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Mating and virulence of *Candida albicans*

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Recently, it was demonstrated that *Candida albicans* contains mating type genes. Since that discovery, it has been demonstrated that the majority of strains contain both **a** and α genes, that a minority are homozygous for **a** or α and can undergo fusion, and that the cytological stages of fusion and zygote development are similar to the stages of mating in *Saccharomyces cerevisiae*. However, it has recently been demonstrated that in order to mate, homozygous **a** and homozygous α cells must undergo phenotypic switching from the white to opaque phase, adding a developmental step to mating that might be related to pathogenesis.

Keywords: *Candida*, mating locus, meiosis, phenotypic switching, virulence

Until 1998, scientists had considered and ultimately dismissed the possibility that the major fungal pathogen *Candida albicans* underwent mating. Indeed, up until that time, although mating-associated genes like the pheromone receptor-coupled G protein had been identified (Sadhu *et al*., 1992), genes homologous to the actual mating type genes *MAT***a***1*, *MAT*α*1* and *MAT*α*2* of *S. cerevisiae* had not been identified. In addition, population studies indicated that the population structure of *C. albicans* was for the most part clonal, implying that recombination of genes due to sex was rare (Anderson *et al*., 2000; Gräser *et al*., 1996; Pujol *et al*., 1993). However, this all changed in 1998 when Hull and Johnson identified the mating type-like locus of *C. albicans* in the laboratory strain SC5314 (Hull & Johnson, 1998). The yeast *Saccharomyces cerevisiae* has a mating cassette system in which mating type genes are only expressed when they are moved by a specific recombination event into an expression site in the genome. This yeast, therefore, has two silent loci and one expression locus containing mating type genes (Haber, 1998). In contrast, diploid *C. albicans* possessed one mating type-like (*MTL*) locus that contained an *MTL***a** allele and an *MTL*α allele on homologous chromosomes. In *S. cerevisiae*, the two silent loci *HML* and *HMR* contain a copy of *MAT***a** and *MAT*α, respectively, and the expression locus *MAT* contains either *MAT***a** or *MAT*α (Haber, 1998). A cell switches between **a** and α by recombining at the *MAT* locus with a copy of the silent **a** or α gene. It is, therefore, the genotype of the expressed *MAT* allele that dictates mating type. In contrast, *C. albicans* possesses only one *MTL* locus and must become homozygous for **a** or α by mitotic recombination or gene conversion. In contrast to *S. cerevisiae*, an **a** strain of *C. albicans* (**a**/**a**) cannot convert to α and an α strain (α/α) cannot convert to **a**, since each homozygous strain has lost the alternate *MTL* allele.

Having identified and characterized the mating type-like locus, it was then incumbent to test whether *C. albicans* could in fact mate. Hull *et al*. (2000) generated from the heterozygous strain SC5314 (**a**/α), a hemizygous *MTL***a** (**a**/-) and a hemizygous *MTL*α (α/-) strain. By deleting one of the two *MTL* alleles, they created strains with the appropriate genotypes for mating. They then crossed the **a**/- and α/- strains by inoculation into a mouse. The hemizygous strains were each auxotrophic for a different genetic marker, and mated parents were selected based on complementation of these markers. Hull *et al*. (2000) presented data indicating that cell fusion occurred only between **a**/- and α/- cells. Magee and Magee (2000) similarly generated auxotrophic hemizygous strains by inducing loss of one or the other chromosomal homolog that encoded the *MTL*. They then crossed the **a**/- and α/- strains in culture at 25°C, 30°C or 37°C. They also presented data suggesting that cell fusion occurred only between **a**/- and α/- cells. In both studies cell fusion was demonstrated, but appeared to be a rare event, resulting in apparent tetraploids. Hull *et al*., (2000) provided evidence that

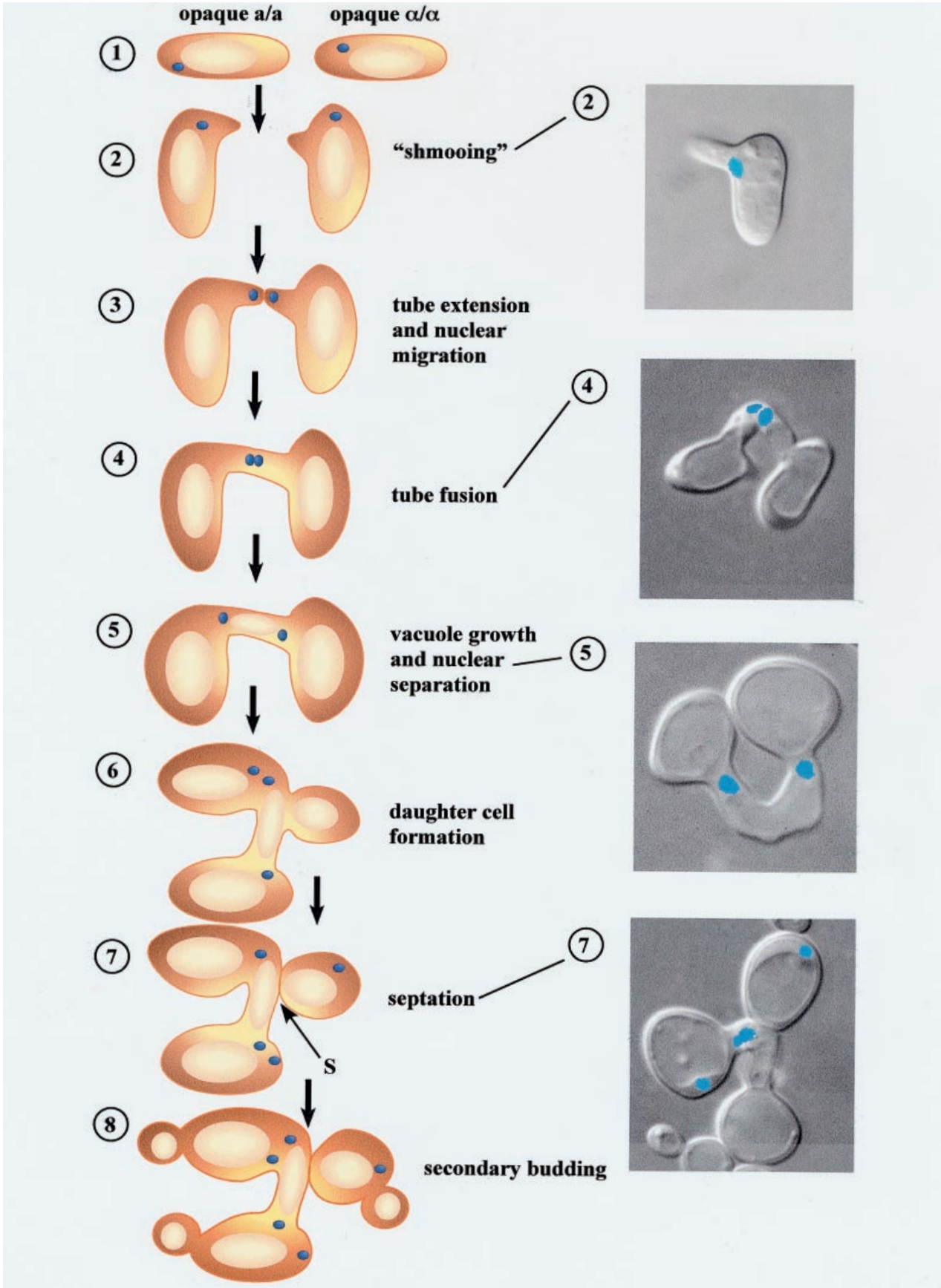

Fig 1 Stages in the mating process of *Candida albicans*. To the left is the interpreted sequence of stages, and to the right select images of cells at particular stages (see text for description). Nuclei in the model are color-coded blue, and nuclei in the images identified by DAPI staining are also color-coded blue. See Lockhart *et al*., 2003, for details.

nuclear fusion occurred. In neither study, however, was meiosis demonstrated, suggesting that the pathway to full sexual recombination was still blocked at a stage after mating.

Hence, in a relatively short period of time, our view of the basic biology of *C. albicans* was complicated (in a good way) by the discovery of a mating system. Not only did *C. albicans* undergo the developmental programs of the bud-hypha transition and high frequency phenotypic switching, but also the complex process of mating. High frequency phenotypic switching is a phenomenon shared by many strains of *C. albicans*, which is manifested as conversion between a fixed repertoire of colony forms each with a distinct pattern of gene expression. In addition, the configuration of the mating loci differed markedly from the cassette system of *S. cerevisiae*. But the story did not rest there. In January of 2002, Miller and Johnson announced at the ASM-sponsored *Candida* meeting in Tampa, FL, the extraordinary observation that both the **a**/- and α/- derivatives of strain SC5314 underwent switching from white to opaque colony forms. This was remarkable since the heterozygous **a**/α parent strain SC5314, like the majority of *C. albicans* strains, did not normally undergo the white-opaque transition (Srikantha *et al*., 1998). Johnson and co-workers further announced that the opaque phase phenotype increased mating frequency around a million fold. These observations were recently published in the journal *Cell* (Miller & Johnson, 2002). To test whether the dependency of switching on *MTL*-homozygosity demonstrated in genetically engineered hemizygotes of strain SC5314 (Miller & Johnson, 2002) could be generalized to clinical isolates that were naturally homozygous at the mating locus, Lockhart *et al*. (2002) identified seven white-opaque switchers in a large collection of clinical isolates of *C. albicans* and tested *MTL*-zygosity. They found that all of the selected white-opaque switchers were *MTL*-homozygous (**a**/**a** or α/α). They then performed the reverse experiment and screened a large collection of clinical isolates for *MTL*-homozygosity. They found that 3% were naturally *MTL*-homozygous, and that the majority of these underwent the white-opaque transition. The great majority of *MTL*-heterozygotes, in contrast, did not undergo white-opaque switching. Those few *MTL*-heterozygous strains that did (one in ten) were found to generate *MTL*-homozygotes at moderate to high frequency, and that it was the *MTL*-homozygous progeny that underwent white-opaque switching (Lockhart *et al*., 2002). In summary, only homozygous **a**/**a** and α/α strains could undergo fusion, and only when they were both in the opaque phase (Miller & Johnson, 2002; Lockhart *et al*., 2003). Observations by Miller & Johnson (2002) and by Lockhart *et al*. (2002, 2003) revealed a number of characteristics of, and relationships between, mating, switching and zygosity. First, they demonstrated that the majority of natural strains of *C. albicans* was heterozygous, but a minority was homozygous. Second, they suggested that most strains of *C. albicans* could undergo the unique white-opaque transition if they became homozygous at the *MTL* locus. Previously, it was believed that the capacity to undergo the white-opaque transition was limited to a small minority of strains. Third, they suggested that one role of the opaque phase phenotype was to facilitate mating. Fourth, they suggested that unlike *S. cerevisiae*, homozygosity at the *MTL* locus was not sufficient for mating competence. This would suggest that *C. albicans* has interjected an additional complex cellular differentiation, the transition from white to opaque, in the acquisition of mating competence.

Having demonstrated that *C. albicans* could mate, it was next incumbent to visualize the mating process that had eluded one hundred years of previous experimental observation by the *Candida* research community. To this end, Lockhart *et al*. (2003) employed a variety of 2D and 3D imaging, reconstruction and fluorescent microscopy techniques to describe the steps in the mating process of *C. albicans* in vitro at 25°C. The steps they described (Fig 1) were remarkably similar to those of *S. cerevisiae* mating (Cross, 1988). When opaque **a**/**a** and opaque α/α cells were mixed, they extended 'fusion tubes' (Fig 1, stages 1 through 4). Tubes of the opposite mating type grew toward each other, and fused at the ends to form a 'fusion bridge' (Fig 1, stage 4). The nuclei of the mother cells moved to the ends of the tubes, and upon tube fusion, became juxtaposed, but did not undergo karyogamy (nuclear fusion) (Fig 1, stages 1 through 4). The nuclei were then separated through growth of a bridge vacuole (Fig 1, stage 5). A daughter cell then formed from the fusion tube (Fig 1, stage 6), the nuclei in the zygote divided, and one or two of these nuclei then entered the daughter cell (Fig 1, stages 6 and 7). Secondary buds then formed on the daughter cell, bridge and mother cells (Fig 1, stage 8). During fusion and daughter cell development, the only septum that formed was at the fusion bridge-daughter cell junction (Fig 1, stage 7). The steps in the mating process of *C. albicans* (Fig 1) were cytologically similar to those in the mating process of *S. cerevisiae*. However, Lockhart *et al*. (2003) found no cytological or genetic evidence for meiosis or segregation under the conditions they employed.

Hull *et al*. (2000) presented evidence that the fusants they selected by complementation were mononucleate, and Magee & Magee (2000) briefly noted the same, although the latter did not provide corroborating cytological evidence to this effect. Lockhart *et al*. (2003) however, found no evidence for karyogamy. The difference between observations may be due to differences in the experimental approaches. Specifically, Johnson and co-workers (Hull *et al*., 2000; Miller & Johnson, 2002) and Magee & Magee (2000) selected through complementation of genetic markers for fusants that were either binucleate or that had undergone karyogamy. The fusants they identified represented a small minority of cells. In contrast, Lockhart *et al*. (2003) observed cellular fusions at a far higher frequency and, therefore, may have missed a minority of cells that had undergone both cellular fusion and nuclear fusion. An alternative explanation might be that incompatibility may have evolved in natural strains through mutation of one or a number of genes essential for karyogamy. In the filamentous ascomycetes, incompatibility between natural strains is the norm (Sauke, 2000), and in *S. cerevisiae*, mutations in at least 13 genes lead to a block in karyogamy (Rose, 1996). None of the studies on cellular fusion in *C. albicans* reported so far, however, have identified a reduction division (meiosis) from tetraploid to diploid, but one study has demonstrated random chromosome loss, that would return a cell to the diploid state (Bennett and Johnson, 2003). However, given the pace of discovery in this area of *C. albicans* biology, evidence for meiosis is probably just around the corner.

In summary, in the last few years we have been witness to the startling discovery of an apparently complete mating system in *C. albicans*, beginning with the identification of the *MTL* locus by Hull & Johnson (1999). Now, instead of emphasizing that the population structure of this pathogen is primarily clonal, we must consider the small but ever present indication of recombination in population studies based on molecular epidemiology (Anderson *et al*., 2000; Gräser *et al*., 1996; Pujol *et al*., 1993). It has been demonstrated that *C. albicans* contains a mating system that is suppressed in the majority of strains heterozygous at the *MTL* locus. Because of the homology of the *MTL***a** and *MTL*α alleles of *C. albicans* with the *MAT***a** and *MAT*α alleles of *S. cerevisiae*, it has been suggested that an Mtl**a**1p/Mtlα2p complex homologous to the Mat**a**1/Matα2 protein complex in *S. cerevisiae* (Herkowitz *et al*., 1992; Johnson, 1995) suppresses mating in *C. albicans* (Miller & Johnson, 2003). However, the regulation of mating in *C. albicans* appears to have an additional developmental component making it ostensibly more complex (Fig 2). In *C. albicans*, homozygosity at the mating locus alone is not sufficient for mating competency. *MTL*-homozygous strains acquire the capacity to switch, and must switch from the white to opaque phase (Slutsky *et al*., 1987; Anderson *et al*., 1987) to acquire mating competence. The transition from white to opaque involves the up-regulation and down-regulation of a number of phase-specific or phase-regulated genes (Soll, 2003) (Fig 2). It is therefore reasonable to suggest that in *C. albicans*, an Mtl**a**1/Mtlα2 protein complex may be involved in suppressing switching, but cannot be the only factor involved in suppressing mating. A white phase-specific factor in addition to the Mtl**a**1p/Mtlα2p complex must also be involved in the suppression of mating in *C. albicans*.

One must wonder why in *C. albicans* the complexity of mating regulation has been increased by adding the additional step of the switch from white to opaque (Fig 2). The answer may be related to the unique phenotype of opaque phase cells and *C. albicans* pathogenesis. Opaque phase cells are stable at 25°C, not at 37°C (Slutsky *et al*., 1987). In addition, opaque phase cells are far more efficient than white phase cells in colonizing the surface of skin (Kvaal *et al*., 1999), which is at a lower temperature than internal regions of the body. If opaque phase cells are shifted from low temperature (~ 25°C) to high temperature (~ 37°C), they spontaneously and efficiently convert to the white phase after two cell doublings (Srikantha and Soll, 1993; Soll, 2001). Hence, *C. albicans* mating must occur outside the human body (*i.e.*, on skin or in an environmental reservoir), or during a two-generation time window prior to the opaque to white transition in the human body. The reason for this added limitation is not obvious, but exist it must. Elucidating it, as well as the molecular pathways involved in the newly demonstrated dependencies of mating on switching and switching on homozygosity, and the mechanisms involved in reducing tetraploids to diploids (meiosis?) after the mating event, represent the immediate challenges in this newly and rapidly evolving story.

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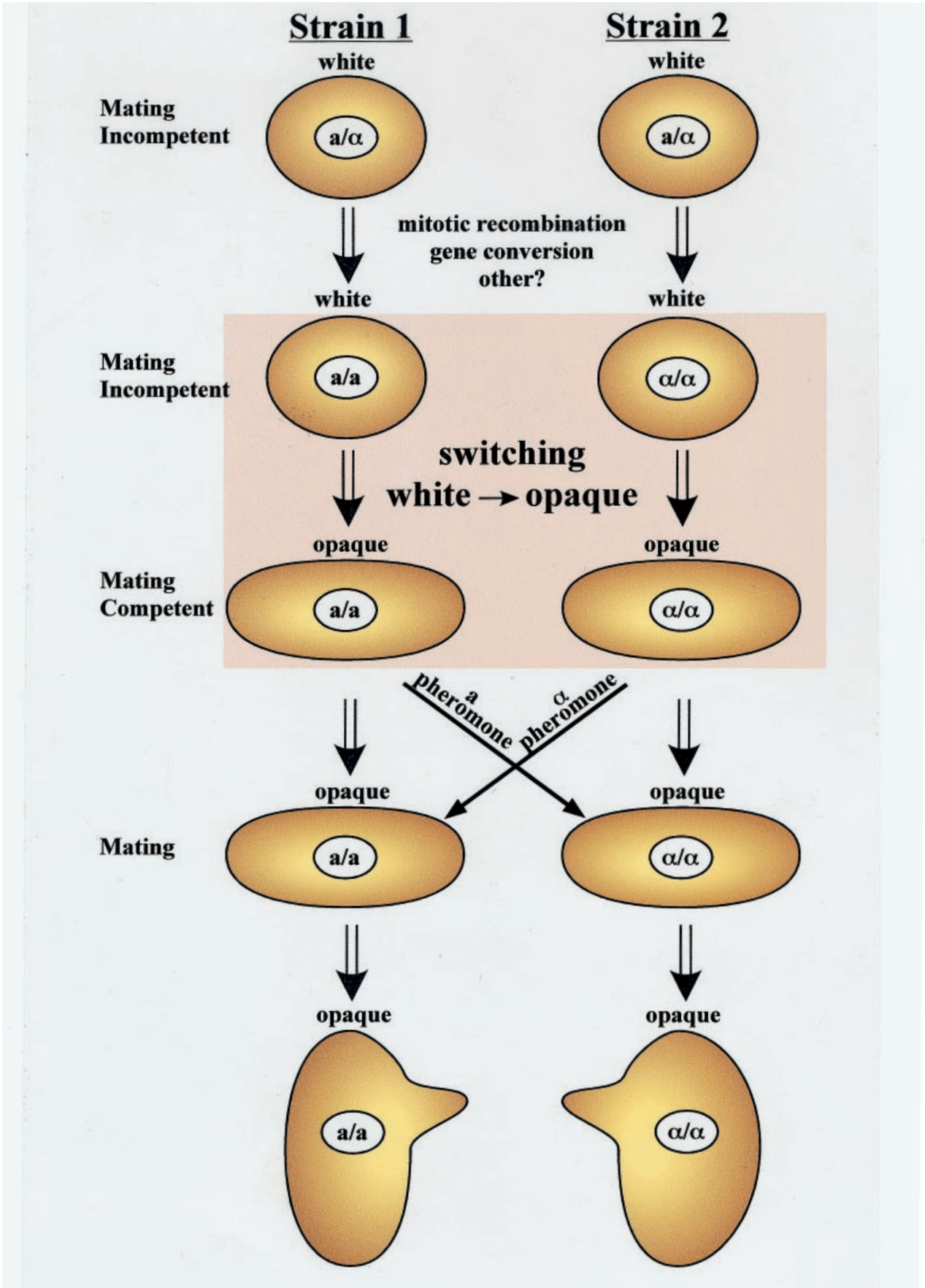

Fig 2 The sequence of genetic and phenotypic changes necessary for mating in *Candida albicans*. The white and opaque cells are drawn as rounded and elongated ovals, respectively.

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