INTERCONVERSION OF YEAST MATING TYPES II. RESTORATION OF MATING ABILITY TO STERILE MUTANTS IN HOMOTHALLIC AND HETEROTHALLIC STRAINS

JAMES B. HICKS*

AND

IRA HERSKOWITZ

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

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ABSTRACT

The two mating types of the yeast *Saccharomyces cerevisiae* can be interconverted in both homothallic and heterothallic strains. Previous work indicates that all yeast cells contain the information to be both **a** and α and that the *HO* gene (in homothallic strains) promotes a change in mating type by causing a change at the mating type locus itself. In both heterothallic and homothallic strains, a defective α mating type locus can be converted to a functional **a** locus and subsequently to a functional α locus. In contrast, action of the *HO* gene does not restore mating ability to a strain defective in another gene for mating which is not at the mating type locus. These observations indicate that a yeast cell contains an additional copy (or copies) of α information, and lead to the "cassette" model for mating type interconversion. In this model, *HM***a** and *hm*α loci are blocs of unexpressed α regulatory information, and *HM*α and *hm***a** loci are blocs of unexpressed **a** regulatory information. These blocs are silent because they lack an essential site for expression, and become active upon insertion of this information (or a copy of the information) into the mating type locus by action of the *HO* gene.

THE specificity of mating by the yeast *Saccharomyces cerevisiae* is determined by the mating type locus, which exists in two allelic forms, **a** or α (MORTIMER and HAWTHORNE 1969). Although the structure of the mating type locus itself is not known, recent evidence indicates that the α locus specifies at least two cistrons (STRATHERN, HICKS and HERSKOWITZ, in preparation). It has been proposed (MACKAY and MANNEY 1974) that the mating type locus controls expression of unlinked genes essential for mating. Genes essential for the mating process have been identified by the isolation of eleven different classes of nonmating (*ste*$^-$) mutants (MACKAY and MANNEY 1974). Unlinked to the mating type locus are classes which confer the mating-deficient phenotype to both **a** and α cells, and classes which confer the mating-deficient phenotype specifically to either **a** or α cells (MACKAY and MANNEY 1974). Two classes of *ste* mutants, both derived from α strains, carry mutations inseparable from the mating type

* Present address: Department of Genetics, Development & Physiology, School of Agriculture, Cornell University, Ithaca, New York 14850.

locus. In these cases, α *ste*$^-$/**a** *ste*$^+$ diploids yield no α *ste*$^+$ recombinants (MACKAY and MANNEY 1974; see below). Since these mutations are recessive to α^+, they may affect a positive regulator of α specific functions (STRATHERN, HICKS and HERSKOWITZ, in preparation).

The stability of mating type specificity is influenced by the *HO* gene (MORTIMER and HAWTHORNE 1969). In *ho* (heterothallic) strains, mating type specificity is stable, although rare switches from **a** to α and from α to **a** do occur (HAWTHORNE 1963a; RABIN 1970; HICKS and HERSKOWITZ 1976a; this paper). In *HO* (homothallic) strains, cells of one mating type switch frequently to the other mating type (HAWTHORNE 1963b; OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1976a), with the switch occurring as often as every cell division (STRATHERN, in preparation). Previous work has shown that the *HO* gene is necessary for the high frequency interconversion of mating types but is not necessary for the *maintenance* of the changed mating type (OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1976a). That is, an **a**′ cell—derived from an α cell by action of the *HO* gene—behaves as a stable **a** cell when the *HO* gene is removed by genetic crosses. The mating type of strains which have sustained the rare mating type change observed in the absence of the *HO* gene is likewise stable (HAWTHORNE 1963a; RABIN 1970; this paper). These observations indicate (i) that all cells contain the information to be both **a** and α, and (ii) that the *HO* gene promotes a change in mating type by causing a change at the mating type locus itself.

In order to learn more about the relationship between the **a** and α mating type loci and the mechanism by which **a** and α alleles are interconverted, we have asked whether α strains with defects at the mating type locus can be switched to become functional **a** strains. In addition, we have asked whether these **a** strains can then be converted to functional α strains. We show here that these switches occur in both homothallic and heterothallic strains. DR. DONALD HAWTHORNE (personal communication) has made similar observations on the ability of homothallic strains to restore mating ability to α strains with a defective mating type locus.

MATERIALS AND METHODS

Media. YEPD agar, SPOR agar, YM-1 broth and SD (minimal medium) plates for scoring nutritional markers have been described in HICKS and HERSKOWITZ (1976a). Agar slabs for dissection, α-factor assays and pedigree analyses are YM-1 plus 3% agar. Cryptopleurine resistance (Cry^r) was scored on YEPD plates supplemented with 1 mg/L cryptopleurine (SKOGERSON, MCLAUGHLIN and WAKATAMA 1973) which was obtained from CHEMASEA Pty., Ltd., Sydney, N.S.W., Australia.

Strains. Strains are described in Table 1.

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. Other matings were performed by prototroph selection as follows. Parent strains containing complementary nutritional markers were suspended in sterile water, and $10^5 - 10^7$ cells of each were spread together on a YEPD plate. After incubation overnight at 30°, the YEPD plate was replica plated onto SD agar. Colonies appearing on the SD plate after two to three days at 30° were purified to single colonies on SD and sporulated.

TABLE 1A

Strain list

Strain	Genotype	Source or reference
X10–1B	**a**/α *HO/HO his5–2/his5–2 ade5/ade5 ura4/ura4 met4/met4 met13/met13*	HICKS and HERSKOWITZ (1976a)
70	α *thr3–10*	F. SHERMAN
73	**a** *ilv3–10*	F. SHERMAN
XT1172–S245c	α *ade6 his6 gal2 leu1 met1 trp5 can1*	MACKAY and MANNEY (1974)
VC2	α*ste1–2 ade6 his6 gal2 leu1 met1 trp5 can1*	MACKAY and MANNEY (1974)
VN33	α*ste1–5 ade6 his6 gal2 leu1 met1 trp5 can1*	MACKAY and MANNEY (1974)
VP1	α*ste1–4 ade6 his6 gal2 leu1 met1 trp5 can1*	MACKAY and MANNEY (1974)
VI14	α *steX ade6 his6 gal2 leu1 met1 trp5 can1*	MACKAY (1972)
X50–2D	α *cry1–3 ade6 his4 leu2–1 lys2*	This laboratory
X57–1B	**a** *cry1–3 ade6 his4 leu2–1 lys2*	This laboratory
XJ24–40B	α *cry1–3 trp1–1 tyr7–1*	J. STRATHERN
205	α *cry1–3 his1–1*	C. MCLAUGHLIN

Assays for mating type and sporulation. Mating type was determined by the prototroph complementation assay (HICKS and HERSKOWITZ 1976a).

Sporulation was assayed on SPOR agar. After incubation for three to seven days at 30°, cells from each isolate were suspended in a small drop of water on a glass slide and observed microscopically. Isolates which formed asci containing mature spores were scored as Spo+.

Assays for homothallism. Various aspects of the homothallic phenotype were used to score the presence of the homothallic allele *HO* (see HICKS and HERSKOWITZ 1976a). These were (1) the ability to segregate α-factor sensitive cells in the progeny of a haploid cell insensitive to α factor, (2) zygote formation among progeny of a single haploid spore, and (3) sporulation of cells in a colony derived from a single haploid spore. A positive response in any of these assays was considered sufficient to score an isolate as *HO*.

α-factor assays. α-factor production and response were assayed by confrontation tests (DUNTZE, MACKAY and MANNEY 1970). To assay α-factor production, a dense streak of cells of the strain to be tested was applied to the edge of a slab of dissection agar placed on a glass cover slip. The

TABLE 1B

Diploids	Haploid parents
X18	VC2 × X10–1B–1 (haploid segregant derived by sporulation of X10–1B)
X18	VN33 × X10–1B–2 (haploid segregant derived by sporulation of X10–1B)
X38	VI14 × X10–1B–3 (haploid segregant derived by sporulation of X10–1B)
X91	X50–2D × X18–0 (**a**′ *HO*)
X93	X50–2D × X78–15 (**a**′ *HO*)
X95	X57–1B × X18–0 (α′ *HO*)
X96	X57–1B × X78–15 (α′ *HO*)
X22	VC2 × 205
X65	X50–2D × XT1172–S245c
X66	X50–2D × VN33
X71	X50–2D × VC2
X72	X50–2D × VP1
X97	X57–1B × X22–23–2C (**a**′ *ho* segregant of X22)
X98	X57–1B × X66–37–9A (**a**′ *ho* segregant of X66)
X106	X57–1B × X65–67–11B (**a**′ *ho* segregant of X65)

cover slip was then inverted over a dissection chamber and 20–30 individual mating type **a** cells (strain 73) were placed approximately .25 mm from the streak by micromanipulation. The chamber was sealed and incubated at 30°. Under these conditions, production of α-factor by the cells in the streak caused the **a** assay cells to cease division and change their shape (DUNTZE, MACKAY and MANNEY 1970) within 5 hours. In order to test strains for response to α-factor, an α strain (70) was used in the dense streak, and individual cells of the strain to be tested were arranged nearby and observed as described above.

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 concentrated to 10^9 cells per ml by centrifugation. Less than 5% of the cells in suspension were in aggregates larger than two cells. In the *rare mating test*, 0.2 ml of each suspension to be tested (Parent 1, Tables 4 and 8) was mixed with 0.5 ml (5×10^8 cells) of a tester strain (Parent 2) of the *same* mating type and spread on a YEPD plate. The plates were incubated at 30° for 24 hours, then replica-plated onto minimal medium (SD) and incubated for two to five days at 30°. The rare mating parameter, Prototrophs/Parent 1, was the ratio of colonies on SD agar to the total number of Parent 1 cells initially added to the mixture. *Efficiency of mating tests* were done in similar fashion, except that the Parent 1 suspension was diluted 10^{-5} and mixed with approximately 10^7 tester cells of the *opposite* mating type (Parent 2'). The mixture was spread on a YEPD plate, incubated for 24 hours at 30°, and then replica-plated onto SD agar. Total colony-forming units (of Parent 1) were assayed on YEPD plates. The efficiency of mating was the ratio of the number of prototrophic colonies appearing on SD agar after two to five days at 30° to the total number of Parent 1 cells initially added to the mixture. Efficiency of mating by normal laboratory **a** and α strains was between .29 and .88 (Table 4; HICKS and HERSKOWITZ 1976a).

Barrier effect. The barrier test is described in HICKS and HERSKOWITZ (1976b). The Bar+ phenotype is an inhibition of response to α factor and is exhibited by **a** cells.

Other techniques. Procedures for tetrad analysis and pedigree analysis of homothallic cells are described in HICKS and HERSKOWITZ (1976a).

RESULTS

1. *Construction of* αste⁻ HO: *formation of diploids between a homothallic strain and strains defective at the mating type locus*

The homothallic diploid X10–1B (**a**/α *HO/HO*) has been described previously (HICKS and HERSKOWITZ 1976a). This strain was induced to sporulate, and the resultant spores were mated with strains VC2 (*αste1–2*) and VN33 (*αste1–5*) by prototroph selection as described in MATERIALS AND METHODS. Prototrophs were selected from each cross, purified to single colonies on SD plates and sporulated. Greater than 90% of the cells sporulated in all cultures after three days at 30° and yielded greater than 90% viable spores. Representative diploids from the two crosses were chosen for further study and should have the following genotype:

$$\frac{\mathbf{a}}{\alpha ste}\ \frac{HO}{ho}\ \frac{b}{+}\ \frac{c}{+}\ \frac{+}{d}\ \frac{+}{e} \qquad (b, c, d, e \text{ are auxotrophic mutations})$$

The selective mating method used to form the diploid does not preclude the introduction of mutations affecting mating type, such as reversions to *STE*+ or suppressors of the *ste* mutation (HICKS 1975). Thus, analysis of the segregation data from such a cross must provide evidence that the diploid is indeed of the genotype expected. Specifically, tetrad analysis must account for the **a** allele, the sterile allele, and *HO* as well as the auxotrophic markers present in the parent strains.

The segregation data for X18 (X10–1B × VC2) and X78 (X10–1B × VN33) are presented in Table 2. The inferred genotypes for each type of tetrad with regard to mating type and homothallism are shown along with their observed and expected frequencies based on nonlinkage of *HO* to the mating type locus or to its centromere (HARASHIMA, NOGI and OSHIMA 1974). These data show that the homothallic phenotype segregates 2:2 despite the presence of the sterile mutation. In addition, auxotrophy for uracil, tryptophan, and leucine segregate 2:2, as expected. Most importantly, no heterothallic segregants of mating type α were observed. Consequently, these diploids do not contain *STE*$^+$ back mutations or sterile-suppressor mutations.

A striking result from the tetrad analysis is that even though the α*ste*$^-$ allele is segregating in the crosses, two of the four spores always give rise to colonies containing sporulated cells. This result indicates that the α*ste*$^-$ mutation does not affect the ability of the *HO* gene to produce diploids capable of sporulating. For example, consider nonparental ditype tetrads, which contain two heterothallic (*ho*) **a** spores. The other two spores, of genotype α*ste*$^-$ *HO*, grow into colonies containing cells capable of sporulating. If these sporulating diploids are formed in the manner of ordinary homothallic diploids, *i.e.*, by mating of siblings, we expect that the α*ste*$^-$ allele is being converted to functional **a** and α alleles by *HO*.

TABLE 2

Segregation of mating type and homothallism from **a** HO × αste$^-$ ho *crosses*

Tetrad type		Mate with α	Mate with **a**	Sporulation	Inferred genotype of spore	Tetrads observed X18	Tetrads observed X78	Tetrads expected
PD	A	+/—*	+/—	+	**a** *HO*			
	B	+/—	+/—	+	**a** *HO*	.18	.22	.17
	C	—	—	—	*ste ho*	(10/57)	(11/50)	
	D	—	—	—	*ste ho*			
NPD	A	+/—	+/—	+	*ste HO*			
	B	+/—	+/—	+	*ste HO*	.18	.20	.17
	C	+	—	—	**a** *ho*	(10/57)	(10/50)	
	D	+	—	—	**a** *ho*			
T	A	+/—	+/—	+	† *HO*			
	B	+/—	+/—	+	† *HO*	.65	.58	.67
	C	+	—	—	**a** *ho*	(37/57)	(29/50)	
	D	—	—	—	*ste ho*			

Diploids were formed between X10–1B spores and α*ste1–2* (X18) or α*ste1–5* (X78) cells as described in MATERIALS AND METHODS. Mating type was assayed by the prototroph complementation test. Segregants capable of sporulation after seven days at 30° on SPOR agar (MATERIALS AND METHODS) were scored as *HO*. Expected frequencies of Parental Ditype (PD), Nonparental Ditype (NPD) and Tetratype (T) asci were calculated assuming nonlinkage of *HO* to the mating type locus or to its centromere (HARASHIMA, NOGI and OSHIMA 1974).

* "+/—" indicates variable mating with both **a** and α tester strains (HICKS and HERSKOWITZ 1976a).

† Original mating type of spore cannot be determined by these tests.

2. *Microscopic observation of mating type switching in spores from X18 and X78*

a. *Homothallic switching and zygote formation*: In order to determine the mechanism by which spores of the presumed genotype *αste⁻ HO* gave rise to diploid colonies, tetrads from X18 and X78 were dissected on agar slabs and observed microscopically in order to determine which were *αste⁻* (see also, HICKS and HERSKOWITZ 1976a). Twelve tetrads from X18 were dissected on an agar slab in the presence of α-factor supplied by a dense streak of α cells. In 10 of 12 tetrads, all four spores germinated within five hours at 30°. In each complete tetrad, two spores were observed to exhibit the typical mating type **a** response to α-factor (aberrant cell morphology and no cell division), whereas the other two (presumed to be *αste⁻*) budded normally. Such behavior conforms to the prediction drawn from earlier tetrad analysis that X18 should segregate 2**a** : 2 *αste⁻* spores.

The progeny of the twenty α-factor insensitive cells (*αste⁻* spores) were subjected to pedigree analysis by separating individual cells after each round of cell division by micromanipulation (HICKS and HERSKOWITZ 1976a). The appearance of α-factor sensitive cells in the progeny of an insensitive cell indicates a switch to mating type **a** and, hence, the presence of the *HO* allele.

In 6 of the *αste⁻* pedigrees, **a′** cells were apparent at the four-cell stage. The pattern of switching was identical to that reported for normal homothallic cell lines (HICKS and HERSKOWITZ 1976a) in that when a change occurred, only the original spore cell and its immediate daughter were observed to switch. After four cell divisions, 10 of the 20 *αste⁻* pedigrees showed **a′** cells and were scored as *HO*. The other pedigrees were presumed to be derived from *αste⁻ ho* spores. **a′** *HO* and *αste⁻ ho* cells were then transferred to an agar slab without α-factor for further observation and growth into colonies. Zygotes appeared in each of the *HO* clones within three generations of the transfer. These observations on *αste⁻ HO* cells indicate that *HO* is capable of converting *αste⁻* to a functional **a** ("**a′**") and subsequently to a functional α ("*α′*") at high frequency. The mating behavior of these presumed **a′** and *α′* strains is described below.

The combined data for microscopic observation and plate assays of mating and sporulation performed on the tetrads of X18 described above are presented in Table 3. These data confirm the correlation between the appearance of sporulating diploids in segregants from X18 and X78 and the ability to undergo a homothallic interconversion of mating type.

b. *Mating behavior of* αste⁻ HO *spores*: The analysis in the preceding section assumed that the α-factor insensitive spores capable of switching to **a′** were initially *αste⁻*, that is, sterile at the time of germination. Two observations indicate that this is indeed the case.

(1) In spores from **a**/α *HO/HO* strains, one observes zygote formation by the four cell stage more than 50% of the time (HICKS and HERSKOWITZ 1976a). Although α-factor insensitive homothallic spores from X18 (**a**/*αste⁻ HO/ho*) frequently segregate **a′** cells at the four cell stage, mating is not observed to occur at that stage. Among 40 α-factor insensitive clones from each of the diploids X18 and X78, none was observed to form zygotes prior to the eight cell stage. This

TABLE 3

Segregation of sensitivity to α-factor, zygote formation and mating ability from X18

(**a**/αstel-2 ho/HO)

Tetrad type		Microscopic observations		Plate assays			Genotype of spore	Number observed
		Response to α-Factor	Zygote formation or mating type switch	Mate with α	Mate with **a**	Sporulation		
PD	A	+	+	+/—	+/—	+	**a** *HO*	1/10
	B	+	+	+/—	+/—	+	**a** *HO*	
	C	—	—	—	—	—	*αste⁻ ho*	
	D	—	—	—	—	—	*αste⁻ ho*	
NPD	A	+	—	+	—	—	**a** *ho*	1/10
	B	+	—	+	—	—	**a** *ho*	
	C	—	+	+/—	+/—	+	*αste⁻ HO*	
	D	—	+	+/—	+/—	+	*αste⁻ HO*	
T	A	+	+	+/—	+/—	+	**a** *HO*	8/10
	B	+	—	+	—	—	**a** *ho*	
	C	—	+	+/—	+/—	+	*αste⁻ HO*	
	D	—	—	—	—	—	*αste⁻ ho*	

Symbols and procedures are as described in the Legend to Table 2 and in MATERIALS AND METHODS.

observation suggests that the *αste⁻* genotype of the original spore in these clones is maintained by two of the cells at the four cell stage, hence mating does not occur. We suggest that *α′* cells are subsequently derived from the **a′** cells and are not formed directly by correction of the *αste⁻* lesion by *HO*.

(2) It is well known that individual yeast spores are capable of mating with vegetative cells of the opposite mating type prior to the initiation of the first cell division cycle (HAWTHORNE 1963a; OSHIMA and TAKANO 1971). This so-called spore-cell mating is very efficient; with mating-proficient **a** and α strains, zygote formation occurs in approximately 80% of the spore-cell pairs prior to germination of the spore (HICKS and HERSKOWITZ, unpublished observations). We have used this technique to test whether any of the spores from X18 are phenotypically *αste⁺* before they have undergone cell division. Twelve tetrads from X18 were dissected on an agar slab, and each spore was immediately placed in direct contact with a budding, heterothallic **a** cell (strain 73). Forty-five of 48 spores tested in this manner germinated, and *no* mating was observed through the first cell division. Therefore, tetrads from X18 behave as if they contain no mating-competent α spores prior to cell division.

In summary, these observations suggest that a spore initially of genotype *αste⁻ HO* can be converted to a functional **a′** cell within two cell divisions, and that the **a′** cell thus created can be subsequently converted to a functional *α′* cell through the action of the homothallism genes. Our analysis provides no information as to whether these **a′** and *α′* cells are ever converted to *αste⁻*.

3. *Behavior of heterothallic* **a′** *and* α′ *strains derived from* αste HO *cells*

In order to study further the properties of the **a′** and α′ alleles derived from *αste⁻* by action of the *HO* gene, we have removed the *HO* gene by genetic crosses. Heterothallic strains carrying **a′** and α′ alleles derived from *αste1–2* and *αste1–5* were constructed by crossing **a′** *HO* (or α′ *HO*) with α *ho* (or **a** *ho*). The origin of these **a′** and α′ cells and the crosses used to remove *HO* are described in the Appendix. These **a′** *ho* and α′ *ho* strains show α-factor production and response characteristic of ordinary heterothallic strains (data not shown), and mate as efficiently with strains of opposite mating type as do ordinary heterothallic strains (Table 4). Furthermore, **a′** and α′ mating types are stable, since the frequency at which they form prototrophs with tester cells of the same mating type is low (Table 4). This frequency is comparable to that previously reported for **a** × **a** and α × α matings (HICKS and HERSKOWITZ 1976a). By these criteria, **a′** and α′ mating types derived from *αste⁻* strains are indistinguishable from the mating types of normal laboratory heterothallic **a** and α strains. As in the case of **a′** and α′ strains derived from α^+ strains (HICKS and HERSKOWITZ 1976a), the *HO* gene is not necessary for the maintenance of the new mating type.

4. *Effect of HO on a nonspecific sterile mutation unlinked to the mating type locus*

In order to test the possibility that restoration of mating ability to *αste⁻* homothallic cells occurred by an *HO*-controlled bypass of the normal mating control system, we asked whether the *HO* gene could restore mating ability to mutants with a defect not at the mating type locus.

TABLE 4

Stability of **a′** *and* α′ *mating types derived from* αste⁻ *in homothallic strains*

		Rare mating			Efficiency of mating		
Parent 1	Mating type	Parent 2 (Tester strain)	Proto-trophs	Prototrophs / Parent 1	Parent 2′ (Tester strain)	Proto-trophs	Efficiency of mating
X91-31B	**a′**	**a** (73)	120*	3.1×10^{-5}	α (XJ24-40B)	71*	.18
X91-30C	**a′**	**a** (73)	112	3.2×10^{-5}	α (XJ24-40B)	98	.27
X93-31C	**a′**	**a** (73)	148	3.2×10^{-5}	α (XJ24-40B)	101	.22
X93-13B	**a′**	**a** (73)	134	5.2×10^{-5}	α (XJ24-40B)	69	.29
none		**a** (73)	0	—	α (XJ34-40B)	0	—
73	**a**	—	—	—	α (XJ24-40B)	34	.29
X95-8B	α′	α (XJ24-40B)	340	1.3×10^{-4}	**a** (73)	35	.58
X95-8A	α′	α (XJ24-40B)	102	2.5×10^{-5}	**a** (73)	292	.70
X96-1B	α′	α (XJ24-40B)	43	1.1×10^{-5}	**a** (73)	201	.50
X96-6D	α′	α (XJ24-40B)	43	0.8×10^{-5}	**a** (73)	284	.52
none		α (XJ24-40B)	0	—	**a** (73)	0	—
XJ24-40B	α	—	—	—	**a** (73)	80	.80

*Numbers represent the average of two separate platings from the same parent 1 culture. Other details are described in MATERIALS AND METHODS and in HICKS and HERSKOWITZ (1976a).

Strain VI14 carries a sterile mutation which affects both **a** and α cells and which is unlinked to the mating type locus (MacKay 1972). Diploids formed by mating between vegetative cells of VI14 and spores of X10–1B were isolated by prototroph selection (Cross X38). Six prototrophs were isolated, purified and sporulated.

The presumed genotype of the prototrophs from this mating, with regard to the mating type and homothallism, was:

$$\frac{\mathbf{a}}{\alpha}\ \frac{ho}{HO}\ \frac{ste^-}{+}$$

From a diploid of this type, one of two possible modes of segregation might be expected. If *HO* can bypass the sterile mutation, then two segregants capable of sporulation would be expected in each tetrad. The nonsporulating (*ho*) segregants would be 50% Ste$^+$ and 50% Ste$^-$. However, if the sterile mutation prevents zygote formation regardless of the action of the *HO* gene, then two nonmating, nonsporulating segregants would be expected in each tetrad. The remaining spores would display either normal heterothallic or normal homothallic phenotype in a ratio of 1:1. The segregation data for X38 (Table 5) clearly show that the latter possibility is observed. The sterile phenotype appears in two out of four segregants in 46/48 tetrads and thus is epistatic to *HO*.

Preliminary evidence (Hicks, unpublished observations) indicates that the homothallic system also cannot correct the mating defect in cells containing **a**-specific (*ste2–1*) and α-specific (*ste3–1*) mutations. Homothallic cells carrying these mutations undergo mating type interconversion at normal frequencies; however, since one of the two mating types is sterile in each case, mating is prevented. The phenotype of the colony produced is that of a haploid of the mating type not affected by the particular *ste*$^-$ mutation present. For example, a spore of genotype α *HO ste3–1* (α specific) is insensitive to α-factor, but after several divisions segregates α-factor sensitive progeny. The colony resulting from this clone consists of **a** cells and sterile cells and thus exhibits the **a** phenotype.

These results indicate that action of the *HO* gene cannot supplant the entire mating system. Rather, we interpret the recovery of α' cells from α*ste1 HO* and

TABLE 5

Segregation of the sterile and homothallic phenotypes in tetrads from X38
(**a**/α HO/ho ste$^+$/steX)

Tetrad type*	Phenotypes of spores	Tetrads observed
Parental ditype	2 sterile : 2 homothallic	12/48
Nonparental ditype	2 sterile : 2 heterothallic	11/48
Tetratype	2 sterile : 1 homothallic : 1 heterothallic	23/48
**	1 sterile : 2 homothallic : 1 heterothallic	1/48
**	3 sterile : 1 homothallic	1/48

* X38 was formed by mating of **a** *HO ste*$^+$ (X10–1B–2) with α *ho steX* (VI14).
** Aberrant tetrads probably resulting from gene conversion of the *steX* mutation.

αste2 HO cells to be the result of some action by *HO* at the mating type locus itself, which then controls the normal mating pathway of heterothallic strains.

5. *Recovery of functional* **a** *and* α *from heterothallic αste⁻ strains*

Heterothallic strains can switch mating type at low frequency. Such rare events can be identified by selecting prototrophs from mass matings between two strains of the same mating type which carry complementary nutritional mutations (HAWTHORNE 1963a; RABIN 1970). Such diploids presumably arise through the spontaneous interconversion of mating type in a single cell followed by mating between that cell (or one of its progeny) and a member of the population which has retained the original mating type. Diploids formed in this fashion are capable of sporulation and segregate 2**a**:2α spores. [Other types of diploids are also formed (RABIN 1970; HICKS and HERSKOWITZ, unpublished observations), but these will not be discussed here.] The newly converted mating type allele, which we will designate **a′** or α′ by analogy to those formed by homothallic interconversion, can thus be isolated from the diploid by sporulation and tetrad analysis. We have used this technique to compare the interconversion characteristics of sterile strains VC2 (*αste1–2*), VN33 (*αste1–5*), and VP1 (*αste1–4*) with their common parent, XT1172–S245c. Specifically we wished to determine whether the presence of a sterile mutation at the mating type locus would inhibit conversion of that strain to **a′** and subsequently to α′ in the absence of the homothallic system.

a. *Conversion from* αste⁻ *to* **a′**: α^+ (XT1172–S245C) or α^- (VP1, VC2, VN33) strains (Parent 1) were mated with α^+ strains (Parent 2) carrying complementary nutritional requirements, and prototrophs selected as described in MATERIALS AND METHODS. Table 6A summarizes these rare matings and the types and frequencies of prototrophs obtained in each cross. Note that Parents 1 and 2 differ in their sensitivity to the drug cryptopleurine, which is determined by a gene (*CRY*) closely linked to the mating type locus (2.1 centimorgans; SKOGERSON, MCLAUGHLIN and WAKATAMA 1973). In each sporulating prototroph (i.e., **a′**/α diploid), the mating type locus (from Parent 1 or Parent 2) which had undergone conversion to **a′** could be determined by observing the coupling of **a′** to the cryptopleurine resistance marker (*cry1–3*).

From each cross an **a′**/α diploid in which the **a′** allele showed tight linkage to CRY^S, and which was therefore derived from the *αste⁻* strain, was picked for further study. Representative diploids formed by matings between α^+ cry^R and CRY^S strains carrying *αste1–2*, *αste1–5*, or α^+ were X22–23, X66–37 and X65–67 respectively. Linkage data for *cry1–3* and mating type are presented in Table 6B. Tetrad analysis of these diploids showed that **a′** and α segregated 2:2 (as did all heterozygous auxotrophic markers). Several **a′** segregants from each diploid were tested for response to α-factor, barrier effect, efficiency of mating and rare mating ability and were found to be indistinguishable from normal laboratory **a** haploids by these criteria (data not shown).

These results show that strains carrying the *ste1–2* and *ste1–5* alleles can be converted to **a′**. The observation that no **a′** CRY^S/a cry^r diploids were isolated

TABLE 6

Isolation and characterization of **a**′/α *diploids from* $\alpha \times$ *aste*$^-$ *matings*

A. Summary of matings

Mating	Strains crossed: Parent 1	Strains crossed: Parent 2	Prototrophs per *aste*$^-$ cell	a/α per Total prototrophs*
X22	VC2 (*aste1–2 CRY*^{S})	205 (α *cry1–3*)	2.8×10^{-5}	5/70
X65	XT1172–S245c (α *CRY*^{S})	X50–2D (α *cry1–3*)	9.2×10^{-5}	18/240
X66	VN33 (*aste1–5 CRY*^{S})	X50–2D (α *cry1–3*)	1.7×10^{-4}	8/120
X71	VC2 (*aste1–2 CRY*^{S})	X50–2D (α *cry1–3*)	1.8×10^{-4}	9/126
X72	VP1 (*aste1–4 CRY*^{S})	X50–2D (α *cry1–3*)	2.5×10^{-4}	0/200

B. Coupling of *CRY* and mating type

Diploid isolate	Genotype	Segregation of mating type and *cry1–3*: PD	NPD	T
X22–23	**a**′ *CRY*^{S} / α *cry1–3*	6	0	0
X66–37	**a**′ *CRY*^{S} / α *cry1–3*	12	0	1
X65–67	**a**′ *CRY*^{S} / α *cry1–3*	10	0	0

A. *Summary of matings*: Matings were performed by prototroph selection as described in the text and MATERIALS AND METHODS. All Parent 1 strain except VC2 were irradiated with ultraviolet light (to 50% survival) before mating to increase the frequency of mating type interconversion in these strains. The apparent stimulation of mating type interconversion by UV irradiation is under study.

* Prototrophs were assayed for mating and sporulation as described in MATERIALS AND METHODS. Isolates which sporulated but did not mate were scored as **a**′/α.

B. *Coupling of* CRY *and mating type in representative diploids*: The genotype of the diploids were inferred from the observed linkage of the *CRY*^{S} and **a**′ alleles.

from cross X72 involving VPI (see Table 6A) may indicate that the *ste1–4* allele carried by VP1 cannot be converted to **a**′, and thus differs from *ste1–2* and *ste1–5*. VP1 differs from strains carrying *ste1–2* and *ste1–5* also in that it exhibits at least one **a**-specific phenotype (see HICKS and HERSKOWITZ 1976b).

b. *Conversion of* **a**′ *(derived from aste*$^-$*) to* α: X22–23–2C, X65–67–3C and X66–37–11B are **a**′ haploid strains isolated as meiotic segregants from the **a**′/α diploids selected in the previous experiment. A number of independent single colony isolates of each **a**′ strain were mated separately with the cryptopleurine-resistant **a** strain X57–1B by prototroph selection as described in MATERIALS AND METHODS (see Table 7A). All prototrophs isolated from these crosses were nonmaters capable of sporulation, and 25 of 28 yielded 2**a**:2α spores. (The three exceptional diploids are described in the legend to Table 7.) A number of these independently isolated diploids were then subjected to tetrad analysis to confirm their **a**/α character and to determine which of the two parental mating type **a** alleles had been converted to α′. As before, this determination was made by following the coupling of the new α mating type allele to *cry1–3* (Table 7B). The data presented in Table 7 show that in each cross, diploids in which *cry*^{r} is linked to α and those in which *cry*^{r} remains linked to **a** occur with approximately equal frequencies. Thus the **a**′ alleles derived from *aste*$^-$ backgrounds have approximately the same potential for conversion to α′ as does an **a** allele from a normal haploid background. The efficiency of mating and stability of representative α′ strains from X97, X98 and X106 are presented in Table 8.

TABLE 7

Characterization of diploids formed by **a** $\times$ **a′** *matings*

A. Summary of matings

Mating	Diploids formed between:	a′ strain derived from:	Number of diploids obtained of type:* α' *CRY*^{S} **a′** *cry*^{r}	α' *cry*^{r} **a′** *CRY*^{S}
X97	X22–23–2C (**a′** *CRY*^{S}) $\times$ X57–1B (**a** *cry*^{r})	VC2 (*αste1–2*)	8	3
X98	X66–37–9A (**a′** *CRY*^{S}) $\times$ X57–1B (**a** *cry*^{r})	VN33 (*αste1–5*)	3	5
X106	X65–67–11B (**a′** *CRY*^{S}) $\times$ X57–1B (**a** *cry*^{r})	XT1172–S245c (*α STE+*)	3	3

B. Coupling of *CRY* and mating type

Diploid isolate	Genotype	Segregation of mating type and *cry*^{r}: PD	NPD	T
X97–1	α' *cry*^{r} / **a′** *CRY*^{S}	4	0	0
X97–2	α' *CRY*^{S} / **a** *cry*^{r}	4	0	0
X97–3	α' *cry*^{r} / **a′** *CRY*^{S}	5	0	1
X97–4	α' *CRY*^{S} / **a** *cry*^{r}	5	0	2

A. *Summary of matings*: **a′** *CRY*^{S} strains (X22–23–2C, X66–67–9A, X65–67–11B) are haploid segregants from **a′**/α diploids X22, X66 and X65 (described in Table 6). Diploids X97, X98 and X106 were isolated by prototroph selection (MATERIALS AND METHODS) and each isolate was assayed for linkage of α' and *CRY*^{S} by sporulation and subsequent tetrad analysis.

* Three exceptional diploids (one obtained from X98 and two from X106) yielded occasional nonmating segregants and thus were not scored as **a**/α.

B. *Coupling of* CRY *and mating type in representative diploids*: Segregation of cryptopleurine resistance and mating type from four of the diploids formed in mating X97.

TABLE 8

Stability of α' *mating types derived from* αste$^-$ ho

Parent 1	Mating type	Rare mating: Parent 2 (Tester strain)	Rare mating: Prototrophs	Rare mating: Prototrophs / Parent 1	Efficiency of mating: Parent 2′ (Tester strain)	Efficiency of mating: Prototrophs	Efficiency of mating: Efficiency of mating
X97–4C	α'	α (XJ24–40B)	18	9×10^{-6}	**a** (73)	191	.53
X97–2B	α'	α (XJ24–40B)	—	—	**a** (73)	104	.59
X98–1D	α'	α (XJ24–40B)	25	8×10^{-6}	**a** (73)	330	.59
X98–2B	α'	α (XJ24–40B)	—	—	**a** (73)	66	.75
X106–1D	α'	α (XJ24–40B)	42	2.3×10^{-5}	**a** (73)	183	.65
X106–3A	α'	α (XJ24–40B)	11	8×10^{-6}	**a** (73)	137	.68
none		α (XJ24–40B)	2	—	**a** (73)	0	—

Procedures as in Table 4.

DISCUSSION

The observation that mating types can be interconverted indicates that all yeast cells contain information for both mating type alleles, but that only one is expressed. The two pathways of interconversion—the rare interconversion in heterothallic strains and the frequent interconversion in homothallic strains—act similarly. Both yield stable **a′** and α' cells, and both can convert α^- to **a′** and subsequently to α'. Since the *HO* gene is necessary for the establishment but not the

maintenance of the new mating type, it is likely that this gene controls functions which facilitate the ordinarily rare interconversion event.

In this paper we show that a defective α mating type locus can be converted to a functional **a** locus and subsequently to a functional α locus in both homothallic and heterothallic strains. Specifically, homothallic spores carrying either of two α mating type sterile mutations, *ste1–2* or *ste1–5*, can give rise to mating proficient **a′** cells within a few divisions after germination in the manner of normal α homothallic cells. Further observation of the **a′** cells thus obtained indicates that after several more divisions, they in turn can segregate α' cells which are indistinguishable in mating behavior from normal α cells. The newly arisen α' strains maintain their α character even after removal of the *HO* gene.

The recovery of mating-proficient segregants from cells containing sterile mutations through the action of *HO* appears to be limited to defects at the mating type locus itself. *HO* is unable to restore function to the unlinked nonspecific sterile mutation (*steX*) carried by strain VI14. Although *HO* may be able to promote interconversion at the mating type locus in a cell containing the *steX* mutation, the cell remains sterile. No mating occurs among its progeny, and the homothallic phenotype is masked. Thus, it is likely that recovery of mating proficiency in cells originally carrying a sterile mating type allele results from action of the homothallic system at the mating type locus itself rather than bypassing the normal regulatory pathway.

The recovery of a functional α locus from an αste^- mutant is not a special property of the *HO* gene since it occurs in heterothallic strains. Heterothallic **a′** strains were selected from strains carrying either *ste1–2* or *ste1–5* by UV-stimulated mating type conversion. These **a′** strains were found to have approximately the same capability for spontaneous conversion to a functional α' state as **a′** strains similarly selected from a nonmutant α background. We therefore conclude that the α^- mutation does not affect the **a** information in the cell. Furthermore, the observation that **a′** cells derived from an αste^- background can be converted to functional α' with or without *HO* leads to the conclusion that the switching process includes a mechanism for circumventing the mutant α information.

One would like to know whether the homothallic system can restore **a′** function to a strain carrying a defective **a** allele. Selections and screens for *ste*$^-$ mutants have not yielded mutations at the **a** mating type locus (MacKay and Manney 1974; Hicks, unpublished observations). Several possible explanations for the lack of **a***ste*$^-$ mutations at the mating type locus can be proposed. One possibility is that more than one copy of the **a** allele is normally expressed in a haploid cell, thereby masking any recessive sterile mutations. Another possibility is that mutations in the **a** allele may not be capable of forming a sporulating diploid, thereby precluding genetic analysis. The strains assigned to Class 9 by MacKay and Manney (1974) fall into this category. A further possibility is that the **a** mating type locus does not contain regulators of **a**-specific mating functions (J. Strathern, personal communication); that is, these **a** functions may be constitutively produced in **a** cells and repressed in α cells.

How does *HO* stimulate a switch between **a** and α? The answer to this question requires some understanding of the structure of the mating type locus, about which little is known. Since **a** and α alleles are codominant (**a**/α strains having properties different from **a**/**a** and α/α strains), **a** and α must code for different functions. We imagine that **a** and α alleles are blocs of DNA which specify one or more regulatory proteins. Two kinds of models for interconverting mating types involve either modification or recombination of the DNA at the mating type locus. In the modification model, the information for both **a** and α is present at the mating type locus, and expression of either the **a** or α information is determined by a promoter (or other regulatory site) subject to specific methylation or sequence modification (H. LODISH, personal communication; D. HAWTHORNE, personal communication; MACKAY 1972; see also HOLLIDAY and PUGH 1975). For example, an unmodified promoter might direct transcription leftwards (to produce an **a** cell), and a modified promoter might direct transcription right-

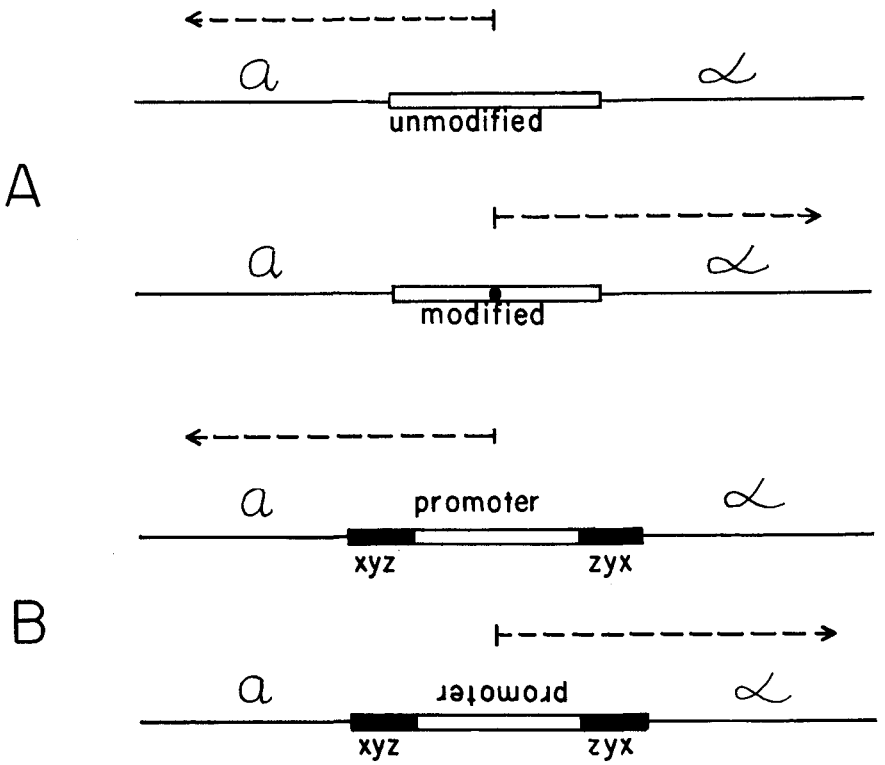

FIGURE 1.—Modification and recombination models for mating type interconversion.

The figure shows the mating type locus, which contains regulators for both **a** and α mating type behavior and a regulatory site between these regulators. In model A, based on HAWTHORNE's proposal (personal communication; see also HOLLIDAY and PUGH 1975), a promotor has two forms—for example, modified or unmodified—which direct RNA polymerase rightwards or leftwards. In model B, intramolecular recombination within the regions (*xyz* . . . *zyx*) flanking the promoter inverts the promoter and thus changes the direction of transcription. See also Figure 4. Dashed lines indicate the direction of transcription.

wards (to produce an α cell) (Figure 1A). The enzyme responsible for modification of the DNA and, hence, control of transcription would be under the control of the *HO* gene. This model easily accounts for the observation that the **a** information is not affected by a *ste*$^-$ mutation in α, but is not sufficient to account for the recovery of α function at high frequency in $\alpha ste^- HO$ strains.

In the recombination model, expression of the information for **a** or α is determined by which information is adjacent to essential controlling sites. For example, Figure IB shows one such possibility in which intramolecular recombination occurring at specific recognition regions places either **a** or α information in position to be transcribed. The recombinational event would be controlled by the homothallism gene, which perhaps codes for a site-specific recombination enzyme (SIGNER and WEIL, 1968). A similar recombinational model has been

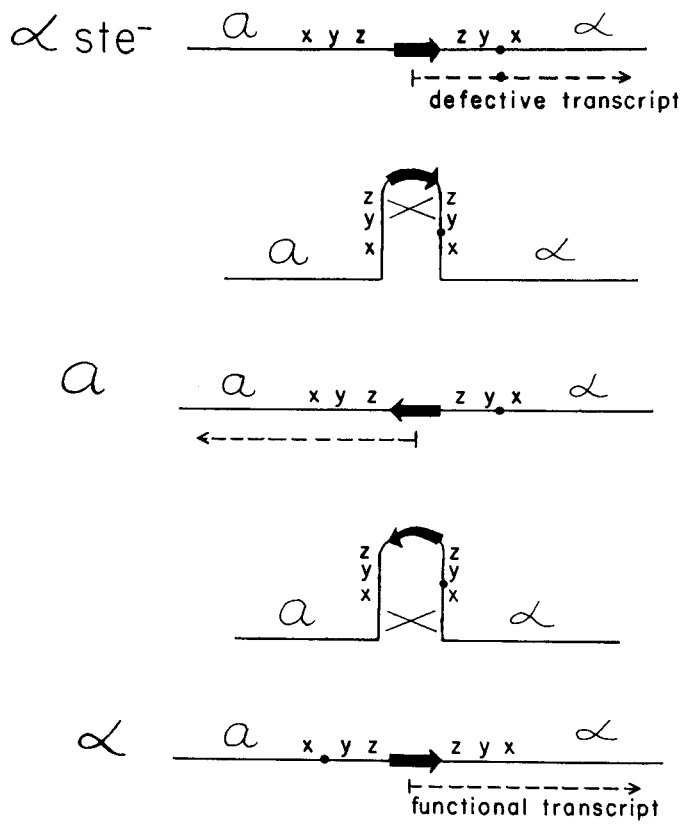

FIGURE 2.—Recovery of functional α from αste^- by recombination within a reverse-repeated DNA region.

The mating type locus is shown as in Figure 1B. $xyz \ldots zyx$ are reverse-repeated DNA regions which can recombine with each other to invert the promoter (indicated by arrow). The dot indicates the position of the ste^- mutation. The top line indicates the structure of ste^- mating type locus. Intramolecular recombination between y and z generates a functional **a** mating type locus (middle line). A second crossover below x generates a functional α mating type locus (bottom line).

proposed by OSHIMA and TAKANO (1971; see also HARASHIMA, NOGI and OSHIMA 1974) in which *HO* controls insertion of a regulatory element, perhaps analogous to bacterial IS elements (HIRSCH, STARLINGER and BRACHET 1972; FIANDT, SZYBALSKI and MALAMY 1972) or controlling elements in maize (MCCLINTOCK 1956). The model depicted in Figure 1B can account for the ability of α^- to be converted to **a**, but does not readily explain the mechanism by which this **a** locus can subsequently be switched to a functional α. We shall discuss possible explanations for the recovery phenomenon in the context of these models of mating type interconversion.

Recovery of a functional α from a defective α suggests that the cell contains an additional copy (or copies) of the α information, at least that part of α information defective in mutations *ste1–2* and *ste1–5*. This additional α information may be present within the mating type locus itself or be located elsewhere. To explain how α^- can be converted to α^+, DAVID BOTSTEIN (personal communication) has suggested that if the αste^- mutations occur in the stem of a recombination region, two successive crossovers could generate a functional α (see Figure 2). Although we do not know the nature of mutations *ste1–2* or *ste1–5*, D. HAWTHORNE (personal communication) has found that an α^- nonsense mutation can be converted to **a** and then to α in homothallic strains. This observation suggests that a recombinational event associated with interconversion must include the α information coding for a protein. Another explanation for recovery of α^+ from α^-

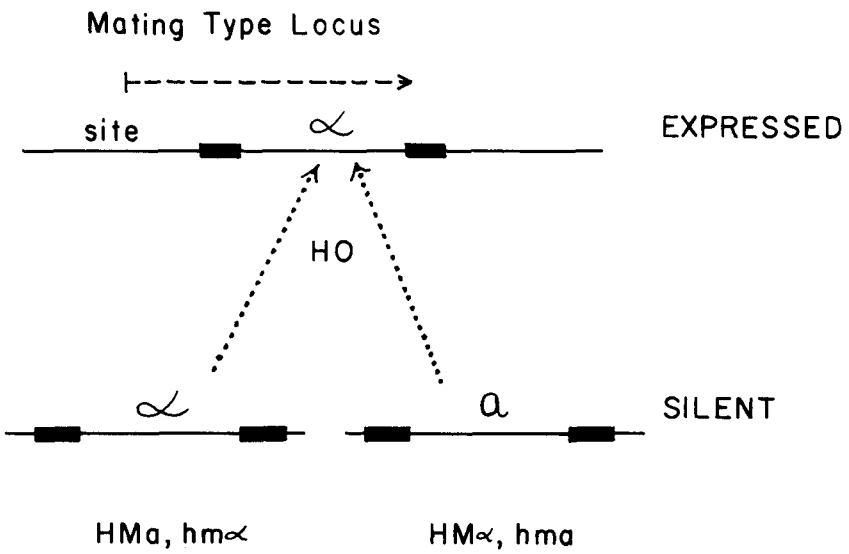

FIGURE 3.—Cassette model for mating type interconversion.

Boxes represent DNA sequences involved in the recombination event mediated by the *HO* gene. The bloc of DNA between these boxes is a "cassette" of information containing genes which control **a** or α mating type behavior. The mating type locus contains an essential site—for example, a promoter or ribosome binding site—which allows expression of adjacent mating type information. The dashed line indicates, for example, transcription. Dotted lines indicate insertion of information into the mating type locus.

is that additional α information exists as a complete copy (or copies) of the α information. As a unifying hypothesis for the structure of the mating type locus and the mechanism of interconversion, we propose that the mating type locus contains a bloc of information, either **a** or α, which is adjacent to a promoter and consequently expressed (Figure 3). Elsewhere in the genome are other blocs, either **a** or additional copies of α, which are not adjacent to a promoter and hence are not expressed. We propose that the information of these silent blocs can be substituted for the resident information at the mating type locus by action of the *HO* gene.

Work by HARASHIMA, NOGI and OSHIMA (1974) indicates that there are two genetic systems of homothallism, type II homothallism with genotype *HO HM***a** *HM*α and type I homothallism with genotype *HO hm***a** *hm*α, and that most heterothallic strains are *ho HM***a** *HM*α. Our homothallic strains are of type II, and our heterothallic strains are *ho HM***a** *HM*α (HICKS and HERSKOWITZ 1976a). In type II homothallism, *HO* is necessary but not sufficient for conversion from **a** to α and from α to **a**. *HM***a** is required in addition for conversion from **a** to α, and *HM*α is required in addition for conversion from α to **a**. It has been proposed (HARASHIMA, NOGI and OSHIMA 1974; see also NAUMOV and TOLSTORUKOV 1973) that "the association of an *HM*α-element with the mating-type locus would form the **a** mating-type allele and the association of an *HM***a**-element with the mating-type locus would give rise to the α mating-type allele." More specifically, we propose (Figure 3) that the *HM***a** and *hm*α loci are silent blocs of α information, that the *HM*α and *hm***a** loci are silent blocs of **a** information, and that the *HO* gene is responsible for inserting the **a** or α information into the mating type locus.

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APPENDIX

Origin and isolation of heterothallic **a**′ *and* α′ *strains derived from* α*ste*⁻ *cells*

The crosses used to remove *HO* were initiated by dissecting tetrads from X18 and X78 on agar slabs in the presence of α-factor (see Figure A1). α-factor insensitive spores were allowed to divide until a pair of **a**′ (α-factor sensitive cells) were segregated (Figure A1-A). These **a**′ cells were then removed to agar lacking α-factor. From each pair of **a**′ cells, one was immediately subjected to cell-cell mating with a heterothallic α cell (strain X50-2D) (Figure A1-B). In most cases zygote formation took place between these cells without further division. Diploids formed between X50–2D and **a**′ segregants of X18 and X78 were designated X91 and X93 respectively

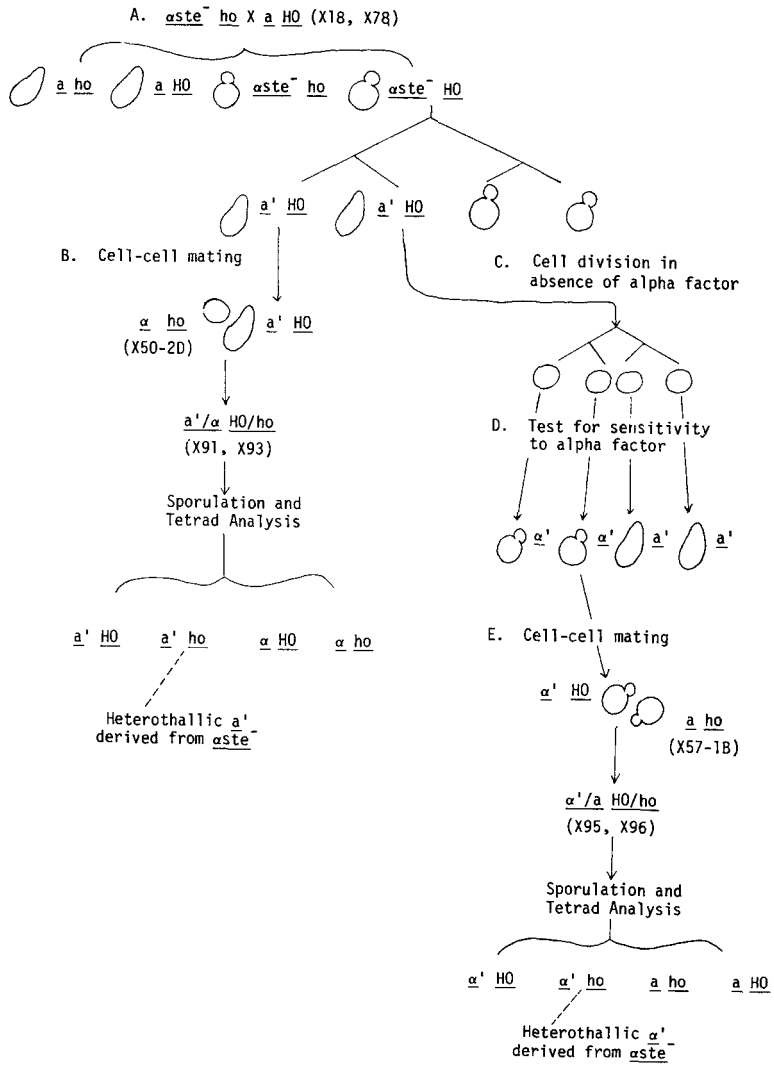

FIGURE A1.—Isolation of heterothallic **a**′ and α′ strains from *αste⁻ HO*.

Procedures A and D were performed in the presence of α-factor. Other procedures were performed in the absence of α-factor. Descriptions of strains and other procedures are in the text.

and will be discussed below. The remaining **a**′ cell of each pair (Figure A1-A) was allowed to continue division in the absence of α-factor (Figure A1-C). Progeny cells were prevented from mating by micromanipulation. After 3 or 4 divisions the progeny were subjected to α-factor (Figure A1-D). At this point approximately one-half of the progeny were observed to be α-factor sensitive. The rest had presumably been converted from **a**′ to α′ by the *HO* gene. These newly created **a**-factor insensitive cells were then removed from α-factor and individually mated with heterothallic **a** cells (strain X57-1B) (Figure A1-E). In 14 of 22 cell-cell pairs (pooled data from 2 clones each from X18 and X78), mating occurred within one round of cell division. Representative diploids from these crosses were designated X95 and X96.

Tetrad analysis of sporulated cultures of X91 and X93 (**a**′ *HO* × α *ho*) are summarized in Table A1 and those of X95 and X96 (α′ *HO* × **a** *ho*) in Table A2. The segregation of mating type and homothallism indicates that all four diploids are of the general genotype: **a**/α *HO*/*ho*. Furthermore, the fate of each mating type allele present in the original zygote can be followed by observing its segregation with respect to cryptopleurine resistance which was contributed by the heterothallic strain in each cross. As expected, cryptopleurine resistance exhibited tight linkage to the α allele in X91 and X93 and similar linkage to **a** in X95 and X96 (see Tables A1 and A2).

TABLE A1

Segregation data from α ho × **a**′ HO (*derived from* αste1–2 *and* αste1–5)

	Tetrad type	Mate with α	Mate with **a**	Sporulation	Inferred genotype of spore	Tetrads observed		Tetrads expected
						X91	X93	
PD	A	+/—	+/—	+	**a**′ *HO*	.14 (5/38)	.22 (8/37)	.17
	B	+/—	+/—	+	**a**′ *HO*			
	C	—	+	—	α *ho*			
	D	—	+	—	α *ho*			
NPD	A	+/—	+/—	+	α *HO*	.26 (10/38)	.07 (3/37)	.17
	B	+/—	+/—	+	α *HO*			
	C	+	—	—	**a**′ *ho*			
	D	+	—	—	**a**′ *ho*			
T	A	+/—	+/—	+	* *HO*	.61 (23/38)	.70 (26/37)	.67
	B	+/—	+/—	+	* *HO*			
	C	—	+	—	α *ho*			
	D	+	—	—	**a**′ *ho*			

Coupling of *CRY* and the mating type locus

	Fraction recombinant spores†	$\frac{(\alpha\ CRY^S) + (\mathbf{a}\ cry^r)}{\text{Total}}$
X91	.08	7/86
X93	.07	4/58

a′ *CRY*^{S} *HO* strains were formed by homothallic conversion of *αste1–2* (X91) and *αste1–5* (X93). The α *cry1–3 ho* parent was X50–2D. Other details are described in the Legend to Table 2.

* Original mating type of spore cannot be determined by these tests.

† Only heterothallic segregants were scored.

Two heterothallic *CRY*^{S} segregants of mating type **a** were chosen from both X91 (strains X91-31B and X91-30C) and X93 (strains X93-31C and X93-13B) for further study (Table 4). Heterothallic *CRY*^{S} segregants of mating type α from X95 (strains X95-8B and X95-8A) and X96 (strains X96-1B and X96-6D) were chosen for further study (Table 4).

TABLE A2

Segregation data from **a** ho $\times$ α' HO (*derived from* αste1-2 *and* αste1-5)

	Tetrad type	Mate with α	Mate with **a**	α Factor sensitivity of spore	Homothallic zygote formation	Genotype of spore	Tetrads observed		Tetrads expected
							X95	X96	
PD	A	+/—	+/—	—	+	α' *HO*			
	B	+/—	+/—	—	+	α' *HO*	.19	.17	.17
	C	+	—	+	—	**a** *ho*	(3/16)	(3/18)	
	D	+	—	+	—	**a** *ho*			
NPD	A	+/—	+/—	+	+	**a** *HO*			
	B	+/—	+/—	+	+	**a** *HO*	.06	.33	.17
	C	—	+	—	—	α' *ho*	(1/16)	(6/18)	
	D	—	+	—	—	α' *ho*			
T	A	+/—	+/—	—	+	α' *HO*			
	B	+/—	+/—	+	+	**a** *HO*	.75	.50	.67
	C	+	—	+	—	**a** *ho*	(12/16)	(9/18)	
	D	—	+	—	—	α' *ho*			

Coupling of *CRY* and the mating type locus

	Tetrad type		
	PD	NPD	T
X95	13	0	3
X96	18	0	0

α *CRY*^{S} *HO* strains were formed by homothallic conversion in progeny of *αste1-2* (X95) and *αste1-5* (X96). The **a** *cry1-3 ho* parent was X57-1B.