A NEW GENE AFFECTING THE EFFICIENCY OF MATING-TYPE INTERCONVERSIONS IN HOMOTHALLIC STRAINS OF SACCHAROMYCES CEREVISIAE

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ABSTRACT

Homothallic strains of Saccharomyces cerevisiae are able to switch efficiently from one mating genotype to another. From a single haploid spore arise both $a$ and $\alpha$ mating type cells, which then self-mate to produce a colony consisting almost exclusively of nonmating $a/\alpha$ diploid cells. We have isolated a mutant homothallic strain that gives rise to colonies that show bisexual mating behavior. The mating reaction is always asymmetric, that is, in some colonies $a$ mating is much stronger than $\alpha$ mating, while others show greater $\alpha$ than $a$ mating. This mating phenotype arises from the presence of three cell types in a colony: some $a/\alpha$ nonmating diploids and an unequal number of $a$ and $\alpha$ haploid cells. The predominant haploid type is that of the original cell that gives rise to the colony. This mixture of cell types arises from a very reduced efficiency of homothallic mating-type interconversions in the mutant strain.---The mutation, designated switch ($swt-1$), behaves as a single genetic locus. The mutation is centromere linked, but not linked to the mating type locus or to any of the homothallism genes: $HO$, $HM\alpha$ and $HMa$. The switch mutation does not affect the efficiency of self-mating, but rather directly affects the frequency of interconversion of mating types.

SEXUALITY in the yeast Saccharomyces cerevisiae is primarily determined by a single genetic locus. Haploid strains may be either of $a$ or $\alpha$ mating type. Cells of opposite mating type readily fuse to form diploids of $a/\alpha$ genotype that are nonmating and able to undergo meiosis (MORTIMER and HAWTHORNE 1969).

In most laboratory strains of S. cerevisiae, haploid strains are quite stable, remaining haploid and of one mating type. These heterothallic strains do exhibit rare interconversions of mating type at a normal mutation frequency (HAWTHORNE 1963; HICKS and HERSKOWITZ 1977). In contrast, homothallic strains of yeast exhibit a frequent conversion of one mating type to the other (WINGE and ROBERTS 1949; OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1976). Thus, a haploid homothallic cell of $a$ mating type will give rise to offspring of both $\alpha$ and $a$ mating types. The offspring of opposite mating type may then conjugate to form an $a/\alpha$, nonmating diploid isogenic for all loci other than the mating type. The interconversion of mating type occurs often enough that a colony derived from a single haploid homothallic spore will consist almost exclusively of nonmating diploids.

The process by which one mating type is substituted for the other is not yet fully understood, but both genetic and physiological studies have provided a sound basis for further study of the switching of mating types. Genetic analyses have indicated that homothallism is controlled by a dominant gene unlinked to mating type and most commonly designated HO (Oshima and Takano 1971). In addition there are two other important homothallism genes, one of which permits the switching of a mating type spores to \( \alpha \) cells (designated \( HMa \)) and a second gene which permits conversion of \( \alpha \) mating type cells to a (\( HMa \)) (Harashima, Nogi and Oshima 1974; Naumov and Tolstrovukov 1973). Data from Oshima's laboratory show that \( HMa \) and \( HMa \) are both on chromosome III, but at the opposite ends of the chromosome and not closely linked to the mating-type locus (Harashima, Nogi and Oshima 1974; Harashima and Oshima 1976). When these homothallism alleles are all present, a homothallic diploid

\[
\begin{align*}
& \text{S} \quad \text{S} \quad \text{S} \quad \text{S} \\
& \text{a} \quad \text{a} \quad \text{a} \quad \text{a} \\
& \text{Separate} \\
& \text{S} \quad \text{D1} \\
& \text{a} \quad \text{a} \\
& \text{D2} \quad \text{D2-I} \\
& \text{Separate} \\
& \text{D3} \quad \text{D1} \quad \text{D1-2} \\
& \text{a} \quad \text{a} \quad \text{a} \quad \text{a} \\
& \text{D2-I} \quad \text{D2} \quad \text{D1-I} \quad \text{D1-I-1} \\
\end{align*}
\]

**Figure 1.**—Homothallic mating type conversions. A generalized picture of mating type interconversions, as observed by Hicks and Herskowitz (1976). A spore cell of one mating type—in this case a—germinates and divides mitotically to produce an identical daughter cell. At the second cell division, the original spore cell and its second daughter undergo a highly efficient interconversion of mating type, while the first daughter and its daughter remain unchanged in mating type. Thus at the four cell stage there are 2 a and 2 a cells in close proximity and two zygotes form. The colony then becomes composed of a/a diploid cells isogenic for all loci except mating type.

The vertical line of this figure illustrates the process of mating-type interconversion when cells are separated from each other to prevent mating. Under these conditions one can see that mating-type switching will continue to occur in every generation (Hicks and Herskowitz 1976).
yields two α and two a spores, all of which grow into clones of nonmating a/α diploid cells.

A more detailed description of homothallic mating type switching has been provided by the observations of Hicks and Herskowitz (1976), who followed the pedigree of individual spores and their offspring to determine the nature of mating-type switching (Figure 1). A haploid spore was found to give rise to a daughter cell of identical mating type. In the next generation, however, the original spore cell and its next daughter (D2) change to the opposite mating type, while the first daughter (D1) and its offspring remained unchanged. This switching was found to occur at a frequency of greater than 80%. Thus, at the four-cell stage, there are usually two α and two a cells, which are in contact and easily able to mate and form two a/α diploids. Apparently a cell must first go through one round of mitosis before it is able to undergo homothallic mating-type conversion. Switching may then occur in each successive generation if mating is prevented.

We initiated a search for mutations of homothallic strains that would be altered in mating-type switching. Our basic approach was to look for strains whose spores gave rise to colonies that still contained a significant number of cells able to mate, in contrast to normal homothallic colonies that are essentially nonmating. If homothallic mating-type conversion were completely prevented by some mutation, then a colony grown from a single spore would consist only of haploid cells of the same mating type as the spore.

On the other hand, it is possible that a mutation would not entirely prevent homothallic mating-type conversion, but rather make such interconversions infrequent. In this case, a spore will germinate and begin to divide, eventually forming a colony consisting mostly of haploid cells of the original spore genotype. However, as illustrated in Figure 2, infrequent mating type interconversions will occur, giving rise to a few cells of opposite mating type. Such cells would be able to conjugate with haploids of the original mating type to form nonmating a/α diploid cells. If such a colony were tested for mating phenotype by confrontation with complementary α and a cells, there would be extremely efficient mating with the many haploid cells of one mating type in the colony, but also less extensive mating with the few cells of opposite (interconverted) mating type that had not yet self-conjugated. Thus, the colony would appear to mate bisexually, but with one mating type much stronger than the other. Because some a/α diploids are formed, the colony would also be able to sporulate.

In this paper we describe a mutation unlinked to mating type or to the known homothallism genes that makes homothallic mating-type switching inefficient.

**MATERIALS AND METHODS**

**Strains:** A wild type Saccharomyces strain, Y55, originally isolated by Wing and obtained from the collection of Dr. H. O. Halvorson, was used to isolate homothallism mutations. Spontaneous auxotrophic mutations for histidine and tryptophan were independently isolated in this background, as was canavanine resistance. The histidine mutation has been shown by complementation analysis with standard laboratory strains of *S. cerevisiae* to be hisΔ. The trp mutation
FIGURE 2.—A model for the effect of a “switch” (swi) mutation. At the four-cell stage, when normally a switch of mating type in the original spore and its second daughter ought to have occurred (see Figure 1), no such event takes place, leading to 4 cells of one mating type. In later cell generations an interconversion of mating type does occur, but at a low frequency. Consequently, the colony will contain some nonmating \( a/a \) diploids, but also a large fraction of cells of the original mating type and a few cells of opposite mating type.

is not \( trp1, trp2 \) or \( trp5 \). When spores of Y55 were crossed with homothallic strains, provided by Dr. Oshima, all of the segregants were consistent with the assumption that the homothallism genes \( HO, HMa \) and \( HMa \) of Y55 are at identical genetic locations and have an equivalent function to those described by Harashima, Nogi and Oshima (1974). For the testing of homothallism genes, Dr. Oshima kindly provided T-1059-18B (\( a HO HMa hma ade1 gal1 his4 leu2 thr4 \) and C-18-16D (\( HO hma hma arg4 lys2 \)). Heterothallic strains (all of which have the genotype \( ho HMa HMa \)) were derived from strains originally obtained from the Yeast Stock Center in Berkeley, California.

**Growth conditions:** Cells were grown either in liquid or on agar plates with YEPD medium (1% yeast extract, 2% bactopeptone, 2% dextrose). Growth was always at 30° unless otherwise specified.

**Genetic analysis:** Cells were grown on YEPD agar plates at 30° for two days before replica plating to sporulation plates containing 1% potassium acetate supplemented with 0.04% dextrose as well as amino acids, adenine and uracil (Sherman, Fink and Lukins 1970). After 3 days, the cell walls of asci were digested with the enzyme preparation Glusulase (Endo Labs) and tetrads were then microdissected on agar slabs as described by Mortimer and Hawthorne (1969). After germination and growth, the colonies were picked onto master plates and scored for nutritional requirements by replica plating to nutritional drop-out plates (Sherman, Fink and Lukins 1970). Further genotypic characterization by complementation analysis was carried out when necessary by crossing the meiotic segregants with tester haploid strains. Testing for mating type is described below.
SWITCHING OF YEAST MATING TYPE

Mating-type tests: Tests of mating type were basically those described by Haber (1974). Strains to be tested carried at least one auxotrophic marker and were replica plated to a fresh YEPD plate and cross streaked with cells of a or α mating type auxotrophic for some complementary nutritional requirement. The plate was incubated overnight to permit mating between cells at the intersection of the two streaks, and then replica plated to minimal medium. In this way only prototrophic diploids formed by successful matings would grow. Under these conditions, normal homothallic strains derived from single spores exhibit no mating with cells of either mating type.

Pedigree analysis: A pedigree analysis of the conversion of mating type in the mitotic offspring of one cell or spore was carried out essentially as described by Hicks and Herskowitz (1976). Individual spores were placed at well-separated locations on an agar plate by micromanipulation with a glass needle under a microscope. When they had budded to become two daughter cells, the mother and daughter cells were separated from each other. Further separations of the offspring of these cells were also performed. The cells were then allowed to grow to colonies which were then tested for mating behavior.

In order to determine whether the mating behavior of a colony derived from a single spore was consistent with the original mating type of the spore, the spores were first germinated on medium containing α factor which arrests the growth of a cells but does not affect a cells. It was then possible to determine by direct observation which spores initially were a and which were α (Hicks and Herskowitz 1976). The cells were then transferred to agar medium not containing α factor, so that all cells could grow to form colonies. These colonies were then tested for mating behavior.

Partially purified a factor was prepared according to the method of Dunze et al. (1973). The medium from a 48 hr stationary phase culture of α cells was passed over an Amberlite CG-50 column and then eluted with 0.01 N HCl in 80% ethanol. The eluate was then neutralized, concentrated, lyophilized and resuspended in H2O.

Selection of strains altered in homothallic mating-type switching: To enhance the isolation of spontaneous mutants altered in homothallic mating-type interconversion, we allowed spores that could undergo normal switching to conjugate and form nonmating diploids, so that only cells that could not efficiently switch mating types would remain able to mate. Sporulated cultures of Y55-2 (HO HMa HMa can+ trp) and Y55-3 (HO HMa HMa can+ histo) were separately treated with the enzyme preparation Glusulase to break the ascus wall surrounding four spores of one tetrad. The spores were then freed from each other by gentle sonication. The spores from each strain were inoculated into YEPD liquid medium at a density of approximately 1 x 10⁶ spores per ml and allowed to germinate and conjugate for 18 hours with extremely gentle stirring. During this time virtually all cells which were capable of efficient homothallic self-mating should have done so. The two cultures were then mixed together and pelleted by centrifugation. The cells were resuspended in a small volume of sterile water and spread at a density of approximately 1 x 10⁷ cells on YEPD plates. After overnight growth, the cells were replica plated to minimal medium, to select complementing diploids formed by mating Y55-2 and Y55-3. Colonies able to grow in minimal medium were then picked, purified, and sporulated. Tetrad dissections were carried out to isolate segregants which might show altered homothallic properties.

RESULTS

When wild-type homothallic spores are allowed to germinate and grow, mating type conversion is so efficient that haploid cells in close proximity are able to mate to form nonmating diploids. We allowed spores to go through this process of mating-type interconversion and mating before confronting the cells with a complementing strain to select cells that might be altered in homothallic mating-type switching (see Materials and Methods). Some of the complementing diploids selected in this manner seemed to be formed by the mating of two homothallic cells that simply, by chance, had not yet mated with a cell of opposite
mating type; and when these diploids were sporulated and dissected, all four segregants of one tetrad grew to form apparently normal, nonmating homothallic colonies. Among the first 10 diploids tested, one yielded tetrads from which there were only two nonmating colonies and two that exhibited substantial mating as both mating types. In every case, one mating type was much stronger than the other, as measured by the growth of complementing cells on minimal medium. Examples of this asymmetric dual mating behavior of colonies are shown in Figure 3. In some segregants, the colony exhibited much more $a$ mating type than $a$; in approximately an equal number of segregants, $a$ mating type predominated over $a$. Despite exhibiting strong mating, these "bisexual" colonies were also able to sporulate, in contrast to wild-type yeast strains that either mate but do not sporulate or else sporulate but are nonmating (Fris and Roman 1968). 

Characterization of the bisexual mating phenotype: It was important to determine whether the bisexual mating behavior was a property of each individual cell in the colony or rather was a reflection of a mosaic nature of the clone itself. To this end, one bisexual segregant designated 1–3C, was subcloned from single cells and the colonies arising from individual cells were then tested for

Figure 3.—Mating behavior of subclones of a bisexual ($a > a$) colony. A bisexual strain, 1–3C, which mates more strongly as an $a$ than as an $a$ was spread for single colonies on a YEPD plate. Thirty-four colonies were arranged on a YEPD and cross streaked with $a$ and $a$ tester strains. After overnight growth, this mating plate was replica plated to two minimal medium plates. The plate on the right was incubated for about 20 hr. One sees bisexual mating in some colonies and nonmating in others. The plate on the left was incubated for about 12 hr. Here, where less growth has occurred, one can see that bisexual mating is extremely asymmetric. While most bisexual colonies show the same asymmetry ($a > a$) as the original clone, one subclone, in the fourth row, shows a distinct reversed ($a > a$) mating pattern.
mating behavior and sporulation. We found that the clones, indeed, contained a mixture of cell types as illustrated in Figure 3.

Strain 1–3C exhibited much stronger α mating type than α mating type (designated α>α). Three types of mating behavior appeared in the subclones. Approximately half the colonies retained the same mating genotype as the original clone, that is, α>α. Most of the remaining subclones were entirely nonmating in character, as would be expected for a normal homothallic diploid. A small number of subclones exhibited reversed bisexual mating behavior, i.e., greater α than a mating behavior (α>a). All colonies were able to sporulate. When nonmating subclones were streaked to give new colonies derived from single cells, all of these colonies were nonmating. The α>α colonies again gave a mixture of subclones distributed essentially as in Figure 3. However, those rare subclones that showed a reversed asymmetric mating (α>a) gave rise to a set of colonies in which approximately half of the colonies showed more α mating than a mating and only a very few colonies in which a mating was stronger than α mating. The remainder were nonmating.

Thus, it seems that asymmetric mating behavior is not a property of every cell in a clone. Some cells give rise to colonies in which there is far more α mating type than α mating (α>α), whereas a few cells from the same colony give rise to exactly opposite mating behavior (α>α). Still other cells are nonmating. These results are consistent with those expected for a strain showing the inefficient homothallic mating type switching shown in Figure 2. If a single haploid cell of mating type a were grown to a colony, the colony should exhibit an (a>α) mating type, but also should contain some nonmating cells. However, if a (relatively infrequent) haploid cell of α mating type—from the same original colony—were chosen, an exactly opposite (α>a) mating phenotype would be found.

If the mating behavior of colonies of this mutant strain is to be explained by an assumption of inefficient mating-type switching, we would predict further that the nonmating cells would be a/α diploid cells, while the cells still able to mate would be haploid. Furthermore, as the nonmating a>α diploid cells must have arisen in a colony by conjugation of cells isogenic at all loci except the mating type, we would expect that the four spores of a tetrad of such a nonmating cell would all give rise to colonies that again show an asymmetric bisexual mating type and would be homozygous for all auxotrophic markers.

Analysis of nonmating colonies: Nonmating cells in the mixed colonies of strain 1–3C are, indeed, diploid and apparently homozygous for the mutation affecting homothallic switching. Subclones of strain 1–3C (a>α) that showed no mating were sporulated, tetrads dissected and the four colonies grown from spores of each tetrad were then tested for mating phenotype. In Figure 4 we show a time course for the appearance of complementary diploids formed by matings between meiotic segregants of strain 1–3C and either a or α tester strains. Cells were allowed to conjugate on a YEPD plate overnight. The extent of mating was then monitored by examining growth on a minimal medium replica plate photographed at intervals. After 12 hr incubation, the complementation pattern
shows two $\alpha$ and two $\alpha$ mating colonies in each tetrad, as would be seen for a heterothallic diploid. However, with longer incubation of the mating test plates, a pronounced but less strong growth begins to appear at the intersection of opposite mating type. Each tetrad in fact gives rise to two ($\alpha>\alpha$) and two ($\alpha>\alpha$) bisexual mating colonies. Thus, a diploid homozygous for the mutation affecting interconversion is itself nonmating, as are wild type $\alpha/\alpha$ diploids; however, the altered homothallism phenotype is re-expressed in colonies derived from haploid meiotic segregants of the diploid. These observations confirm that nonmating cells within a colony of strain 1–3C are $\alpha/\alpha$ diploids and that each haploid spore gives rise to either an ($\alpha>\alpha$) or ($\alpha>\alpha$) colony, depending on its mating genotype.
In fact, we were able to confirm more directly that the asymmetry of mating is a direct reflection of the original mating genotype of the spore. This was accomplished by dissecting spores from one tetrad onto agar containing $a$ factor (Hicks and Hershkowitz 1976). The two cells carrying the $a$ mating type allele are allowed to germinate and to divide, whereas the two $a$ cells become arrested in cell division and can readily be distinguished. After the apparent genotype was determined, the cells were micromanipulated onto another piece of agar not containing $a$ factor and allowed to grow into colonies. We found that the two clones derived from $a$ mating type spores exhibited an asymmetric bisexual mating pattern with much greater $a$ mating character than $a$ mating, whereas the reverse was true for the two colonies derived $a$ spores.

Characterization of cells able to mate: By mating a colony of cells exhibiting ($a>a$) mating type with both $a$ and $a$ haploid strains, we could demonstrate that those cells in the mutant homothallic colony that actually mated were indeed still haploid. The bisexual ($a>a$) strain 1–2A ($HO\, HMa\, HMa\, his6\, trpx$) was mated with heterothallic haploid strains A88 ($a\, HMa\, HMa\, his4\, ade1\, leu2\, lys2$) and A90 ($a\, ho\, HMa\, HMa\, thr4\, leu2\, lys2$) and complementing wild-type conjugants were selected on minimal medium. The purified wild-type cells were then sporulated and dissected for tetrad analysis. All of the nutritional markers tested in these crosses segregated $2$ wild type: $2$ auxotroph, characteristic of the segregation of heterozygous markers in diploid cells. These results demonstrate that both the $a$ mating type and $a$ mating type cells in a colony of strain 1–2A are haploid. Thus, a clone derived from a single spore contains nonmating $a/a$ diploid cells as well as both $a$ and $a$ haploid cells. Again, these observations are consistent with the idea that strains 1–3C and 1–2A are mutant homothallic strains in which mating type interconversion is very inefficient.

The segregation of mating phenotypes in meiotic tetrads of the diploids 1–2A/A88 and 1–2A/A90 is consistent with the notion that strain 1–2A carries both a normal $HO$ gene and some other unlinked, modifying mutation. Each tetrad contains two apparently heterothallic ($ho$) segregants, with either $a$ or $a$ mating type. These two segregants do not sporulate. The other two segregants are either nonmating or exhibit asymmetric bisexual mating, and all of these segregants sporulate. It seems most likely that both the nonmating and bisexual colonies from segregants carry the $HO$ allele. Those colonies that exhibit either an ($a>a$) or ($a>a$) mating phenotype must also carry a mutation affecting mating type interconversion. We assume that the mutation is an allele of a gene essential for normal homothallic mating type interconversion that we have designated "switch" (swi1). The mutation has no apparent effect on heterothallic ($ho$) strains.

Genetic analysis of the "switch" gene: The diploids constructed between strains 1–2A and A88 or A90 appear to be heterozygous for both homothallism ($HO/ho$) and the switch mutation (swi1−1/+). Based on the assumption that the asymmetric dual mating type arises in cells carrying $HO$ and swi1−1, whereas nonmating colonies are $HO$ and wild type for the switch gene, the data from Table 1 can be used to determine the linkage of $HO$ and swi1. The ratio
TABLE 1

Segregation of bisexual mating in diploids heterozygous for mating and heterozygous for the homothallism gene HO

<table>
<thead>
<tr>
<th>Diploid strain*</th>
<th>a</th>
<th>α</th>
<th>a</th>
<th>a</th>
<th>α</th>
<th>a</th>
<th>a</th>
<th>a</th>
<th>a</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2A/A88</td>
<td>a a</td>
<td>α a</td>
<td>a a</td>
<td>a a</td>
<td>α a</td>
<td>a a</td>
<td>a a</td>
<td>a a</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>1-2A/A90</td>
<td>3 3</td>
<td>1 1</td>
<td>4 1</td>
<td>2 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6 2</td>
<td>7 6</td>
<td>3 3</td>
<td>1 1</td>
<td>17 20</td>
<td>3 4</td>
<td>3 4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrad mating type†</td>
<td>PD</td>
<td>NPD</td>
<td>TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2A/A88</td>
<td>3 3</td>
<td>1 1</td>
<td>4 1</td>
<td>2 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td></td>
</tr>
<tr>
<td>1-2A/A90</td>
<td>3 3</td>
<td>1 1</td>
<td>4 1</td>
<td>2 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
<td>1 1</td>
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</tr>
<tr>
<td>Total</td>
<td>6 2</td>
<td>7 6</td>
<td>3 3</td>
<td>1 1</td>
<td>17 20</td>
<td>3 4</td>
<td>3 4</td>
<td>3 4</td>
<td>3 4</td>
<td></td>
</tr>
</tbody>
</table>

* Two diploid strains were constructed by crossing a bisexual strain 1-2A (bisexual, HO HMα HMα his6 trp) with haploid strains A88 (a ho HMα HMα adel leu2 his4 lys2) and A90 (a ho HMα HMα adel leu2 his4 thr4). Ascii were dissected and the mating behavior of each segregant was then determined.

† Nonmating colonies are designated by −. Bisexual colonies exhibiting greater a mating type than α are indicated by a>a, while the reverse bisexual phenotype is given as α>a.

of parental ditypes: nonparental ditypes: tetratypes is 8:15:39, which is consistent with a 1:1:4 ratio expected if HO and swil are unlinked.

From these data we also conclude that bisexual mating behavior is not closely linked to the mating type locus. The predominant mating type of the bisexual colony reflects the original spore mating type. As there are as many (a>a) segregants as (α>a) segregants in both the tetrads from the diploid 1-2A/A88 and those from 1-2A/A90, we conclude that the swil-1 allele is as likely to segregate with a as with α.

A more detailed genetic analysis of the swil-1 mutation was accomplished by constructing a diploid homozygous for HO and heterozygous for swil. The diploid was constructed by mating spores of homothallic strain exhibiting normal mating-type switching with a bisexual homothallic (swil) strain. The genotype of this diploid, designated 6A/22B is:

\[
\begin{align*}
\text{a} & \quad \text{HO} \quad \text{HMα} \quad \text{HMα} \\
\text{α} & \quad \text{HO} \quad \text{HMα} \\
\text{leu2} & \quad + \\
\text{his4} & \quad \text{his6} \quad \text{lys2} \\
\text{swil-1} & \quad +
\end{align*}
\]

All segregants carry the homothallism genes HO HMα and HMα. The bisexual mating behavior appears to segregate as a single trait, as 34 of the 38 tetrads gave 2 nonmating: 2 bisexual segregants (Table 2). It is also possible to determine the linkage of swil to the mating type locus and to the centromere-linked marker leu2, both of which are on chromosome III. From the fact that the number of parental ditypes and nonparental ditypes are equal, we conclude that swil is not linked either to the mating type locus or to leu2. On the other hand, the swil mutation appears to be tightly linked (within 10 cM) to some other
SWITCHING OF YEAST MATING TYPE

TABLE 2

Genetic analysis of the switch (swi) mutation

A. Segregation of bisexual mating

<table>
<thead>
<tr>
<th>Tetrad mating type*</th>
<th>a&gt;a</th>
<th>a&gt;a</th>
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<th>a&gt;a</th>
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</tr>
<tr>
<td>Number</td>
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<td>11</td>
<td>8</td>
<td>2</td>
<td>2</td>
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</table>

B. Segregation of swi and chromosome III markers

<table>
<thead>
<tr>
<th>Markers analyzed</th>
<th>PD</th>
<th>NPD</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>swi : leu2</td>
<td>15</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>swi : mating type</td>
<td>15</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>leu2 : mating type</td>
<td>20</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

A diploid colony homozygous for the homothallism gene HO and heterozygous for bisexual mating (swi) and several nutritional markers including leu2, was sporulated and dissected. The mating behavior of each segregant and the segregation of bisexual mating relative to other markers are given in parts A and B, respectively. 

* The mating behavior of the 4 members of a tetrad is given. Two types of bisexual mating of a colony are distinguished: that with much more a mating type than α (a>a) and the reverse (α>a). Nonmating colonies are designated by –.

centromere, as the percent tetratype asci in segregations with leu2 is approximately 20%, as opposed to 67% which would be expected if swi1 were not centromere linked. That swi1 itself does not alter normal meiotic linkage relations is seen from the fact that the segregation of leu2 and the mating type locus gives a pattern which is expected for markers which are approximately 25 cM distant on the same chromosome. We also concluded from an analysis of the tetrads of 1–2A/A88 that swi1 is not linked to the centromere-linked marker ade1 on chromosome I (data not shown). Further, because the two homothallism genes HMα and HMα have been shown to be on chromosome III, but not centromere linked, (Harashima and Oshima 1976), swi1 cannot be closely linked to either of these homothallism genes.

Characterization of the delay in homothallic mating type switching: The delay in normal homothallic switching could be determined from the frequency with which one obtains a reversal of the mating asymmetry in subclones derived from a bisexual colony. In wild-type homothallic strains, mating-type interconversions occur in nearly one out of two cell divisions (Hicks and Herskowitz 1976), so that a colony of cells, if prevented from conjugating, would consist of a nearly equal number of cells of both mating types. As we discussed above, if mating-type interconversion occurs only infrequently, a colony will contain many more haploids of one mating type than the other. The ratio of the number of cells of each mating can be used to determine how frequently mating-type switching occurs.

Rather than examine the mating types of individual cells in a clone, we have taken advantage of the colonial mating phenotypes of cells carrying the swi1–1 mutation. As shown in Figure 3, if an a spore is germinated, the resulting colony
J. E. HABER AND B. GARVIK exhibit an \((a>a)\) mating phenotype. The colony actually consists of many \(a\) haploids, a few \(a\) haploids and some \(a/a\) nonmating diploids. When subcloned, a single \(a\) haploid cell will give rise to an \((a>a)\) colony. Thus, the ratio of \(a\) haploids to \(a\) haploids in a particular colony can be measured by determining the proportion of \((a>a)\) and \((a>a)\) subclones derived from the original colony. Even though the mating tests used here do not examine the mating behavior of one cell, the colonial phenotype gives a clear indication of the mating type of the original single cell that formed the colony. For this determination, strain 1–3C was sporulated and dissected to give tetrads each of which contain four bisexual clones (two \(a>a\) and two \(a>a\)). Colonies from four tetrads were subcloned to obtain 100 single colonies which were then tested for mating. In each case there were many more subclones of one asymmetric mating type than of the other (Table 3; cf. Figure 3). The ratio of the infrequent, reversed mating pattern to the predominant asymmetric mating phenotype in these subclones is approximately 0.007 in the first experiment and 0.03 in the second. The difference in these results between experiments 1 and 2 in Table 3 may be attributed to the

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Spore</th>
<th>(a&gt;a)</th>
<th>(a&gt;a)</th>
<th>Nonmating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>91</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1B</td>
<td>1</td>
<td>91</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1C</td>
<td>0</td>
<td>92</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1D</td>
<td>86</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>0</td>
<td>87</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>87</td>
<td>0</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>2C</td>
<td>91</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2D</td>
<td>1</td>
<td>90</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>85</td>
<td>1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>3B</td>
<td>88</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3C</td>
<td>0</td>
<td>91</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3D</td>
<td>0</td>
<td>95</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4A</td>
<td>92</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>4B</td>
<td>0</td>
<td>88</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4C</td>
<td>0</td>
<td>80</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4D</td>
<td>53</td>
<td>2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>5A</td>
<td>2</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>6B</td>
<td>55</td>
<td>1</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>7B</td>
<td>2</td>
<td>55</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>8D</td>
<td>66</td>
<td>1</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Tetrads of strain 1–3C were dissected and tested for mating after growth of the colonies. Two colonies in each tetrad exhibited bisexual mating behavior in which there was much more mating as an \(a\) strain than as \(a\) \((a>a)\). The other two members of the tetrad showed much more mating as \(a\) cells than as \(a\) \((a>a)\). Each colony was then subcloned, and approximately 100 subclones were tested for mating.

---

**TABLE 3**

*Mating behavior of subclones of bisexual segregants of strain 1–3C*
time of cultivation of the colonies prior to subcloning. If colonies remain at stationary phase, there is an increase in the proportion of nonmating subclones. From these data we can estimate the efficiency of mating-type interconversions in swil-1 strains. A switch once in every 26 cell divisions would give a ratio of one mating type to the other of 0.016, similar to the ratios in Table 3. For wild-type homothallic strains, Hicks and Herskowitz (1976) found that a switch occurred at a frequency of approximately 0.4, as only the mother cell and not its daughter was able to switch in the next generation. Thus, the swil-1 mutation appears to reduce mating-type interconversions to about 4% of the wild-type frequency.

An alternative way of estimating the efficiency of mating-type switching is to measure the ratio of nonmating colonies to the total number of colonies. Here we assume that the frequency of conjugations to form nonmating diploids is limited by the number of mating-type interconversions to the infrequent mating type. In experiment 1 of Table 3, 171 of 1,572 subclones are nonmaters. In some of these cases, the nonmating colony may have arisen from an event in the generation after plating single cells, so that a frequency of 171/3144, or 0.055, switching events is calculated.

Thus, by either criterion, the swil-1 mutation reduces the efficiency of mating-type interconversions to less than 10% of the wild-type rate.

Pedigree analysis of mating type switching in swil-1 strains: By carrying out a pedigree analysis similar to that described by Hicks and Herskowitz (1976), we confirmed by direct microscopic examination that mating-type switching in homothallic strains carrying the mutation swil-1 was significantly delayed. Individual spores from tetrads of strain 1–3C were micromanipulated to separate locations on an agar plate. After germination and cell division, the original spore cell and its daughter were separated; and as growth continued, this process was repeated for one or more generations. The separated cells were then allowed to grow up into colonies and were tested for mating behavior. The results for one complete tetrad analyzed in this way are shown in Table 4. Each of the offspring of one segregant exhibited the same mating pattern. If a normal switch of mating

<table>
<thead>
<tr>
<th>Spore</th>
<th>Colony origin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>A</td>
<td>a&gt;α</td>
</tr>
<tr>
<td>B</td>
<td>a&gt;α</td>
</tr>
<tr>
<td>C</td>
<td>a&gt;α</td>
</tr>
<tr>
<td>D</td>
<td>a&gt;α</td>
</tr>
</tbody>
</table>

* Four spores of one tetrad were placed on an agar slab by micromanipulation and allowed to germinate. The original spore cell (S) was then separated from its first daughter (D1). After another generation of growth, the original spore cell was separated from its next daughter (D2), while the first daughter cell and its daughter (D1-1) were also separated. A third separation was also carried out in some cases. Each separated cell was allowed to grow into a colony and was then tested for mating.
type had occurred, we would have found some colonies with an asymmetric mating pattern opposite from that of the other colonies. Finding a consistent mating pattern confirms that normal homothallic switching, at least to the stage of separating four cells, must be delayed by at least one or two cell divisions.

We carried out such pedigree analysis for 15 spores. In no case did we find that the original spore cell, even after giving rise to several daughter cells, had an opposite asymmetric mating pattern from that of any of its daughters. There were two cases in which a mating-type switch may have occurred. In two instances we found nonmating colonies from the original spore (S) cell after separation of S and D2. These may represent a normal homothallism event beginning with the S cell in its third cell division and leading to only diploid cells.

This cell lineage analysis also allows us to reject the possibility that those cells which do convert mating type are preferentially inviable, leading to many more cells of the original genotype. Were this hypothesis true, we should have found that some cells observed in following cell lineage would not have grown or formed colonies. All of the cells separated by micromanipulation during the first few cell divisions following germination were viable and grew normally to form colonies.

Effect of other homothallism genes on the swi phenotype: We were also able to demonstrate that the asymmetric bisexual mating phenotype of homothallic haploid strains carrying the swi1-1 mutation depends not simply on the presence of the HO allele, but on the ability of a homothallic spore actually to interconvert mating type. In homothallic (HO) strains, the gene HMa is apparently required for the switching of an a cell to α (Harashima, Nogi and Oshima 1974). An alternative allele, hma, has been shown not to permit this conversion. Thus, a strain of genotype a HO HMa hma remains a haploid of a mating type, while cells of a HO HMa hma genotype are able to undergo the normal homothallic mating-type interconversion and conjugation. To see whether the asymmetric mating pattern observed in swi1-1 strains depends on the ability of a cell to switch in both directions, we constructed a HO HMa hma swi1-1 and a HO HMa hma swi1-1 strains from meiotic segregants of a cross between the bisexual homothallic strain 1-3C and strain T-1059-19C, a haploid homothallic strain unable to convert mating type from a to α (a HO HMa hma). Colonies from spores of a HO HMa hma swi1-1 genotype exhibited only a mating type, as we would expect for a strain unable to interconvert mating type. Colonies of a HO HMa hma swi1-1 strains always exhibited (a>α) mating pattern. When such a colony was subcloned, however, no rare reversed (a>α) mating patterns were found; rather, there were colonies with only strong a mating type and no α mating conversion. We feel that these results support our conclusion that the asymmetric bisexual mating pattern found in swi1-1 strains does indeed arise from the greatly reduced efficiency of mating-type interconversion.

The swi1-1 mutation probably does not affect mating efficiency: We have also tried to rule out the possibility that the bisexual mating observed in homothallic strains carrying swi1-1 resulted from impaired mating between the opposite
mating-type cells formed by normal homothallic switching. Such a possibility seems unlikely because bisexual mating of these clones is invariably asymmetric, indicating the presence of more cells of one mating type than the other. If mating were inefficient, but mating-type interconversions normal, we would expect to see equal numbers of both mating phenotypes. The subclone analysis given in Table 3 and shown in Figure 3 confirms the disproportionate number of one mating type over the other.

The *swi1*-1 mutation does not affect the mating of heterothallic (*ho*) haploid strains. A number of different mutations affecting mating have been described by MacKay and Manney (1974). These sterile (*ste*) mutants give very poor mating in complementation tests, whereas *swi1*-1 haploids are indistinguishable from *SWII* strains.

To confirm further that the asymmetric bisexual mating pattern found with *swi1*-1 is not due to inefficient mating of haploid cells, we have also examined the mating behavior of wild-type homothallic colonies in which mating itself is made inefficient. We based our experiment on the observation of Pallaroni (1961) that homothallic spores germinated on acetate media fail to form zygotes in microcolonies. We dissected and germinated wild-type homothallic spores of strain Y55-2 on yeast extract-peptone medium containing glycerol (YEPG) instead of glucose and then streaked the colonies on YEPG. After overnight growth, the colonies were replica plated to a YE PD plate for a standard mating test. Treated in this way, the colonies derived from wild-type homothallic spores had not already conjugated to form nonmating diploids, but exhibited extensive mating with both mating-type testers, whereas spores germinated on YEPD gave nonmating colonies. Invariably, the extent of mating with a and α strains was equal, in contrast to the asymmetric mating behavior caused by the *swi1*-1 mutation. The *swi1*-1 mutation does not primarily prevent a or α cells from mating.

A further demonstration that *swi1*-1 does not affect mating efficiency was carried out by comparing the extent of zygote formation between homothallic spores and a or α ade1 haploid strains. Spores of homothallic strains, wild type or carrying the *swi1*-1 mutation, were germinated on YE PD plates in the presence of haploid cells of one mating type. After 24 hr, cells were resuspended and plated on minimal medium to assess the formation of complementary diploids and on complete medium minus adenine to determine the fraction of homothallic cells that had mated with the adenine requiring haploid. The results in Table 5 show that the efficiency of mating of *swi1*-1 strains is comparable to that in wild-type homothallic strains. In fact, the *swi1*-1 strain seems to mate somewhat more efficiently; however, this is most likely due to the decreased probability of self-conjugation.

**Isolation of other switch mutants:** The procedure described in this paper to isolate mutants with impaired homothallic mating-type interconversion has been used to isolate other mutants, as well as *swi1*-1. A second switch mutant, *swi2*-1, has been found that acts substantially like *swi1*-1 (Garvik and Haber, manu-
TABLE 5

**Efficiency of wild type and swi1-1 homothallic spores in mating with haploid heterothallic cells**

<table>
<thead>
<tr>
<th>Homothallic strain</th>
<th>Heterothallic strain</th>
<th>Number of colonies growing on minimal*</th>
<th>Fraction homothallic cells that have mated with ade1 haploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3C (swi1-1)</td>
<td>a ade1</td>
<td>8.6 x 10^5</td>
<td>0.65</td>
</tr>
<tr>
<td>Y55-3 (SW11)</td>
<td>a ade1</td>
<td>1.0 x 10^6</td>
<td>0.39</td>
</tr>
<tr>
<td>1-3C</td>
<td>a ade1</td>
<td>2.9 x 10^5</td>
<td>0.55</td>
</tr>
<tr>
<td>Y55-3</td>
<td>a ade1</td>
<td>2.0 x 10^5</td>
<td>0.10</td>
</tr>
</tbody>
</table>

The extent of mating of wild-type and "switch" homothallic cells with heterothallic haploid strains was measured. Separated spores of both Y55-3 (HO HMa HMa trp can+ SW11) and 1-3C (HO HMa HMa trp can+ swi1-1) were prepared after sporulation as described in MATERIALS AND METHODS. Approximately 4 x 10^6 spores of either Y55-3 or 1-3C were mixed with about 6 x 10^6 haploid heterothallic cells (a ho ade1 or a ho ade1) and spread on a YEPD plate to germinate and conjugate. After overnight growth at 30°C, about 1 x 10^6 cells were resuspended in sterile H2O and plated after dilution on minimal medium or complete medium minus adenine to measure the fraction of homothallic cells that had mated with the adenine requiring haploid.

* Colonies growing on minimal medium are wild type diploids formed by mating of the homothallic and heterothallic parents.

† Colonies growing on complete medium lacking adenine (ade- plates) are either wild-type diploids derived from mating of the homothallic strain or the homothallic strain that has not mated with an ade1 haploid. This plate reflects the total number of colonies derived from the homothallic spores.

The switch (swi1-1) mutation is present in a homothallic haploid spore, the efficiency of switching from one mating type to the other is greatly reduced. Rather than forming a colony composed almost exclusively of nonmating, diploid cells, homothallic spores carrying swi1-1 grow to yield mixed colonies containing many haploid cells of the original mating genotype, nonmating diploid cells, and a few cells of opposite mating type. The frequency with which mating-type switching appears to take place is much greater than the rare events with heterothallic strains (Hicks and Herskowitz 1977), but it is less than 10% of the rate in normal homothallic strains. The swi1-1 mutation affects mating-type switching both from a to α and from α to a.

**Relation of swi1-1 to other homothallism genes:** The swi1-1 phenotype is only manifest when a homothallic spore is actually able to undergo mating type switching. Thus, a strain α HO hma HMa swi1-1 will show α>α mating in a colony formed from a single cell, while a colony of aHO hma HMα swi1-1 shows only α mating.

A similar mating phenotype has been reported by Naumov, Kondratieva and Tolstoraiov (1974) for strains carrying an altered allele of HO from another Saccharomyces species. The switch mutant reported here clearly differs from a
direct mutation of the HO gene, as swit–1 segregates completely independent of HO (Table 2). Further, because swit–1 is apparently centromere-linked, while neither HMa nor HMα is, swit–1 is not allelic with either HMa or HMα.

It is also most likely that swit–1 represents a different gene from cmt (change of mating type) isolated by Hopper and Hall (1975). The cmt mutation makes haploid heterothallic strains diploid and isogenic, but is not allelic to HO. This mutation has no apparent phenotype in strains carrying HO (Hopper and Hall 1975), just as swi does not affect heterothallic strains (ho). It would be interesting to see if a heterothallic strain carrying both swi and cmt showed an asymmetric mating pattern.

The role of the switch gene in homothallism: The switch mutation (swit–1) apparently interferes with the normal way in which homothallic mating-type interconversions are carried out. The mechanisms by which the set of one mating-type information is substituted for the other at the mating-type locus are not yet clear. It now seems likely that there must be silent copies of both a and α information in the cell that can be "plugged in" to the mating-type locus. The evidence that such additional information is present in the cell comes from the observations of Hicks and Herskowitz (1977), who found that α sterile mutants at the mating-type locus could be converted either by the homothallic genetic system or by rare spontaneous mutations to a functional α genotype. This correction of an α sterile mutation to a functional α allele could occur only if some other copy of α information was present. A similar "curing" of an α mutant has also been found (Wygal and Haber 1977).

The mechanisms by which mating-type information is actually interconverted is not yet understood. We believe that the switch mutation represents an alteration in a gene essential to this process.

Deborah Wygal kindly prepared the α factor. We are grateful for stimulating conversations with Jeff Straathorn and Ira Herskowitz and for the comments of Anita Hopper, Amar Singh and Jeff Hall. Jim Hicks gave an excellent critical review of this paper and his suggestions about the calculation of the frequency of mating-type switching have been incorporated. The work was supported by Public Health Service grant GM 20056.

LITERATURE CITED


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