PERSPECTIVES: MICROBIOLOGY

Candida's Arranged Marriage

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It has always been presumed that the fungus *Candida albicans*, a common human pathogen, only reproduces asexually (*1*). This organism is naturally diploid (that is, it retains a full complement of paired chromosomes) and has not been observed to undergo mating spontaneously. However, two reports in this issue (*2*, *3*) by Hull *et al.* (page 307) and Magee and Magee (page 310) reveal that *C. albicans* does have a sex life after all. Apparently, this organism can be "forced" to mate, suggesting that mating may occur naturally (albeit rarely).

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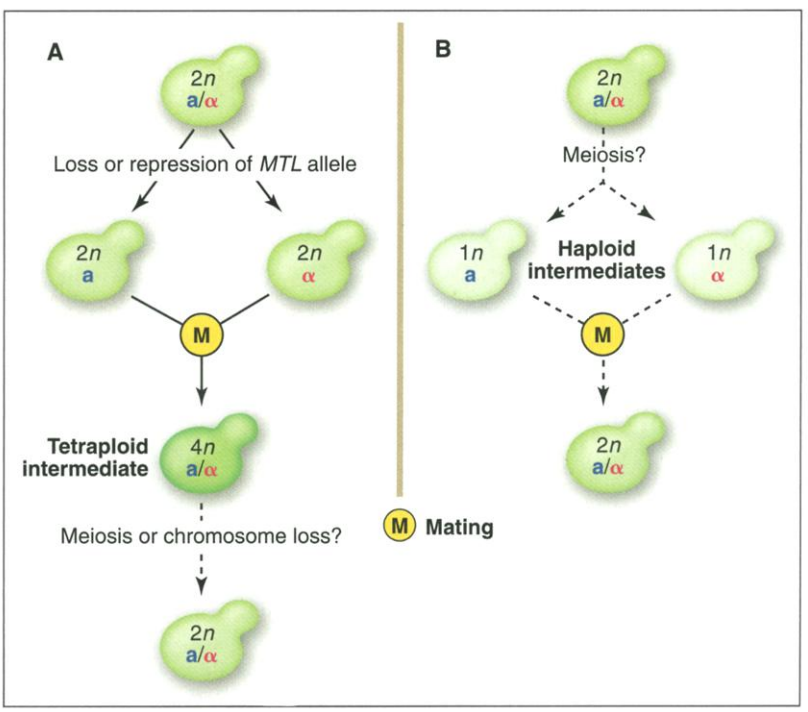
Diploid organisms (with two copies of every gene) are still viable even if one copy of a gene is inactive or imperfect. However, meiotic cell division, generating haploid cells (with only one copy of every gene), unmasks any balanced (lethal) mutations. It has been argued that balanced lethal mutations would preclude the formation of viable haploids and a full sexual cycle in *C. albicans*. Recently, sequencing of the complete genome of this fungus led to the surprising finding that a mating type–like locus (*MTL*)—whose genes encode mating type specificity—exists on chromosome 5. This locus is similar to the mating type (*MAT*) locus in the closely related yeast, *Saccharomyces cerevisiae* (*4*). *C. albicans* is heterozygous at the *MTL* locus: that is, one chromosome carries the *MTL***a***1* allele encoding one mating type, whereas the other chromosome carries the opposite mating type alleles, *MTL*α*1* and *MTL*α*2*. In *S. cerevisiae*, *MAT***a***1* and *MAT*α*2* encode subunits of a heterodimeric protein a1/α2, which represses mating in diploid yeast. But, pseudohaploid *S. cerevisiae* mutants, which are diploid but have only one functional mating type gene—either *MAT***a** or *MAT*α (expressed as *MAT***a***/mat*α or *mat***a***/MAT*α, respectively)—will mate with each other (*5*). Therefore, although the *MTL***a***1/MTL*α*2* genotype of *C. albicans* probably precludes mating because it encodes the a1/α2 repressor protein, sex could be arranged if the *MTL* locus were engineered to create pseudohaploid *MTL***a***/mtl*α and *mtl***a***/MTL*α mating partners (*4*, *6*).

Hull *et al.* and Magee and Magee (*2*, *3*) confirm this possibility. They forced mating between compatible diploid strains rendered haploid at the *MTL* locus (through two different strategies). The matings created progeny that carried complementary genetic markers from both parents and that had tetraploid nuclei (or nuclei with a higher than normal DNA content). The specificity of the process was confirmed by showing that mating could be induced only when an "*MTL***a**" strain was mated with an "*MTL*α" strain. Previous work on the segregation patterns of alleles at certain loci suggests that *C. albicans* is largely clonal (the progeny are identical to their parents), an indicator of asexual reproduction (*7*). But some degree of recombination between loci hints at a cryptic sexuality in this yeast [see discussion in (*7*)]. These earlier findings suggested that a full sexual cycle including meiosis might be discovered in *C. albicans*.

The surprise finding by Hull *et al.* and Magee and Magee that *C. albicans* mates is heightened by the long history of attempts to demonstrate mating and meiosis in this yeast. New names for the genus such as *Syringospora* (*8*) and *Saccharomycopsis* (*9*) were proposed following discovery of *C. albicans* isolates with a reduced (*9*, *10*) or increased (*8*, *10*) number of chromosomes. Organisms with increasing numbers of chromosomes (created by protoplast fusion) seemed to undergo sequential and spontaneous chromosome loss to regenerate the original diploid state (*11*) in the absence of an ordered program of reductive division (reminiscent of meiosis). Other studies showed that single *C. albicans* chromosomes could be eliminated to generate viable strains (aneuploids)—for example, by treatment with the microtubule inhibitor benomyl (*12*), which induces loss of one copy of chromosome 3, or growth on sorbose, which induces loss of one copy of chromosome 5 (*13*). Thus, *C. albicans* can tolerate the loss of one copy of at least two of its eight chromosomes. This does not support the idea that *C. albicans* is a constitutively diploid organism.

By growing *C. albicans* on sorbose, Magee and Magee were able to create artificial pseudohaploid *MTL***a** and *MTL*α yeast strains. In a separate approach, Hull *et al.* deleted specific *MTL* sequences to create compatible *MTL***a***/mtl*α and *mtl***a***/MTL*α mating partners. The alternative approaches taken for constructing compatible mating partners probably account for some of the differences in results between the two studies. Hull *et al.*



The mating game. Sexual reproduction in the yeast *C. albicans*. There are at least two possible pathways, depending on whether the intermediates are tetraploid (A) or haploid (B). (**A**) Compatible diploid (2*n*) mating partners a (blue) and α (red) could be generated by either loss or repression of genes at the mating type-like (*MTL*) locus that carries genes encoding proteins involved in mating; the tetraploid (4*n*) progeny could then be rendered diploid through meiosis or chromosome loss. (**B**) Alternatively, compatible haploid (*n*) mating partners a (blue) and α (red) could be generated by meiosis. (Solid arrows, experimentally proven steps; dotted arrows, steps that remain to be confirmed.)

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showed that compatible partners mated effectively within a mammalian host during an experimental infection, but they were unable to demonstrate mating in vitro on agar plates. In contrast, Magee and Magee showed that mated progeny arose spontaneously, albeit more slowly, on selective agar plates (they did not investigate mating of *C. albicans* in animal hosts).

One explanation for the differing results could be that non-*MTL* genes on chromosome 5 might directly or indirectly affect mating. The *C. albicans MTL* locus is much larger than the *S. cerevisiae MAT* locus, and contains several additional genes that encode two phosphoinositol kinases, two oxy-sterol binding proteins, and two polyadenylate polymerases. Deletion of these genes along with the *MTL* genes seems to reduce mating efficiency (*2*). Chromosome 5 genes located outside the *MTL* locus (of which the Magee and Magee organisms had only one copy) may also affect mating frequency. Also, the different temperatures and the contrasting environmental conditions in vitro and in vivo may account for the observed differences in the efficiency and rate of mating of *C. albicans* between the two studies.

Does mating in *C. albicans* occur at any meaningful frequency in nature, and if so, does it generate a tetraploid intermediate (see the figure)? Aneuploid organisms may arise from time to time in the human host, but possibly not with sufficient frequency for compatible mating partners to meet. Hull *et al.* report that a tetraploid cell, generated by one of the induced mating sessions, underwent spontaneous random chromosome loss. Thus, a tetraploid cell might undergo chromosome loss or meiosis to restore the diploid condition. Alternatively, *C. albicans* might undergo meiosis naturally to generate true haploids; these haploids might exist only transiently and only under favorable conditions. Completion of the sexual reproductive cycle might depend on the rapid mating of such haploids during infection of a mammalian host, before the possible deleterious consequences of the entire genome becoming haploid take effect. There are many examples in nature of haploid gametes that are not independently viable and exist only as a transient stage of the life cycle.

It will be now important to examine the formal possibility that *C. albicans* not only mates, but also undergoes meiosis. Significantly, the *Candida* genome sequencing project has revealed homologs of many of the genes in *S. cerevisiae* that are required for meiosis (*14*). The discovery of a full sexual cycle in *C. albicans* will be important for understanding how this fungus has coevolved with its human host. It will also have a major impact on the future of molecular genetics in *C. albicans*. Our detailed knowledge of *S. cerevisiae* biology is based firmly on classical genetic methods that exploit the sexual cycle in this yeast.

These reports provide a salutary lesson to us all. As a direct result of genome sequencing, one of the firmest tenets of the biology of a highly studied microorganism is now being questioned. This is yet another striking example of what the study of genomes can teach us about basic biology.

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PERSPECTIVES: DEVELOPMENT

p73—Guilt by Association?

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Just because proteins have similar amino acid sequences does not mean that they do the same job. The rapidly expanding p53 family of transcription factors exemplifies this phenomenon. The founding member of this family, p53, activates the expression of target genes engaged in promoting growth arrest or cell death in response to genotoxic stress (DNA damage). Mutations in p53 are frequently found in human tumors, and so p53 is often called a tumor suppressor protein. Long considered an orphan, the only one of its kind, p53 has now been found to have two close relatives, p63 and p73. The amino acid identities of these two proteins are conserved within critical functional domains suggesting that, like p53, they may also be involved in the regulation of growth arrest and cell death. However, a wealth of new studies, including the report on p73 (*1*) by Pozniak *et al.* (page 304 of this issue), clearly demonstrate that despite being evolutionarily conserved, members of the p53 family have distinct, even antagonistic biological activities.

The diversity in biological activity among p53 family members stems from several sources including differences in mRNA processing. The *p53* gene generates a single species of mRNA with one open reading frame. In contrast, transcription of the *p63* and *p73* genes generates several alternatively spliced mRNA transcripts (*2*, *3*). Point mutations in the *p53* gene have long been known to alter the activity of the p53 protein (especially its DNA-binding capabilities). However, p53 studies provided no foreshadowing of the contribution of alternative mRNA splicing to the biological diversity of p63 and p73.

There is a *p73* mRNA variant, transcribed under the influence of an alternative promoter located in intron 3 of the *p73* gene, that has a truncated amino terminus (*3*). This truncated form of p73 lacks the transactivation domain, which is essential for activating the expression of p73- and some p53-responsive genes. Similarly, a truncated form of *p63* missing its amino terminus can prevent full-length p63 and p53 proteins from activating transcription of their target genes. Thus, p53 family members lacking a transactivation domain are dominant negative inhibitors of p53-dependent processes.

When overexpressed, full-length p73 shares with p53 the ability to activate common target genes, resulting in growth arrest or apoptosis (*4*–*6*). Despite the structural similarities between full-length p73 and p53 and their display of some common characteristics in culture assays, these proteins differ in several important respects. In contrast to mice that are missing p53, animals lacking all forms of p73 do not exhibit an increased propensity to form tumors (*3*). Moreover, p53-deficient mice develop normally, whereas p73-deficient animals have profound defects in brain development. Thus, p53 and p73 may be involved in different aspects of nervous system development.

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