasmid vector pUC119 and to 1–2 kb for cloning into M13mp18. Individual plasmid and M13 clones were picked and grown and template DNA prepared by using the automated instrumentation developed at Stanford University (8). Sequencing reactions were performed on plasmid and M13 template DNA by using BigDye Primer and BigDye Terminator kits from Perkin-Elmer Applied Biosystems according to the manufacturer's specifications, with slight modifications. Sequencing analysis was performed on ABI377-XL Automated Sequencers at 96 lanes/gel. Sequence data were derived by using the basecaller PHRED (9, 10), assembled with PHRAP (Phil Green, University of Washington), viewed with CONSED (11).

Identification of Meiotic Machinery in *C. albicans*. We have used several approaches to identify meiotic homologues in *C. albicans*, focusing mainly on genes critical for mating and meiosis in budding yeast. We also searched for homologues of genes that are regulators of cellular reproduction in other organisms. The *S. cerevisiae* and other sequences were used as query sequences in basic BLAST searches (12); by using the BLASTP program, we searched for similar proteins in the *C. albicans* genome database Ver. 6 assembly. The BLAST output was sorted and top hits ranked by BLAST scores. The E-value cutoff used to assign homologues was $1e^{-6}$. However, each sequence required specific evaluation as there were exceptional instances where biological data indicated the presence of functional homologues, although

Abbreviations: *MTL* locus, mating-type-like locus; MAP kinase, mitogen-activated protein kinase; SC, synaptonemal complexes.

**To whom reprint requests should be addressed. E-mail: agabian@itsa.ucsf.edu.

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these lacked significant sequence homology. In Table 1 (which is published as supplemental data on the PNAS web site, www.pnas.org) the functional groups used in the categories roughly correspond to: (i) mating differentiation; (ii) nutritional control; (iii) cell type control; (iv) initiation of meiosis; (v) checkpoint control and progression through meiosis; (vi) recombination and the formation of synaptonemal complexes; and (vii) spore wall morphogenesis and ascus formation.

Results and Discussion

Using comprehensive genomic comparisons, we have assessed the repertoire of gene homologues in *C. albicans* that in *S. cerevisiae* are required in pathways leading to sexual differentiation. In a genome-wide transcription analysis of sporulation in *S. cerevisiae* (13), nearly 500 genes were expressed; those of particular interest are genes that are either meiosis-specific or have shown meiotic-mutant phenotypes. By using these 500 genes as a reference point, *C. albicans* homologues were identified on the basis of their sequence similarity with *S. cerevisiae* counterparts from 10.4× sequencing data (see *Materials and Methods*). Genes analyzed in these comparisons are listed in Table 1 (www.pnas.org) and categorized on the basis of their presumptive function in meiosis and sporulation. In this study, homologues of *S. cerevisiae* genes involved in chromosome recombination and the formation of synaptonemal complexes (SC) were conspicuously absent from *C. albicans*, although other groups of genes important for meiosis, mating, and sporulation contained many candidates in the *C. albicans* genome.

Mating Differentiation. In *S. cerevisiae*, mating between haploid cells is signaled by binding of pheromones to a cell-type-specific receptor on cells of the opposite mating type (*STE2* expressed in **a** cells recognized by **α**-factor, and *STE3* expressed in **α** cells recognized by **a**-factor). The signal is transmitted by interaction of a heterotrimeric G protein complex composed of G α (Gpa1), G β (Ste4), and G γ (Ste18) through a downstream mitogen-activated protein (MAP) kinase cascade encoded by *STE20*, *STE11*, *STE7*, and *FUS3*. The resulting activation of the transcription factor Ste12p is required for expression of mating type-specific genes, cell cycle arrest, fusion of mating partners, and karyogamy (14). In *S. cerevisiae*, pseudohyphal growth and invasive growth, respectively, are initiated in diploid and haploid cells on nutrient deprivation and signaled by genes shared with this MAP kinase pathway (15). As indicated in Table 1 (www.pnas.org), *C. albicans* homologues for all of these genes in the MAP kinase cascade have been identified, as well as homologues of the pheromone receptor genes *STE2* and *STE3*. Genes involved in pheromone processing in *S. cerevisiae* such as *STE14*, *AXL1*, *STE23*, *RAM1*, *RAM2*, *STE24*, *RCE1*, *KEX2*, *KEX1*, and *STE13* (16) have homologues in the *Candida* genome, although there is no independent evidence that *C. albicans* can produce or respond to pheromones. We also have identified several pheromone-induced genes such as *FIG1*, *FIG3*, and *FIG4* (Table 1, www.pnas.org), which appear to be important for different steps of mating cell differentiation in *S. cerevisiae* (17). Together, these data suggest that *C. albicans* may have preserved the ability to produce and respond to mating pheromones. Although extensive BLAST analysis failed to identify any mating-factor homologues, computer programs that take into account pheromone gene structure have provided us with several candidates for pheromone genes (unpublished results).

In *S. cerevisiae*, α -agglutinin (*SAG1*) provides tight cell–cell adhesion during mating. It has been postulated that in *C. albicans*, the adhesiveness of the homologous *ALS* gene family contributes to its pathogenesis (18). The presence of *FUS1* might suggest the ability of cell fusion for *C. albicans*. A *KAR1*-like

sequence, which in *S. cerevisiae* is critical for nuclear fusion and spindle pole body formation (19), was not found in *C. albicans*.

One of the major outcomes of *CPH1/STE12* activation through the MAP kinase pathway in *C. albicans* is to induce hyphal morphogenesis (20). Virulence of *cst20/ste20* and *cph1/ste12* disruption mutants is attenuated in the mouse model of systemic candidiasis (21, 22), thereby establishing a potential link between pheromone signaling, filamentous growth, and virulence, as found in the pathogenic fungi *Cryptococcus neoformans* and *Ustilago maydis* (23, 24). In the evolution of pathogenesis, perhaps *C. albicans* has used the MAP kinase pathway to strictly control expression of its hyphal phenotype in response to changes in the host environment. The identification of potential ligands and possible environmental cues that are either recognized by the homologues of *STE2*- and *STE3*-like receptors or that stimulate the MAP kinase pathway through alternate receptors could help us understand hyphal induction and pathogenesis in *C. albicans*.

Nutritional Control. In *S. cerevisiae*, meiosis is initiated only by diploid cells deprived of glucose and nitrogen and grown in the presence of a nonfermentable carbon source, whereas other fungi have different and complex nutrient requirements for this process. For instance, nitrogen starvation is required for mating and meiosis in *Schizosaccharomyces pombe* (25). The plant pathogen *U. maydis* enters meiosis only during growth in its host, *Zea mays* (26). *C. albicans* appears to contain homologues of *S. cerevisiae* genes involved in glucose repression and nitrogen metabolism: *MIG1*, *GAT1*, and *UME6*, as well as *RAS/cAMP*, *SNF1*, and *MCK1*, which are involved in the nutrient sensing pathway (27).

The complexity and cross talk between nutritional and meiotic pathways suggest that, although similar genes may be present in both organisms, their participation in these pathways may have different biological consequences. For example, *SNF1* is essential for the viability of *C. albicans* (28) but is not essential in *S. cerevisiae*, where it coordinates glucose and acetate regulation of the early and late meiotic program (29). Another homologue found in *C. albicans*, *MCK1*, encodes a serine-threonine-tyrosine kinase, which functions as a positive regulator of meiotic gene expression in *S. cerevisiae* and is essential for ascus maturation; it governs centromere behavior in mitosis (27). Comparative genomic analyses also reveal the metabolic diversity of *C. albicans* (S.S., unpublished observations) and suggest that the conditions traditionally used in the laboratory for its culture may in part be responsible for the failure to detect a sexual cycle in this organism.

Cell Type Control. In *S. cerevisiae*, only diploid cells that are heterozygous at the *MAT* locus can initiate meiosis and sporulation on nutritional starvation. An important feature of **a/α** cells is the presence of a transcriptional repressor, **a1-α2**, which is a heterodimeric homeodomain protein (30). *RME1*, which encodes a negative regulator of meiosis, is one of the genes turned off by **a1-α2** (31). The product of another gene, *IME4*, mediates both cell type and nutritional activation of *IME1* (see below) (32). No homologue of *IME1* was found in *C. albicans*, although putative counterparts of *RME1* and *IME4* have been identified.

Mating type loci in yeasts are master regulators of cell fate specification and sexual morphogenesis (33). In *C. neoformans*, there is an association between mating type, hyphal phase, and infectivity (34). Recently, a mating-type-like (*MTL*) locus in *C. albicans* with homology to the *MAT* locus of *S. cerevisiae* was identified (35). The *C. albicans MTL* locus is large (approximately 8.8 kb), single copy, and without silent cassettes such as *HML* or *HMR*. Whether there is a correspondence between mating type and virulence in *C. albicans* has not been estab-

lished. However, most clinical isolates of *C. albicans* are heterozygous at *MTL* locus (P. Magee, personal communication) and by analogy would be equivalent to **a/α** cells in *S. cerevisiae*.

Initiation of Meiosis. Initiation of meiosis and sporulation in diploid *S. cerevisiae* by nutritional limitation occurs through a transcriptional cascade with sequentially expressed distinct classes of meiosis-specific genes (36). *UME6* encodes a Zn₂Cys₆ DNA-binding protein that functions as a developmental switch for mitotic repression and meiotic activation of early meiotic genes (37). Interaction between Ume6p and Ime1p, a transcriptional activator, is required for induction of early meiotic gene expression. In *S. cerevisiae*, *RIM11* and *RIM15* kinases are required for Ume6p-Ime1p interaction (38). *RIM101* defines a signaling pathway that activates *IME1*. As shown in Table 1 (www.pnas.org), homologues of *UME6*, *RIM11*, *RIM15*, and genes in the *RIM101* pathway have been identified in *C. albicans*. Experiments have shown that the *Candida RIM101* homologue participates in hyphal growth (39). In *S. cerevisiae*, Ime1p activates expression of *IME2*, a serine–threonine kinase, essential for premeiotic DNA replication (40). Despite extensive BLAST analysis, we have not found counterparts of *IME1* in *C. albicans*. Similarly, efforts to functionally complement an *ime1* null mutant of *S. cerevisiae* with a *C. albicans* genomic library have failed (41), lending further support to the apparent absence of this gene in *C. albicans*. However, we have found downstream targets of Ime1p such as *IME2*. In *S. cerevisiae*, the Ime1p/Ume6p complex and the URS1 consensus-binding site for early meiotic genes play a pivotal role in the cell's decision to enter meiosis. The absence of *IME1* suggests that the switch machinery used in *S. cerevisiae* to effect commitment to the meiotic pathway is missing. It remains possible that a functional analogue of *IME1* is present in *C. albicans*.

Checkpoint Control and Progression Through Meiosis. Mitosis and meiosis, the two fundamental modes of cellular reproduction, have overlapping functions including DNA replication and chromosome segregation, as well as similar but distinct mechanisms to survey the progression of cell cycle events. We have identified *C. albicans* homologues of the mitotic DNA damage checkpoint genes *MEC1*, *RAD17*, *RAD24*, which are also required for meiotic progression (42), as well as meiosis-specific *MEK1* and the chromatin-silencing factors *SIR2* and *DOT1*, which are implicated in pachytene checkpoint control (43). We have also identified a set of gene homologues whose products in *S. cerevisiae* participate in DNA replication and chromosome segregation: *CDC5*, *CDC7*, *CDC14*, *CDC20*, *CDC25*, *CDC28*, *IPL1*, *CDH1* (44), several components of the anaphase-promoting complex (*APC1*, *APC2*, *APC11*, *CDC16*, *CDC23*, *CDC27*), and its meiotic activator *AMA1* (*SPO70*) (45). Overall, homologues for most of the genes that in *S. cerevisiae* participate both in mitosis and meiosis have been identified in *C. albicans*.

Meiosis I (MI) is a reductional division, whereas meiosis II, like mitosis, is equational. The separation of sister chromatids must be tightly regulated during meiosis; homologues of *PDS5*, *SCC2*, *SMC1*, *SMC2*, *SMC3*, which in *S. cerevisiae* function in sister chromatid cohesion, were also found in *C. albicans* (46). A homologue of *YPR007* (*REC8*), proposed to encode a protein mediating meiotic sister chromatid cohesion (47), was identified, as was *NDT80*, a gene that encodes a meiosis-specific transcription factor. In *S. cerevisiae*, Ndt80p is required for expression of middle meiotic genes, meiotic division, and spore formation (48). Transcription of *NDT80* depends on *IME1*, which remains unidentified in the *C. albicans* genomic sequence. Another important gene apparently absent in *C. albicans* is a homologue of *SPO13*. *spo13* null mutants in *S. cerevisiae* eliminate the reductional division (MI) during meiosis to produce diploid

spores (49). The absence of *SPO13* might indicate that the *C. albicans* sexual cycle is one where cells undergo single-division meiosis.

Recombination and the Formation of Synaptonemal Complexes. Meiosis is fundamentally different from mitosis in the occurrence of high-frequency recombination. Meiotic recombination, which is thought to proceed through a double-strand-break (DSB) repair mechanism (50), follows premeiotic DNA replication. More than 10 genes are required to produce DSBs in meiotic cells in *S. cerevisiae*; strikingly, several of these are absent from *C. albicans*: *MER1*, *MER2*, *REC102*, *REC104*, *REC114*, and *MEI4*. The absence of homologues of approximately half of the genes required for the initiation of recombination in *S. cerevisiae* suggests that *C. albicans* may be greatly impaired in this initial step. However, we have identified *SPO11*, a type II topoisomerase implicated in nicking DNA, to generate DSBs (51). Homologues of *SPO11* have also been identified in *Caenorhabditis elegans*, *Drosophila melanogaster*, *S. pombe*, mouse, and human (52).

Homologues of most of the genes involved in strand invasion and Holliday junction formation have been identified as well: *RAD51*, *RAD52*, *RAD54*, *RAD57*, and *DMC1* (53). The *C. albicans DLH1* gene complements *DMC1* null mutants, its meiosis-specific homologue in *S. cerevisiae* (54). Homologues of the meiosis-specific genes *SAE2* and *SAE3* are also absent from *C. albicans*, whereas those of genes that participate in both mitotic and meiotic mismatch repair *MSH2*, *MSH6*, *MSH3*, *MLH1*, and *PMS1*, are present (55).

Many unique characteristics of meiotic recombination are ensured through the formation of synaptonemal complexes (SC) (56). These structures not only exclude recombination between sister chromatids but also control the frequency of crossovers. Homologues of *HOP1* and *ZIP1*, molecular components of the SC, were found in *C. albicans*, whereas those for other proteins, such as *ZIP2* and *HOP2*, involved in synapsis of chromosomes and formation of SC, are absent (57, 58). Homologues of meiosis-specific genes *MSH4* and *MSH5*, required to promote recombination between homologous chromosomes and Holliday junction resolution, were also identified. Together, these findings suggest that *C. albicans* is not competent to make mature SC. However, *C. albicans* may resemble other fungi such as *S. pombe* and *Aspergillus nidulans*, which exhibit meiotic recombination but do not form SC (59).

Spore Wall Morphogenesis and Ascus Formation. In *S. cerevisiae*, the spore wall is formed *de novo* through a complex morphogenetic program depending on the initiation of meiosis. Although there are no detectable meiotic ascospores in *C. albicans*, homologues of genes involved in the sporulation pathway such as *SPS1*, a *STE20*-like protein kinase, and *SMK1*, a MAP kinase, were identified. We also found homologues of a set of mid/late sporulation-specific genes involved in spore wall maturation: *DIT1*, *DIT2*, *SPR3*, and *YDR104*. *DIT1* and *DIT2* are important for the formation of the outer layer of the spore wall. *YDR104* is essential for spore wall formation in *S. cerevisiae* (13). The presence of these genes may reflect a possible MAP kinase pathway that can transmit environmental signals to modify cell wall structures in *C. albicans*. The extent to which homologues of these genes function in yeast or hyphal wall synthesis is not known. The *C. albicans* cell wall contains variable amounts of dityrosine, and its basic structural features are similar but not identical to those of *S. cerevisiae* (60).

Genomic Comparison Between *C. albicans* and Other Organisms. Given that gametogenesis in metazoans and sporulation in yeasts are highly conserved evolutionary processes, it is not surprising that a survey of available databases revealed several genes that

function in meiosis in multicellular organisms and that have homologues in *C. albicans*. Some of the genes analyzed are listed in Table 2 (which is published as supplemental data on the PNAS web site, www.pnas.org). For example, we have found homologues of *pelota* and *DES-1*, which are required for spermatogenesis in *Drosophila*. *DOM34* is the *S. cerevisiae* homologue of *pelota* and is important for meiosis, pseudohyphal growth, and translation (61). *DES-1*, a transmembrane protein, is required for initiation of meiosis in *Drosophila* spermatogenesis. A *DES-1* homologue has been identified in mouse, *S. pombe*, and *Arabidopsis thaliana* (62) but not in *S. cerevisiae*. The *Drosophila* protein is proposed to mediate the interaction between somatic cells and germ cells during development (63), raising the possibility that *DES-1* homologue might be required for communication between *C. albicans* and its environment.

We have also found several *S. pombe* gene homologues (in *C. albicans*) such as *RCD1* (*YNL288*), *NRD1* (*YPL184*), *STE20* (*YER093*), and *MEI4* (*FKH1*), which are involved in sexual differentiation in the fission yeast (25). Their counterparts in *S. cerevisiae* have been identified, but their functional role in meiosis has not been established. For example, *S. cerevisiae* *FKH1* regulates mitotic cell cycle progression and pseudohyphal growth (64) and is expressed early during sporulation (13).

The apparent dissimilarity in meiotic machinery between *S. cerevisiae* and *C. albicans* may reflect their relative phylogenetic distance (6). To investigate this further, we sought potential meiotic homologues in *C. albicans* that may resemble key meiotic regulators or cognates of genes that function in the sexual cycle in other fungi such as *C. neoformans*, *A. nidulans*, *Neurospora crassa*, and *Podospira anserina*. From this analysis, we have identified several meiotic homologues, including *SPO14*, *MCK1*, *MEK1*, and a pH regulatory system with components of *pall*, *pacC*, *palA*, *palF*, and *palB* shared by *C. albicans*, *S. cerevisiae*, and *A. nidulans*. With respect to *pacC*, a homologue of *RIM101*, this gene is conserved in all three of these fungal species, as are other elements of the *RIM101* pathway. In *S. cerevisiae*, the *RIM101* pathway functions both in meiosis and invasive growth. In *C. albicans* it is required for pathogenesis (65).

Asexual sporulation is common among diverse groups of fungi. *C. albicans* can produce structures known as chlamydo-spores under special conditions (5); their biological role is unknown. We have identified a set of *C. albicans* genes that are homologues of those that participate in asexual sporulation in *A. nidulans* (66). These include *C. albicans* genes designated *SST2* (a homologue of *flbA*), *YPR013* (a homologue of *flbC*), *MYB1* (a homologue of *flbD*), *TEC1* (a homologue of *abaA*), *DOP1* (a homologue of *dopA*), and *EFG1* (a homologue of *stuA*). Several of these were also found in *S. cerevisiae*. Characterization of these gene homologues may help to define the role of chlamydo-spores in *C. albicans* cell biology.

In considering alternative reproductive pathways, the identification of *HET-C* and *HET-E-1* homologues in *C. albicans* is particularly interesting. The *HET-C* gene, which encodes a glycolipid transfer protein, is proposed to function in vegetative incompatibility and ascospore formation in *Podospira anserina* (67). *HET-E-1* encodes a β -transducin-like protein (68). No *HET-C* gene homologue was found in *S. cerevisiae*. Homologues of *MOD-D* and *MOD-E*, modifiers of the *het* locus, were also identified in *C. albicans*. *MOD-D* is a homologue of $G\alpha$ subunit, and *MOD-E* is an *HSP90* homologue (69, 70). As the *het* loci appear to regulate self/nonself recognition during vegetative growth in filamentous fungi, heterokaryon formation could provide an opportunity for genetic exchange in imperfect fungi (71).

Conclusion

C. albicans can produce tetraploid progeny in animals and in culture when parental types homozygous at *MTL* locus are

artificially created (3, 4). However, there is no evidence of reductional division from these matings, and it remains to be shown that this fungus has a meaningful sexual cycle. Most clinical isolates of *Candida* are heterozygous at the *MTL* locus, and most proliferation of naturally occurring populations appears to be clonal (72), suggesting that mating is infrequent. Our comprehensive comparison and analysis of the *C. albicans* genome, focusing on the question of sexuality in *C. albicans*, suggests that, whereas this organism suffers a multiple gene defect in a *S. cerevisiae*-like meiotic machinery, it possesses a repertoire of genes composed of homologues found in sexual pathways of *S. cerevisiae*, filamentous fungi, and metazoans, which in aggregate indicate that *C. albicans* has a sexual cycle in nature.

One of the more interesting results of our analysis is the finding that, whereas *C. albicans* appears to lack a homologue of *IME1*, in *S. cerevisiae* the master switch for entry into the meiotic pathway, it does possess a number of homologues of the downstream target genes of the Ime1p/Ume6p regulatory complex in *S. cerevisiae*. As *IME1* is the integration point of genetic and nutritional signals for meiosis in *S. cerevisiae* (73), the regulation of initiation of meiosis might be achieved in *Candida* through an analogue of *IME1*, which integrates a different set of signals to effect commitment to meiosis. We have not been able to identify *IME1* homologues in other organisms despite extensive BLAST analysis, indicating that perhaps the commitment to meiosis effected by the interaction between Ime1p and Ume6p is unique to *S. cerevisiae*. Similarly absent in *C. albicans* and other organisms as well is *SPO13*, which in *S. cerevisiae* is essential for proper execution of meiosis I. Within the meiotic pathway itself, *C. albicans* is missing 6 of 10 homologues of genes that in *S. cerevisiae* are necessary for the initiation of double-strand breaks and others that are involved in chromosome recombination and the formation of SC. Most of the missing genes are related to recombination and SC formation, although in each of these processes, *C. albicans* still possesses a number of homologues that may participate at various stages in the progression of meiosis.

The study of fungal developmental signaling pathways has revealed an intimate relationship between mating and filamentous growth (74) and suggests that different cell types can use shared signaling components to specify cell fate. *C. albicans* is able to proliferate in many forms—as yeast, pseudohyphae, and filamentous hyphae. It is also able to form chlamydo-spores and express different switch phenotypes in response to a variety of environmental cues. Any one of these forms may be capable of cell fusion, a possibility highlighted by the identification of several components of the *het* system as well as gene homologues that play crucial roles in cellular reproduction in various organisms. Our studies suggest the possibility for hyphal fusion and dikaryon formation in *C. albicans* and allude to more complex patterns of both asexual and sexual differentiation similar to those found in some filamentous ascomycetes and basidiomycetes (75, 76).

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