INTERCONVERSION OF YEAST MATING TYPES I. DIRECT OBSERVATIONS OF THE ACTION OF THE HOMOTHALLISM (*HO*) GENE

JAMES B. HICKS[1] AND IRA HERSKOWITZ

Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene, Oregon 97403

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ABSTRACT

The *HO* gene promotes interconversion between **a** and α mating types. As a consequence, homothallic diploid cells are formed by mating between siblings descended from a single α *HO* or **a** *HO* spore. In order to determine the frequency and pattern of the mating-type switch, we have used a simple technique by which the mating phenotype can be assayed without losing the cell to the mating process itself. Specifically, we have performed pedigree analysis on descendants of single homothallic spores, testing these cells for sensitivity to α-factor.

The switch from α to **a** and vice versa is detectable after a minimum of two cell divisions. 50% of the clones tested showed switching by the four-cell stage. Of the four cells descended from a single cell, only the oldest cell and its immediate daughter are observed to change mating type. This pattern suggests that one event in the switching process has occurred in the first cell division cycle. Restriction of the switched mating-type to two particular cells may reflect the action of the homothallism system followed by nonrandom segregation of DNA strands in mitosis.

The mating behavior of cells which have sustained a change in mating type due to the *HO* gene is indistinguishable from that of heterothallic strains.

THE mating type locus of *Saccharomyces cerevisiae* exists in two states, **a** or α, which control the ability of yeast cells to mate (MORTIMER and HAWTHORNE 1969). Cells which are **a** (or **a**/**a** diploids) can mate efficiently with α (or α/α diploids), whereas cells of like mating-type mate with each other only rarely (HAWTHORNE 1963a). Ability of strains to mate is associated with the ability to produce and respond to diffusible, extracellular mating factors (MACKAY and MANNEY 1974a,b). In particular, α cells (but not **a** or **a**/α cells excrete an oligopeptide pheromone "α factor" which causes **a** cells to stop in the cell cycle just before the initiation of DNA synthesis (BÜCKING-THROM *et al.* 1973; HEREFORD and HARTWELL 1974). The structure of the mating-type locus, i.e., the number of genes and controlling sites, is unknown, although mutations inseparable from the mating-type locus which block mating have been described (MACKAY and MANNEY 1974a,b; HAWTHORNE, personal communication). It is likely that the

[1] Present address: Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14853.

mating-type locus specifies control functions for both mating and for sporulation. Indirect support for this view comes from the identification of genes via isolation of mating-deficient mutants (MACKAY and MANNEY 1974a,b). Different classes of mutants carried mutations unlinked to the mating type locus which affect only **a** cells, only α cells, or both **a** and α. It has been proposed that the mating-type locus regulates expression of these mating-specific genes (MACKAY and MANNEY 1974a,b).

When an **a**/α diploid is induced to sporulate, an individual spore gives rise to a culture of haploid cells either of mating type **a** or mating type α, none of which can be induced to sporulate. Some yeast strains ("homothallic" strains) behave differently from the above described "heterothallic" strains in that cultures grown from a single haploid spore contain diploid cells capable of sporulating (MORTIMER and HAWTHORNE 1969). These sporulating cells are formed from siblings descended from a single spore which have mated with each other (HAWTHORNE 1963b; TAKANO and OSHIMA 1967). The genes responsible for homothallism have been in some cases derived from crosses between *Saccharomyces cerevisiae* and other yeasts (HAWTHORNE 1963b; TAKANO and OSHIMA 1967) or derived from heterothallic strains by mutation (HOPPER and HALL 1975). In the case studied in this paper, homothallic and heterothallic strains differ by a single Mendelian gene, *HO*, which is unlinked to the mating-type locus (OSHIMA and TAKANO 1971; this paper). The sequence of events leading to the formation of the diploid is reported to occur as follows (HAWTHORNE 1963b; OSHIMA and TAKANO 1971): A single spore is either of genotype **a** *HO* or α *HO*. After a number of cell divisions, the mating phenotype of some cells changes, and neighboring cells mate to form a diploid of constitution **a**/α *HO/HO*. Such a diploid is able to sporulate and gives rise to four spores each able to repeat the process.

The change in mating phenotype promoted by the *HO* gene appears to be a change at the mating-type locus itself. When the *HO* gene is crossed out of strains which have sustained a change in mating type, the new mating type is maintained (TAKANO and OSHIMA 1970a; this paper). In addition, the new mating type appears to be stable since it segregates normally in the absence of the homothallism genes (TAKANO and OSHIMA 1970b). The homothallism genes therefore cause a *stable inherited change* at the mating-type locus. OSHIMA and TAKANO (1971) have proposed that the homothallism genes are concerned with a "controlling element," analogous to those of maize (MCCLINTOCK 1956), which can reversibly associate with the mating-type locus.

We have undertaken the present work in order to show in as simple a manner as possible the behavior of homothallic strains, with a goal of understanding the mechanism by which mating types are interconverted. We present here a pedigree analysis of descendants of single homothallic spores, in which the individual cells are tested for sensitivity to the mating pheromone, α factor (SCHERER, HAAG and DUNTZE 1974). We have thus been able to determine the mating-type behavior of each cell easily and without losing the cell to the mating process itself. In addition, we demonstrate the stability of the mating-type locus in strains with changed mating type.

MATERIALS AND METHODS

Media. YEPD broth is 1% yeast extract, 2% peptone, and 2% glucose (added after autoclaving). YM-1 broth is 1% succinic acid, .6% NaOH, .5% yeast extract, 1% peptone, .67% Yeast Nitrogen Base (Difco), 2% glucose (added after autoclaving), and adenine and uracil (final concentration 10 μg/ml). YEPD plates contain in addition 2% agar. SD (minimal medium) plates for scoring nutritional requirements are .67% Yeast Nitrogen Base (Difco), 2% glucose (added after autoclaving), 2% agar, and the following additions as necessary: histidine, methionine, tryptophan, adenine, uracil, arginine (20 μg/ml); leucine, lysine, tyrosine (30 μg/ml); phenylalanine (50 μg/ml); threonine (200 μg/ml). SPOR plates (for sporulation) contain 1.5% potassium acetate, .25% yeast extract, and .1% glucose supplemented with additions as needed. Agar slabs for ascus dissection and pedigree analysis contain 4% glucose, 2% peptone, 1% yeast extract, and 3% agar.

Strains. All strains used are described in Table 1. The *HO* strain (X10-1B) used in most experiments was derived by a series of backcrosses between homothallic strain Z140-9A and heterothallic **a** strain X5-39. Diploids were formed by spore to cell matings between spores from a sporulated culture of Z140-9A and a vegetative culture of X5-39. Diploids were sporulated, and *HO* segregants identified by testing colonies for sporulation and mating (by the prototroph complementation assay). The latter test takes advantage of the fact that colonies derived from a homothallic spore contain primarily **a**/α (hence non-mating) diploids. Spores from an *HO* segregant were mated with an X5-39 cell. The resultant diploid was sporulated, and an *HO* spore again mated with X5-39. Strain X10-1B is a homothallic segregant obtained from this last sporulation. Table 2 shows the behavior of the *HO* gene in representative crosses, and demonstrates that homothallic behavior (*HO*) and heterothallic behavior (*ho*) segregate 2:2. The homothallic strain Z140-9A, supplied by G. Fink, carries the *D* ("Diploidizer") gene which confers homothallism (WINGE and ROBERTS 1949; HAWTHORNE 1963b; ESPOSITO *et al.* 1970; ESPOSITO *et al.* 1972). Work by HARASHIMA, NOGI and OSHIMA (1974) indicates that this strain carries genes *HO HMα HM***a**. Since crosses between Z140-9A and standard heterothallic strain X5-39 show 2 homothallic: 2 heterothallic spores, we believe that X5-39 is *ho HMα HM***a** (see also ESPOSITO *et al.* 1972).

TABLE 1

Strain list

Strain	Genotype	Source or reference
Z140-9A	**a**/α *HO/HO lys2-1/lys2-1 met4/met4*	G. FINK (ESPOSITO *et al.* 1970)
X10-1B	**a**/α *HO/HO his5-2/his5-2 ade5/ade5 ura4/ura4 met4/met4 met13/met13*	Z140-9A × X5-39
X5-39	**a** *ho his5-2 ade5 ura4 lys1-1 met13 can*$^{r}_{1-100}$ *ac*$^{r}_{2}$	This laboratory
73	**a** *ilv3-x*	F. SHERMAN
75	**a** *ade6 lys1 ura1*	P. WHITNEY
77	α *lys1 his6 ura1*	P. WHITNEY
91	α *ade2 leu2-1 trp1-1 tyr7-1*	This laboratory
94	**a** *ade2 leu2-1 trp1-1 tyr7-1*	This laboratory
X17-11C	**a** *cry*$^{r}_{1}$ *lys1 lys2 ade6 ura4 his4 thr4*	This laboratory
X48-15	α′ *lys1 lys2 met13 thr4 ho*	X10-1B × X17-11C
X49-14	α *ade6 thr4 lys2 met13 ho*	X10-1B × X17-11C
X47-3D	**a**′ *ade2 leu2-1 tyr7-1 his5-2*	X10-1B × 91
X45-3D	**a** *ade2 ura4 trp1-1 met13*	X10-1B × 91

Matings. Spore-to-cell and cell-to-cell matings were performed by placing cells or spores in direct contact with each other on dissection agar. Zygotes were subsequently isolated by micromanipulation.

Prototroph complementation assay. Mating type was determined by complementation tests as follows: Master plates containing patches grown from 40 spores were grown overnight on YEPD plates at 30°. These plates were then replica-plated onto two SD plates (supplemented as necessary) previously spread with tester cells carrying complementary nutritional requirements (approximately 10^7 cells in 0.4 ml YM-1). Tester cells were heterothallic strains 73(**a**) and 70(α). These mating plates were incubated at 30° for 24 hours. Heterothallic strains yield unequivocal results in this test—a confluent patch of growth when mated with one tester strain, and no prototrophs when mated with the other. Patches grown from a single homothallic spore yielded a variable number of prototrophs when mated with either tester strain. The reason for such behavior is described in the text.

Sporulation and dissection. Master plates (YEPD) containing cells to be sporulated were replica-plated onto SPOR plates and incubated at 30° until asci were microscopically visible (1–3 days). Asci were suspended in a small volume of Glusulase (Endo Laboratories) previously diluted 10x in distilled water, and incubated at room temperature for 5–10 minutes. Dissection was performed on an agar slab with a de Fonbrune micromanipulator. Efficiency of germination by spores from strain X10–1B was greater than 95%.

Pedigree analysis. Three small agar slabs were placed on a 35 mm × 50 mm glass cover slip. *A* contained glusulase-treated asci to be tested. *B* contained a streak of approximately 10^7 α cells spread along one edge to supply alpha factor. *C* (without alpha factor) was used for growth of **a** cells, which are inhibited on slab *B*. The cover slip was inverted over a dissection chamber containing a small dish of water to prevent drying of the agar. Asci were moved onto the agar slab *B*, and the individual spores separated by micromanipulation. The chamber was then sealed and incubated at 30° with periodic openings for observation and separation of cells for pedigree analysis as described in Results. Observations were carried out for 12 to 36 hours.

Rare mating and efficiency of mating tests. Strains to be tested were grown overnight at 30° on YEPD agar, suspended in sterile water at approximately 10^8 cells per ml, and checked microscopically for cell aggregation. In all strains used in this work, less than 5% of the cells in suspension were observed to be in aggregates larger than 2 cells. Suspensions of the tester strains were concentrated to 10^9 cells/ml by centrifugation.

In the rare mating test, 0.2 ml of each suspension to be tested was mixed with 0.5 ml (5×10^8 cells) of a tester strain of the *same* mating type and spread on a dry YEPD plate. A control plate containing tester cells alone was also prepared. The plates were incubated at 30° for 24 hours, then replica-plated onto minimal medium (SD) plates, and incubated for 2–5 days at 30°

Efficiency-of-mating tests were done in similar fashion, except that the suspension of the strains to be tested were first diluted 10^{-5} and then mixed with approximately 10^7 tester cells of the *opposite* mating type. Each mixture was spread on a YEPD plate, incubated for 24 hours at 30°, and replicated onto SD agar. Total colony-forming units were assayed on YEPD plates. The efficiency of mating was calculated as the ratio of the number of prototrophic colonies appearing on SD agar after 2–5 days at 30° to the total number of test cells initially added. Normal laboratory **a** and α haploid strains show efficiencies of 50–90% by this test (see Table 3).

RESULTS

1. *Mating of Siblings Descended from a Single Homothallic Spore*

Spores derived from sporulation of *HO/HO* or *HO/ho* diploids were allowed to germinate on a thin agar slab in a humidified dissection chamber and observed microscopically. From *HO/HO* strains, all spores gave rise to mating pairs (zygotes) after two or more cell divisions. In 21 of 48 cases, two mating pairs were observed after the first two cell divisions (Figure 1). In the other 27 cases,

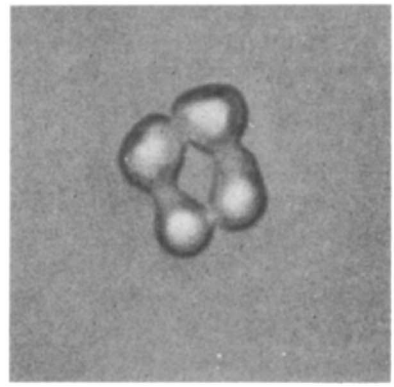

FIGURE 1.—Zygote formation by homothallic strains. Asci from **a**/α *HO/HO* strain X10–1B were dissected, and individual spores allowed to germinate. The figure shows the four-cell stage of a microcolony in which two zygotes have formed simultaneously.

zygotes were observed after more than two cell divisions, at which time the microcolony contained between 8 and 22 cells. From *HO/ho* strains, two spores per tetrad gave rise to zygotes in the 20 tetrads examined. In each case, colonies derived from microcolonies lacking zygotes showed a normal haploid **a** or α

TABLE 2

Zygote formation and segregation of the HO *gene*

Ascus type	Spore	Mating with α	Mating with **a**	Zygote formation and sporulation	Genotype	Cross of spore from X10–1B (*HO/HO* **a**/α) with: X17–11C (**a** *ho*)	91 (α *ho*)
I	A	+++	—	—	**a** *ho*	PD: 2	NPD: 1
	B	+++	—	—	**a** *ho*		
	C	+/—[a]	+/—	+	α *HO*		
	D	+/—	+/—	+	α *HO*		
II	A	+/—	+/—	+	**a** *HO*	NPD: 5	PD: 1
	B	+/—	+/—	+	**a** *HO*		
	C	—	+++	—	α *ho*		
	D	—	+++	—	α *ho*		
III	A	+/—	+/—	+	**a** *HO* α *HO*	T: 13	T: 9
	B	+++	—	—	**a** *HO* **a** *ho* or		
	C	—	+++	—	α *ho* α *ho*		
	D	+/—	+/—	+	α *HO* **a** *HO*		

Results of two crosses (X10–1B × X17–11C and X10–1B × 91) are presented. Zygote formation was scored only for the spores derived from the X10–1B/X17–11C diploid. Numbers indicate the number of PD (parental ditype), NPD (non-parental ditype), or T (tetratype) asci observed in in the crosses. [a] The number of prototrophs observed in mating tests with homothallic strains was usually between — (0 or a few) and +++ (greater than 100). Other details are described in MATERIALS AND METHODS.

phenotype by the prototroph complementation assay (see MATERIALS AND METHODS). Furthermore, only colonies with zygotes contained cells able to sporulate. Segregation data from two *HO/ho* diploids are presented in Table 2. The *HO* gene, as monitored by zygote formation and sporulation, can thus be seen to segregate in Mendelian fashion.

2. *Response of Homothallic Cells to α Factor*

We have just seen that siblings descended from a single homothallic spore can mate with each other. Since mating specificity in heterothallic strains is determined by the mating-type locus, mating between homothallic cells may indicate a change from one mating type to the other. Another possibility is that homothallic strains have an unusual (e.g., universal) mating-type behavior. Consistent with the former hypothesis, it has been shown previously that in any tetrad derived from a homothallic diploid, two of the spores mate with heterothallic **a** cells and two with heterothallic α cells (OSHIMA and TAKANO 1971; HAWTHORNE, personal communication). In order to follow changes in mating-type behavior, we wished to determine the mating-type phenotype without losing the homothallic cell to the mating process. To do this, we have tested whether homothallic cells respond to the mating pheromone, α factor, produced by heterothallic α strains (DUNTZE, MACKAY and MANNEY 1970).

α factor is an oligopeptide (SCHERER, HAAG and DUNTZE 1974) produced by α cells but not by **a** cells or **a**/α diploids (DUNTZE, MACKAY and MANNEY 1970). This factor inhibits growth of **a** cells (but not α or **a**/α cells), causing them to arrest before the initiation of DNA synthesis (BÜCKING-THROM *et al.* 1973; HEREFORD and HARTWELL 1974), and inducing a characteristic change in cell shape (DUNTZE, MACKAY and MANNEY 1970; Figure 2). We have used sensisivity of cells to α factor to indicate mating-type phenotype of **a** cells ("Mt**a**"), and insensitivity to α factor to indicate mating-type phenotype of α cells ("Mtα").

Tetrads from a sporulated **a**/α culture were exposed to α factor as described in MATERIALS AND METHODS. In every case, two of the four spores responded to α factor (Figure 2), regardless of whether the asci were derived from *HO/HO* (94 tested), *Ho/ho* (10 tested), or *ho/ho* (19 tested) sporulated diploids. It was further seen that sensitivity to α factor is perfectly correlated with mating behavior. That is, spores carrying the *ho* allele which were sensitive to α factor were allowed to grow into colonies by removing them from α factor. In the 29 cases examined the colonies displayed an Mt**a** phenotype, as determined by the prototroph complementation assay.

3. *Pedigree Analysis: Watching Mating-Type Change*

Since homothallic strains respond to α factor, we were able to follow the mating-type phenotype of progeny from homothallic spores by monitoring the sensitivity of progeny cells to α factor. In this manner we have directly observed the change in mating behavior. The inferred genotype of cells with a phenotypic change from Mtα to Mt**a** will be denoted as **a′** and the change from Mt**a** to Mtα as α′, as suggested by TAKANO and OSHIMA (1967).

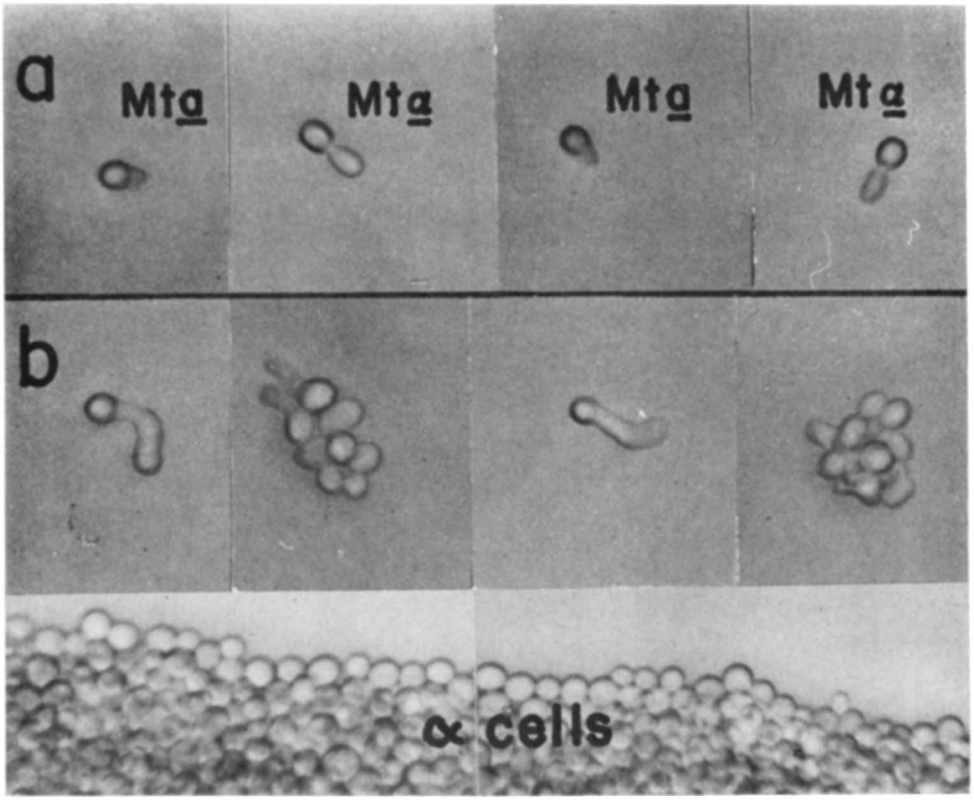

FIGURE 2.—Response of homothallic spores to α factor. Asci from **a**/α *HO/HO* strain X10–1B were dissected and individual spores allowed to germinate on an agar slab in the presence of a dense streak of α cells. The figure is a composite picture of a single tetrad taken at 12 hours (a) and 24 hours (b) after dissection. Mtα response is indicated by the ability to bud. Mta response is indicated by inability to bud and by formation of aberrantly shaped cells.

Tetrads from sporulated cultures of an *HO/HO* strain were displayed on an agar slab and subjected to α factor. The arrangement and response of the spores are shown in Figure 2. As before, two of the spores were sensitive to α factor (Mt**a** phenotype) and two were not (Mtα phenotype).

a. *Mtα to Mt**a** (α to **a**′)*

The Mtα spores were allowed to germinate in the presence of α factor. After cell division was completed, each cell and its bud were separated on the agar by micromanipulation. The progeny were organized into a grid formation, and the behavior of each was noted (Figure 3). In each clone, successive rounds of cell division by different cells were approximately synchronous for the first four generations. Progeny cells are designated as noted in Figures 3 and 4 by their relationship to the original spore (S). Daughters (D1, D2, etc.) are cells budded directly from the spore. Granddaughters (D1–1, D1–2, etc.) are cells budded directly from a daughter.

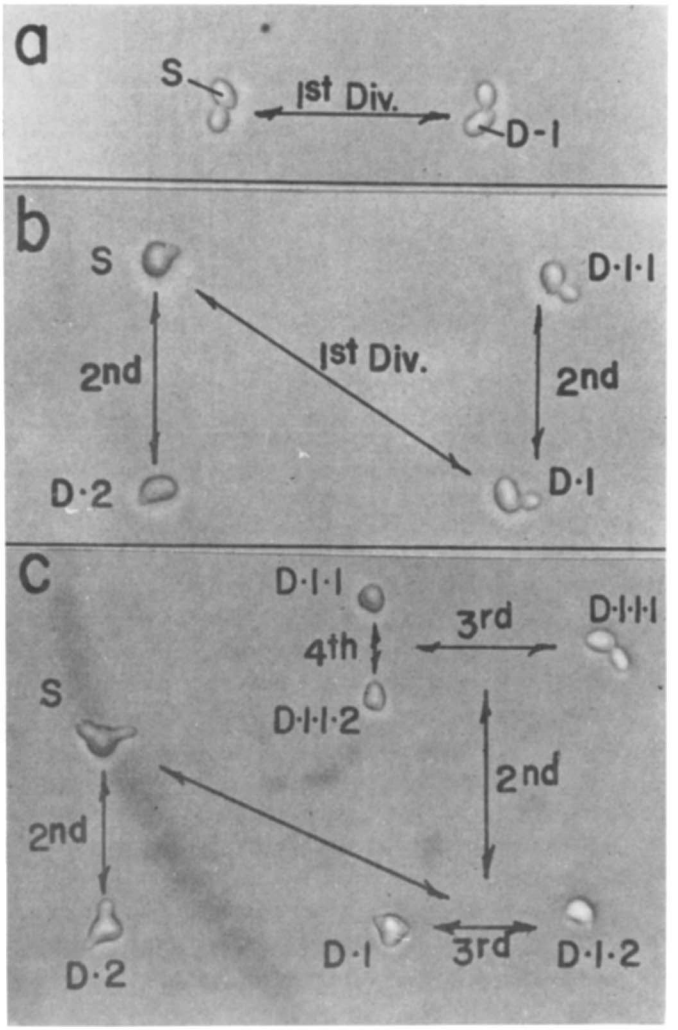

FIGURE 3.—Pedigree analysis of descendants from a homothallic spore. Time lapse photographs of the progeny of a single Mtα spore from X10–1B dividing on an agar slab in the presence of α factor (MATERIALS AND METHODS); (a) after first division; (b) after second division; (c) after fourth division. Cells were separated by micromanipulation after each cell division as indicated by arrows. *S*, spore; *D1* and *D2*, first and second daughters of spore; *D1–1* and *D1–2*, first and second daughters of *D1*; *D1–1–1* and *D1–1–2*, first and second daughters of *D1–1*.

First division. In every case it was observed that after the first round of cell division, both cells (S and D1) immediately initiated another round of budding (Figures 3 and 4). In other words, Mtα phenotype was maintained.

Second division. After completion of the second round of cell division, two different responses were observed. In 37 of 58 cases, S and D2 (the spore's most recent daughter) stopped budding and displayed the morphological change characteristic of response to α factor (see Figures 3B, 4A, 4B). These cells had

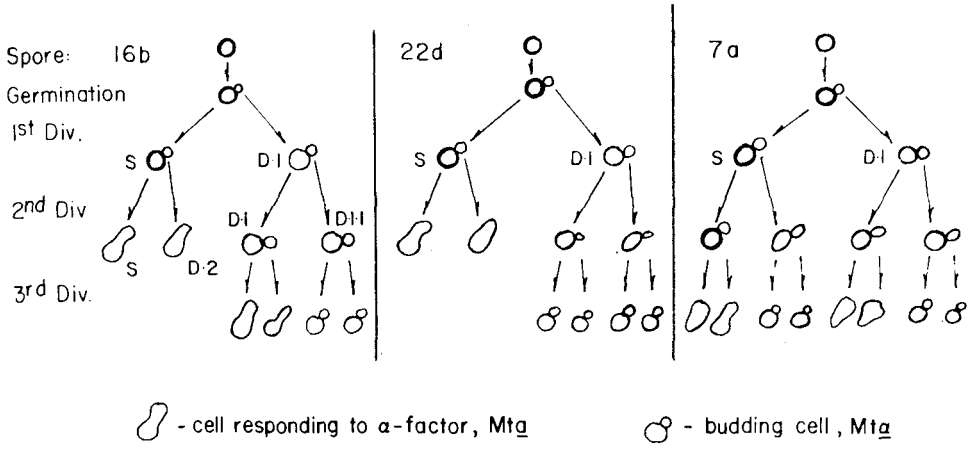

FIGURE 4.—Pedigree analysis of three homothallic spores. Experimental procedure is the same as described in Figure 3 and in MATERIALS AND METHODS.

changed phenotype from Mtα to Mt**a**. The remaining two cells, D1 and D1–1, maintained the Mtα phenotype and continued budding. We have observed no cases in which all four cells at the second division switched phenotype. Cells S and D2 maintained their aberrant shape and were prevented from undergoing cell division for at least eight hours unless removed from α factor (See Figure 3C).

In the other observed response (21/58 cases), all four cells were able to bud (Figure 4C). That is, no change in phenotype had occurred by this time.

Third division. After the third division the unchanged cells in either half of the pedigree were able to exhibit either of the above described responses, giving rise to 2Mtα : 2Mt**a** or 4 Mtα : 0 Mt**a**. As before, the change in phenotype was exhibited by a specific pair of the four cells involved, the oldest cell and its immediate daughter.

Later divisions. Pedigree analysis was carried out for a maximum of 6 generations, and cells were observed to switch throughout this period. In a few cases (4 of approximately 300 cell divisions), Mtα cells were observed to switch to the Mt**a** phenotype singly instead of in pairs.

Summary. In general, four cells descended from any given Mtα cell were either 2 Mtα : 2 Mt**a** or 4 Mtα : 0 Mt**a**. The 2 Mtα : 2 Mt**a** behavior was observed in more than half of such groups of four cells. The change in phenotype was exhibited by a specific pair of these cells, the oldest cells (often the spore cell) and its immediate daughter.

b. *Mt**a** to Mtα (**a** to α')*

Observing the change from **a** to α' is more cumbersome than observing the change from α to **a**$'$ since **a** cells do not grow in the presence of α factor. Each of the spores of a tetrad was grown for nearly two generations, that is, until each had produced two cells with large buds. The cells were then moved into the presence of α factor to observe response. Analysis of one tetrad is shown in

Figure 5. Two spores gave the response shown in Figure 5A, and two the response in Figure 5B. The A response is like that observed in the previous section: the spore cell was apparently Mtα and gave rise to a quartet of cells, of which one pair (cells S and D2) was Mt**a** and the other Mtα. The spores giving response B were apparently Mt**a** and gave rise to two cells of Mtα and two of Mt**a**. Just as in the switch from Mtα to Mt**a**, the switch from Mt**a** to Mtα affects cells S and D2. At present we have insufficient data to determine in how many cases the Mt**a** to Mtα switch has occurred by the four-cell stage or whether it always affects these particular cells. The experiment shown in Figure 5 also demonstrates that the switch from one mating type to the other occurs in the absence of α factor.

c. *Subsequent Changes in Mating Type (α' to **a'**, **a'** to α')*

a' or α' cells, identified as described above, grow into colonies which contain cells capable of sporulating. It is thus likely that the mating type of these cells is subject to change. In the few cases examined, we have observed that the switch from α' to **a'** is similar to the switch from α to **a'**. Specifically, of the progeny derived from an α' cell after two or three cell division cycles, two cells display the response to α factor. The *HO* gene can, therefore, promote successive interconversions between **a** and α mating types.

4. *Stability of Strains with Changed Mating Type*

To determine whether the *HO* gene is necessary for the *maintenance* of the changed response to α factor, it was necessary to cross out the *HO* gene from strains which had sustained a change in α factor sensitivity. **a'** *HO* strains and α' *HO* strains were mated with α *ho* or **a** *ho* strains respectively to form diploids **a'**/α *HO/ho* and α'/**a** *HO/ho*. Heterothallic segregants sensitive to α factor (**a'** cells) were chosen from the first diploid, and heterothallic segregants insensitive to α factor (α' cells) were chosen from the second diploid. The stability of mating type of these strains was assayed by measuring the frequency of cells of opposite mating type in the culture, and by measuring the frequency of cells in the culture which were able to sporulate.

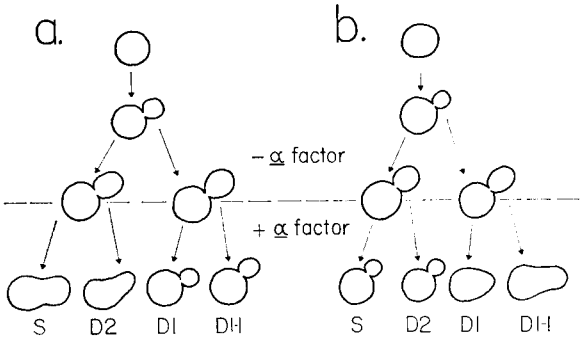

FIGURE 5.—Pedigree analysis of homothallic spores germinated in the absence of α factor. At the four-cell stage, cells were tested for sensitivity to α factor. From a single tetrad, 2 spores gave response A, 2 gave response B. Other details are described in MATERIALS AND METHODS.

The frequency of cells of opposite mating type in various cultures was assayed by a "rare mating" experiment (see MATERIALS AND METHODS). In this technique, a culture of an auxotrophic **a** strain is mixed with a culture of an **a** strain with complementary nutritional requirements. Matings between individuals in the culture yield prototrophic diploids at low frequency (see Table 3), which are able to sporulate and produce two **a** and two α spores (HAWTHORNE 1963a; HICKS and HERSKOWITZ, unpublished observations). One of the parental **a** cultures therefore contained or gave rise to cells of mating type α. The relationship between the low frequency switching event and that promoted by the *HO* gene is under investigation. The frequency of α cells produced in cultures of **a′** cells was determined by the frequency of rare mating with conventional **a** strains. Since approximately the same frequency of **a**/α prototrophs was observed for both **a′** × **a** and **a** × **a** matings (Table 3), the frequency of α cells in **a′** cultures is no greater than in **a** cultures.

Analysis of the stability of α' cells by the "rare mating" technique is the same as described for **a′** cells except that $\alpha \times \alpha$ matings yield two kinds of prototrophic diploids: (1) **a**/α strains, which do not mate with either **a** or α strains, but which are able to sporulate to yield 2**a**:2α spores (HAWTHORNE 1963a; HICKS and HERSKOWITZ, unpublished observations), and (2) α/α strains which can mate with **a** strains, and which do not sporulate (RABIN 1970; HICKS and HERSKOWITZ, unpublished observations). We have not distinguished between these two classes in this experiment. The same frequency of prototrophs was obtained in $\alpha' \times \alpha$ and $\alpha \times \alpha$ matings (Table 3), indicating that the frequency of **a** cells in α' cultures is not greater than in α cultures.

The frequency of cells of changed mating type in a population of **a′** or α' cells can be measured indirectly by determining the number of cells capable of giving rise to spores. We reason that cells which change mating type will often mate with their neighbors to form an **a**/α cell which is capable of sporulating. **a′**, α', **a**, α, and **a**/α cells were grown under conditions which induce sporulation (see MATERIALS AND METHODS). Microscopic examination of these cultures for asci

TABLE 3

Stability of **a′** *and* α' *mating types*

	Rare mating			Efficiency of mating		
Parent 1	Parent 2 (Tester strain)	Prototrophs*	Prototrophs / Parent 1	Parent 2 (Tester strain)	Prototrophs	Efficiency of mating
α	α (77)	180	3.4×10^{-5}	**a** (73)	220	0.88†
α'	α (77)	154	1.6×10^{-5}	**a** (73)	390	0.83†
none	α (77)	0	—	**a** (73)	14	—
a	**a** (73)	120	1.3×10^{-5}	α (77)	610	0.54
a′	**a** (73)	176	1.1×10^{-5}	α (77)	171	0.59
none	**a** (73)	0	—	α (77)	0	—

Parent 1 strains were α (X49-14), α' (X48-15), **a** (X45-3D), and **a′** (X47-3D).
* Numbers represent the average of two separate platings from the same Parent 1 culture.
† Calculated after subtracting (14) prototrophs due to the tester strain. Other details are described in MATERIALS AND METHODS.

showed efficient (greater than 50%) ascus formation by the **a**/α cells but less than .1% asci in the other cultures. By these criteria, **a**′ and α' are as stable as conventional **a** and α mating types.

We have also compared the ability of α' and α strains to mate with **a** strains, as well as **a**′ and **a** strains to mate with α by an efficiency of mating experiment (Table 3). In this procedure (see MATERIALS AND METHODS), α and α' strains and **a** and **a**′ mate comparably well with cells of opposite mating type.

DISCUSSION

The mating-type locus of *Saccharomyces cerevisiae* appears to be a complex locus controlling both mating and sporulation. It is clear that both **a** and α loci specify distinct products since **a**/α diploids, in contrast to **a**/**a** and α/α diploids, sporulate and do not mate. Although the structure of the mating-type locus is not known, we view **a** and α as nonhomologous blocs which specify a number of controlling factors. Preliminary support for this hypothesis comes from our observations that mutations at the mating-type locus may have vastly different consequences (HICKS, STRATHERN and HERSKOWITZ, in preparation).

The genes regulating homothallism (summarized in HARASHIMA, NOGI and OSHIMA 1974) have the remarkable property that they cause a change in mating type which is stable to growth. The *HO* gene appears to promote a change at the mating-type locus itself. We have shown that homothallic cells respond to α factor in a manner similar to heterothallic cells. This fact has allowed the direct observation of change in mating phenotype as cells grow. In particular, the *HO* gene promotes a switch from α to **a**′ often by the second cell division, and likewise a switch from **a** to α'. The mating type of **a**′ and α' cells can also be changed by subsequent action of the *HO* gene. The observed change in mating phenotype, response to α factor and mating behavior, is not merely a change in cell phenotype maintained by the *HO* gene. Rather, the new mating-type behavior is as stable to growth as that of conventional heterothallic **a** and α strains, and does not require the presence of the *HO* gene.

Since the *HO* gene can switch mating types of cells which have probably never before seen the *HO gene* (e.g., recombinants formed between heterothallic and homothallic strains), it seems likely that all cells contain information to be both **a** and α, but only one of these states is expressed. Further support for the view that cells contain both kinds of information comes from the observation that **a** and α can be interconverted in the absence of *HO* at very low frequency (HAWTHORNE 1963a; RABIN 1970; HICKS and HERSKOWITZ unpublished observations). This observation suggests that the *HO* gene controls functions which facilitate the unknown interconversion event. Two of the models proposed to account for the stable switch in mating types involve DNA modification (D. HAWTHORNE, personal communication; H. LODISH, personal communication; see HOLLIDAY and PUGH 1975) or DNA rearrangement at the mating-type locus. OSHIMA and TAKANO (1971) have suggested that the homothallism genes control insertion and removal of a "controlling element" into the mating-type locus, analogous in some respects to controlling elements in maize (MCCLINTOCK 1956) or prophage insertion in lambdoid phages (SIGNER 1968). The interconversion of mating

types appears to be more complex than simply a change between **a** and α. In a subsequent communication, we show that a defective α mating type locus can be converted into a functional **a** locus, and then into a functional α locus in both homothallic and heterothallic strains (HICKS and HERSKOWITZ, in preparation).

Pedigree analysis of cells descended from single homothallic spores shows that expression of the new mating type specificity (sensitivity to α factor) appears in both the mother and daughter bud. If the event mediated by the *HO* gene is a genotypic change, we interpret this observation to mean that the event leading to a mating type change has occurred in the prior cell cycle, e.g., in the S cell at the two-cell stage (Figures 3A, 4A, 4B). The phenotypic delay may occur if the switching event occurs after the point in the cell cycle where cells are sensitive to α factor (BÜCKING-THROM *et al.* 1973; HARTWELL 1972). Another possible explanation is that the switching event occurs before the cell cycle period of sensitivity to α factor and that response to α factor requires degradation of preexisting cellular components or synthesis of new cellular components.

A striking result of the pedigree analysis is that the switching event does not occur at random. For the case studied most extensively (α to **a'**), only two of the four cells descended from a given cell exhibit a changed mating type. Furthermore, in 37/37 α to **a'** events (and 3/3 **a** to α' events), the changed cells are always the oldest cell and its immediate daughter (cells S and D2 in Figure 3A, and D1 and D1–2 in Figure 3C). The restriction of the changed mating type to one pair of cells in a group of four ("exclusion") suggests that one event in the switching process has occurred at the one-cell stage (in cell S, Figures 4A and 4B; and in cell D1, Figure 4C). We suggest that cellular components in this cell are not distributed randomly to the daughter bud. Precedent for such nonrandom distribution comes from autoradiographic analysis of germinating *Aspergillus nidulans* spores, which indicates that DNA strands of identical age segregate together (ROSENBERGER and KESSEL 1968). In *S. cerevisiae* the older DNA strands may remain with the mother cell and be the target of the switching mechanism.

Further understanding of the mechanism of mating-type interconversion will require a better understanding of the physiology of α factor response and of the structure of the mating-type locus.

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