MUTANTS SHOWING HETEROTHALLISM FROM A HOMOTHALLIC STRAIN OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Mutants defective in mating-type conversion were isolated from ascospores of a perfect homothallism strain having the *HO HMR***a** *HML*α genotype. Eighty mutants, including 11 temperature-dependent mutants showing **a** or α mating potency, were isolated from 10,050 colonies derived from spores mutagenized with ethyl methanesulfonate. Of the 80 mutants, 48 were tested by crossing with an *ho HMR***a** *HML*α heterothallic and an *HO HMR***a** *HML*α homothallic strain as standards. The results allowed their division into seven classes. Mutants in the first class were due to mutation of *HO* to an *ho* allele. The second class of mutants, *csm*, lacked the *HO* function as the result of a mutation unlinked with the *HO* locus. Mutants in the third and fourth classes were defective in *HMR***a** and *HML*α gene functions, respectively. Mutants in the fifth and sixth classes were due to a mutation at the mating-type locus showing insensitivity to the function of the homothallic genes. Mutants in the seventh class lacked the expression of mating type as the result of a mutation unlinked to the mating-type locus. All the temperature-dependent mutants lost the mating potency at permissive temperature (25°). One belonging to the last class of mutants was inferred to be a mutation at a locus necessary for the expression of **a** mating type at restricted temperature (35°).

SACCHAROMYCES yeasts are divided into heterothallic and homothallic strains. In a heterothallic strain, cultivation of an ascospore gives rise to a haploid clone having either **a** or α mating type. In a homothallic strain, on the other hand, cultivation of an ascospore gives rise to a diploid clone heterozygous for the mating-type alleles, but homozygous for the other genetic markers. Heterothallic haploid cells of opposite mating type readily conjugate and form diploid cells of **a**/α genotype, which are nonmating and able to sporulate. While a heterothallic haploid cell exhibits a rare interconversion of mating types within normal mutation frequency, a homothallic haploid cell exhibits extremely high frequency of conversion from one mating-type allele to the opposite type by the action of the homothallic genes (HICKS and HERSKOWITZ 1976; TAKANO *et al.* 1977). This conversion gives rise to offspring of both **a** and α mating types in the culture. Offspring of complementary mating type then conjugate to form **a**/α zygotes that produce nonmating diploid cells isogenic for all loci except

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mating type. Genetic analyses have indicated that homothallism is controlled by a dominant gene, *HO*, which is unlinked to the mating-type locus, and by two other genes, *HMα* (or *hm***a**), which specifies the conversion of α to **a**, and *HM***a** (or *hmα*), which specifies the conversion of **a** to α (HARASHIMA, NOGI and OSHIMA 1974). Consequently, homothallic strains can be divided into three different types by the configuration of the homothallic genes, one perfect homothallic (the *Ho* type), which is derived from the **a** or α *HO hmα hm***a** (Type I) or the **a** or α *HO HMα HM***a** (Type II) genotype, and two semi-homothallic (*Hp* and *Hq* types), which are derived from the **a** *HO hmα HM***a** and the α *HO HMα hm***a** genotype, respectively. Recently, KLAR and FOGEL (1977) suggested the equivalence of function of the *hm***a** and *HMα* alleles; results supporting this idea and showing the equivalence of function of *hmα* and *HM***a** alleles have been obtained (HARASHIMA and OSHIMA 1978; ARIMA and TAKANO 1979). These observations indicate that *HMα* and *hm***a** alleles have the concealed ability to impart **a** function and the *HM***a** and *hmα* alleles to impart α function. These facts, together with the finding that the *HMα* and *HM***a** genes are located, respectively, on the right and left arm of chromosome *III* (HARASHIMA and OSHIMA 1976), have lead to proposals for a revised nomenclature for these *HM* genes (*HMR***a** for *HMα*, *HMLα* for *HM***a**, *HMRα* for *hmα* and *HML***a** for *hm***a**: HABER, personal communication; ARIMA and TAKANO 1979). The correspondence between the old and revised genetic symbols for the homothallic genes is listed in Table 1. If the mutant alleles occur in the *HMR***a**, *HMLα*, *HMRα* and *HML***a** loci, the small letters *hmr***a**, *hmlα*, *hmrα* and *hml***a**, respectively, can be assigned.

Functions of the homothallic genes are extremely specific to the mating-type locus, and the mating-type conversion generally takes place in a few generations after spore germination (TAKANO *et al.* 1977) or in cells that have passed through one generation in the cell division cycle (HICKS and HERSKOWITZ 1976). Such a high specific and frequent alternation of the mating-type alleles can not be explained by the function of mutators that cause aberration in the DNA sequence. Therefore, particular mechanisms seem to be involved in the mating-type differentiation by the homothallic genes. Several models have been proposed for the

TABLE 1

Genetic symbols for homothallic genes

Old symbol	Revised symbol*	Remarks
HO	*HO*	Essential for both conversions, **a** to α and α to **a**
ho	*ho*	Recessive allele of *HO*
HMα	*HMR***a**	**a** mating-type function, on right arm of chromosome *III*
hmα	*HMRα*	α mating-type function, alternate allele of *HMR***a**
*HM***a**	*HMLα*	α mating-type function, on left arm of chromosome *III*
*hm***a**	*HML***a**	**a** mating-type function, alternate allele of *HMLα*

* For the mutant alleles of the *HMR* and *HML* loci, small letters *hmr* and *hml* can be assigned, respectively.

differentiation (the controlling element model: OSHIMA and TAKANO 1971, HARASHIMA, NOGI and OSHIMA 1974; the flip-flop model: HOLLIDAY and PUGH 1975, BROWN 1976, and the cassette model: HICKS and HERSKOWITZ 1977), of which the controlling element model or the cassette model seems most plausible, since the *α ste⁻* (*matα*: a sterile mutant of the *α* mating-type locus) and *α-inc* (*matα-inc*: an inconvertible *α* mating-type allele) mutations were effectively healed through mating-type conversion (HICKS and HERSKOWITZ 1977; TAKANO, KUSUMI and OSHIMA 1973).

To investigate the genetic and molecular mechanism of the function of the homothallic genes, we isolated mutants from homothallism to heterothallism. In this paper, we describe the isolation and characterization of mutants defective in the mating-type conversions.

MATERIALS AND METHODS

Strains: Strains used in the present study are listed in Table 2. All strains were selected from our genetic stock cultures. Mutants were isolated from spores of a perfect homothallism strain (Type II *Ho* strain; HARASHIMA, NOGI and OSHIMA 1974), T-1851-2D. For genetic analysis of the isolated mutants, we used four standard heterothallic strains having the *ho HMRa HMLα* genotype and a standard homothallic strain, T-1171-5D, having the *HO HMRa HMLα* genotype. J-1-2B and J-1-5A were used as standards for determination of mating types.

Media: Nutrient medium (YPD) contained 10 g of yeast extract (Daigo Eiyo Chemicals and Co. Ltd., Japan), 20 g of polypeptone (Daigo Eiyo Chemicals and Co. Ltd.) and 20 g of dextrose per liter of deionized water. Minimal medium contained 0.67% of Difco Yeast Nitrogen Base without amino acids and 2% of dextrose in distilled water. For testing auxotrophic genetic markers, omission tests were made with minimal medium supplemented with appropriate nutrients. Sporuation was performed by replicating cells on an agar medium containing 0.5% of anhydrous potassium acetate and incubation for 2 days at 30° or 25°. All solid media were prepared by addition of 20 g of agar per liter.

Isolation of mutants: A perfect homothallic strain, T-1851-2D, was used as parental strain for mutants. Cells of T-1851-2D grown on YPD agar plate for 2 days at 30° were tarnsferred to sporulation agar medium by replication and incubated at 30° for 2 days. More than 95% of the cells formed 4-spored asci. These asci were harvested and suspended in sterile water. After washing twice with 0.1 M phosphate buffer (pH 7.5), the asci were incubated in the same buffer containing 5 mg per ml of Zymolyase- 5000 (Kirin Brew. Co. Ltd., Japan) at 30° for 6 hr to digest ascus walls and vegetative cells. Spores in the digesting solution were dispersed by slight sonication and collected by centrifugation at 3,000 × g for 10 min. After washing twice with sterilized water containing 0.2% of tween 80, the spores were suspended in 0.1 M phosphate buffer (pH 8.0) to give a concentration of 10^7 spores per ml. To induce mutation, 3 ml of the spore suspension was shaken vigorously with 0.1 ml of ethyl methanesulfonate (EMS; Eastman Kodak Co.) at 30° for 1.5 hr. To quench the reaction of EMS, 0.2 ml of the suspension was transferred to 5 ml of sterilized 5% sodium thiosulfate solution. This EMS treatment caused approximately 80% decrease of spore viability. To obtain 100 to 200 colonies per plate, the spore suspension of sodium thiosulfate was diluted with sterilized water, 0.1 ml portions of the diluted spore suspension were spread on the surface of YPD agar plates and the plates were incubated for 3 days at 35°. Colonies appearing on the plates were subcloned on fresh YPD plates at 35° for preservation, and their mating potency at 35° was tested with the standard haploid strain of **a** (J-1-2B) or *α* mating type (J-1-5D). To isolate temperature-dependent mutants, the colonies that showed mating ability at 35° were subcloned on YPD plates at 25° and tested for mating potency and sporulation ability at 25°. Experiments with temperature-dependent mutants (which showed mating ability at 35° but not at 25°) were carried out at 35°, except for sporulation.

TABLE 2

Strains used

Strain no.	Genotype*	Remarks
T-1851-2D	**a** *HO HMRa HMLα ade1 lys2 his4 leu2 trp1 arg4* / *α HO HMRa HMLα ade1 lys2 his4 leu2 trp1 arg4*	Parental strain for mutant isolation
T-1171-5D	**a** *HO HMRa HMLα lys2 thr4* / *α HO HMRa HMLα lys2 thr4*	Standard homothallic strain
T-792-10D	**a** *ho HMRa HMLα lys2*	Standard heterothallic strain
T-794-23D	*α ho HMRa HMLα his4*	Standard heterothallic strain
J-3-5A	**a** *ho HMRa HMLα lys2 thr4*	Standard heterothallic strain
J-3-8D	*α ho HMRa HMLα lys2 thr4*	Standard heterothallic strain
J-1-2B	**a** *ho HMRa HMLα ura3*	Standard strain of **a** mating type
J-1-5A	*α ho HMRa HMLα ura3*	Standard strain of *α* mating type

* Revised genetic symbols (HABER, personal communication; ARIMA and TAKANO 1979) are employed for the homothallic genes (also see Table 1). Genetic symbols for the auxotrophic traits follow the recommendation of the Nomenclaure Committee for Yeast Genetics (PLISCHKE *et al.* 1976).

Mutations were characterized by the segregation patterns of mating types and homothallism in asci of hybrids between the mutants and the standard strain having the *ho HMR***a** *HML*α or *HO HMR***a** *HML*α genotype.

Genetic methods: Mating types were determined by the prototrophic recovery method, *i.e.*, by the appearance of prototrophic growth on a minimal agar plate after replication from a YPD plate on which the standard haploid strain having either the **a** or α mating type and the complementary auxotrophic genetic markers had been cross-streaked. Tetrad dissection was carried out by the method of JOHNSTON and MORTIMER (1959). Diploid hybrids were obtained by the mass-mating method (LINDEGREN and LINDEGREN 1943) for crosses of mutants with the heterothallic strain and by cell-to-spore mating (TAKANO and OSHIMA 1967) for crosses of mutants with the homothallic strain.

RESULTS

Isolation of mutants from HO HMR**a** HMLα *spores:* To obtain mutants defective in the mating-type conversion in the homothallic strain, ascospores from the perfect homothallic diploid strain (T-1851-2D) were subjected to mutagenesis with EMS. After incubation of the treated spores on YPD plates for three days at 35°, colonies appearing on the plates were tested for their mating ability at 35° by the prototrophic recovery method, as described in MATERIALS AND METHODS. Colonies that showed **a** or α mating potency at 35° were isolated and transferred onto fresh YPD plates in duplicate. One of the duplicates was tested for mating potency at 25° and 35° after incubation at 25° to determine the temperature dependency of the mating potency, and the other was preserved as a stock culture after incubation at 35°. From six independent experiments, 10,050 colonies were randomly selected and were examined for their mating ability (Table 3). Of the 10,050 colonies, 38 showed **a** mating type and 42 showed α mating type at 35°. The frequency of appearance of the mutant clones was 0.8% of total colonies tested. Of the 80 mutants, 11 (four of **a** and seven of α mating type) were temperature dependent, *i.e.*, they showed no mating ability at either 35° or 25° when they were grown on YPD plate at 25°. Eight of the 11 temperature dependent mutants grown at 25° had sporulation ability, but the remaining

TABLE 3

*Mutants isolated after testing 10,050 colonies by cultivation of homothallic spores treated with EMS**

Mating type at: 35°	Mating type at: 25°†	Mutants observed‡	Frequency (%)
a	**a**	34	0.34
α	α	35	0.35
a	nonmater	4	0.04
α	nonmater	7	0.07

* EMS-treated spores of *HO HMR***a** *HML*α genotype were incubated at 35° on YPD plates, and colonies showing **a** or α mating type at 35° were isolated as mutants.

† Mating potency at 25° was tested after incubation on YPD plates at 25° for two days. The mating ability of the clones that were nonmating at 25° did not recover even after incubation at 35° for two days.

‡ Figures indicating total numbers in six independent experiments.

three did not. Only one of the potentially informative temperature-sensitive mutants could be classified, as described below (Table 5). All the isolated mutants carried the same auxotrophic genetic markers as the parental strain, T-1851–2D. Forty-eight of the 80 mutants were randomly selected and were subjected to genetic analysis to characterize the mutations.

Classification of mutants: Since cultivation of a spore of a perfect homothallic strain with *HO HMR***a** *HML*α genotype always gives rise to a nonmating diploid clone through the conversion of mating-type alleles by the function of the homothallic genes (HARASHIMA, NOGI and OSHIMA 1974), the appearance of colonies showing **a** or α mating potency strongly suggests that each contains a mutation defective in the mating-type conversion. Two alternative mechanisms are possible for the inconvertibility of the mating-type alleles: (1) defect of homothallic gene function, and (2) insensitivity of the mating-type allele to the homothallic genes. To test these possibilities, 48 of the 80 mutants were crossed with the standard **a** or α haploid cells having the *ho HMR***a** *HML*α genotype and with spores having the *HO HMR***a** *HML*α genotype, and the resultant diploid hybrids were dissected after sporulation. Six types of segregation can be expected in the diploid strains by assuming six possible mechanisms of mutation (Table 4). One of the six possible mutations, *ho*, gives rise to defectiveness in the *HO* allele. The *csm* (*c*ontrolling *s*witch of *m*ating) mutation gives the same phenotype as an *ho* mutation, but the mutant locus is not linked to the *HO* locus. The *hml*α and *hmr***a** mutations indicate the phenotypic changes of the *HML*α and *HMR***a** alleles, respectively; **a**-*inc* and α-inc indicate mutations at the mating-type locus that have, respectively, an inconvertible **a** or α mating-type allele. By comparing the segregation patterns observed in the two types of hybrids (mutant $\times$ *ho HMR***a** *HML*α and mutant $\times$ *HO HMR***a** *HML*α) with the expected patterns listed in Table 4, the 48 mutants tested could be put into seven classes (Table 5). Three mutants, 2–9, 6–26 and 5–17 (mutant class VII: Table 5), gave quite different segregation patterns from those listed in Table 4. These exceptional mutants were thought to have mutations affecting the expression of mating type, as described later (Tables 7 and 8). Two diploid strains constructed by crossing a wild-type spore of the parental strain, T-1851–2D, with the two standard strains having *ho HMR***a** *HML*α and *HO HMR***a** *HML*α genotypes segregated as expected in Table 4, *i.e.*, they gave a 2 homothallic:2 heterothallic (2 homothallic:2**a**, 2 homothallic:2α or 2 homothallic:1**a**:1α) segregation and 4 homothallic:0 heterothallic segregation in asci, respectively (data not shown).

Mutation of the HO *function:* A mutation defective in the *HO* function can be easily identified among the mutants. Since such a mutant should have the equivalent genotype to the *ho HMR***a** *HML*α, diploid strains prepared by crossing the mutant with a heterothallic strain of the **a** or α *ho HMR***a** *HML*α genotype should show a 2**a**:2α segregation in all asci (type I segregation; Table 4). The same mutant should show a 2 homothallic:2 heterothallic segregation when crossed with a spore of the *HO HMR***a** *HML*α genotype. Ten of the 48 mutants tested showed the type I segregation and were deduced to have the *HO* to *ho* mutation (mutant class I; Table 5). Four of the 10 mutants were **a** mating type and the

TABLE 4

*Expected segregations for mating types and homothallism in asci of two types of hybrids obtained by crossing mutants with the standard heterothallic and homothallic strains**

Segregation type	Supposed mutation	Ascospore	Tetrad segregations in hybrid: mutant × *ho HMRa HMLα* strain						Tetrad segregations in hybrid: mutant × *HO HMRa HMLα* strain					
		A	**a**	**a**	**a**	**a**	**a**	**α**	**a**	**a**	**α**	**a**	**α**	hom†
		B	**a**	**a**	**a**	**α**	**α**	**α**	**a**	**α**	**α**	hom	hom	hom
		C	**α**	**α**	hom	**α**	hom	hom	hom	hom	hom	hom	hom	hom
		D	**α**	hom	hom	hom	hom	hom	hom	hom	hom	hom	hom	hom
I	*HO* to *ho*		36	0	0	0	0	0	6	24	6	0	0	0
II	*CSM*† to *csm*‡		6	12	1	12	4	1	6	24	6	0	0	0
III	*HMLα* to *hmlα*		1	12	6	4	12	1	6	0	0	24	0	6
IV	*HMRa* to *hmra*		1	4	1	12	12	6	0	0	6	0	24	6
V	**a** to **a**-*inc*§		6	24	6	0	0	0	36	0	0	0	0	0
VI	*α* to *α-inc*§		6	0	0	24	0	6	0	0	36	0	0	0
	Wild type		0	0	6	0	24	6	0	0	0	0	0	36

* Expected segregations were calculated by assuming that 36 asci of each hybrid were dissected and that the mutant loci and the mating-type locus segregated independently.

† Indicates homothallism.

‡ *csm* indicates a mutation that inhibits the *HO* gene function (*CSM*+ for wild type).

§ **a**-*inc* and *α-inc* indicate, respectively, **a** and *α* mating-type alleles that are insensitive to the function of the homothallic genes.

TABLE 5

Classification of 48 mutants tested

Mutant class	Mutant allele	Mating type of mutant	Number of mutants	Mutant no.
I	*ho*	**a**	4	3–13, 4–7, 4–25, 4–26
		α	6	2–15, 3–6, 4–8, 4–15, 5–7, 5–23
II	*csm**	**a**	3	4–35, 5–4, 5–31
		α	1	4–27
III	*hml*α	**a**	11	1–11, 1–19, 2–8, 3–2 3–3, 4–3, 4–18, 5–6, 5–9, 5–19, 5–29
IV	*hmr***a**	α	14	1–8, 2–11, 3–1, 3–4, 3–12, 4–4, 5–12, 5–16, 5–20, 5–26, 5–28, 6–9, 6–28, 6–33
V	**a**-*inc**	**a**	4	2–6, 3–5, 3–8, 4–28
VI	α-*inc**	α	2	3–7, 5–22
VII	*mex*†	α	2	2–9‡, 6–26
		a	1	5–17

* See footnote ‡ and § of Table 4.
† *mex* indicates a mutant allele that blocks the expression of mating type.
‡ This mutant showed temperature-dependency for mating behavior.

other was α mating type. Two typical segregation patterns in asci of the two types of diploid strains observed in this class of mutation are listed in Table 6. For the auxotrophic genetic markers, a 2+:2− segregation was observed in asci of all the diploid strains, with few exceptions (data not shown). In the case of one of the mutants, 3–13, having **a** mating type, the diploid J-42 obtained by crossing it with the heterothallic standard (*ho HMR***a** *HML*α) strain showed a 2**a**:2α segregation in all 19 asci tested. Diploid J-68 prepared by crossing it with the homothallic standard (*HO HMR***a** *HML*α) showed a 2 homothallic:2 heterothallic segregation in 22 asci of the 23 asci tested and an exceptional 1 homothallic:1**a**:2α segregation in the remaining ascus. Another mutant, 5–7, having α mating type, showed a true segregation of type I (Table 4) in tetrads from two hybrids, J-165 and J-138.

Four of the 48 mutants tested showed characteristic type II segregation, as listed in Table 4 (Table 5). Three of them (4–35, 5–4 and 5–31) showed **a** mating type, and the other (4–27) showed α mating type at both 35° and 25°. Diploid hybrids obtained by crossing these mutants with the *HO HMR***a** *HML*α strain gave a 2 homothallic:2 heterothallic segregation in all asci as if they had mutated from *HO* to *ho*. However, diploid strains obtained by crosses with the *ho HMR***a** *HML*α strain showed distinctly different segregation patterns from the simple

TABLE 6

*Typical data of segregations for mating types and homothallism in asci of the two types of hybrids obtained by crossing mutants with heterothallic and homothallic strains**

Mutant No.	Mutant Mating type	Hybrid no.	Asci tested	Cross: Mutant × *ho HMR***a** *HML*α strain: **a** **a** **a** **a** **a** α / α α hom hom	**a** **a** **a** α α / α hom hom hom	**a** **a** α α / hom hom hom hom	**a** α / hom hom	α / hom hom	Hybrid no.	Asci tested	Mutant × *HO HMR***a** *HML*α strain: **a** **a** α **a** α hom† / hom hom hom hom hom hom	α / hom hom hom	Mutant allele	Estimated Segregation type†
3–13	**a**	J-42	19	19	0	0	0	0	J-68	23‡	3	14	5	0	0	0	*ho*	I
5–7	α	J-165	13	13	0	0	0	0	J-138	18	2	14	2	0	0	0	*ho*	I
5–4	**a**	J-163	34	7	5	4	13	5	0	J-136	22	5	15	2	0	0	0	*csm*†	II
1–19	**a**	J-14	14	1	6	1	0	6	0	J-53	29	4	0	0	19	0	6	*hml*α	III
3–3	**a**	J-33	24	0	8	7	2	6	1	J-65	14	4	0	0	8	0	2	*hml*α	III
3–4	α	J-34	19	1	2	0	4	7	5	J-62	22	0	0	7	0	14	1	*hmr***a**	IV
3–12	α	J-39	13	0	1	0	6	3	3	J-64	19	0	0	2	0	15	2	*hmr***a**	IV
2–6	**a**	J-22	10	2	7	1	0	0	0	J-56	15	15	0	0	0	0	0	**a**-*inc*†	V
3–7	α	J-37	12	2	0	0	8	0	2	J-63	12	0	0	12	0	0	0	α-*inc*†	VI

* All hybrids obtained by crossing the 48 mutants listed in Table 5 with the two standard strains were dissected; typical data are presented for each mutant class, except mutant class VII.

† See Table 4.

‡ One of the 23 asci showed a 1 homothallic:1**a**:2α segregation.

2**a**:2α segregation observed in similar diploid strains of *HO* to *ho* mutants. For example, diploid J-163 obtained by crossing mutant 5–4 with the *ho HMR***a** *HML*α strain gave five different types of asci, while diploid J-136 obtained by crossing with the *HO HMR***a** *HML*α spore showed a 2 homothallic:2 heterothallic segregation in all asci tested (Table 6). The other three mutants, 5–35, 5–31 and 4–27 (Table 5), showed essentially the same segregation in asci of the two types of diploid strains as did mutant 5–4 (data not shown). Although the frequencies of each ascus type in diploid J-163 differed slightly from those expected for segregation type II (Table 4), these segregations suggest that mutations of this class occur at a locus unlinked to the *HO* locus. We designated this mutation *csm* (controlling switch of mating type: *CSM*$^+$ for wild type). No linkage was observed between the *csm* locus, the mating-type locus or the auxotrophic traits so far tested. The *csm* mutation might be different from the *swi1* mutation described by HABER and GARVIK (1977), since *csm* mutant showed stable **a** or α mating type, while the *swi1* mutant showed bisexual mating reaction. No temperature dependency was observed in the *HO* to *ho* and *csm* mutants (mutant classes I and II; Table 5).

Mutation of the HMLα *and* HMR**a** *gene function:* A defect of the *HML*α gene function in the **a** *HO HMR***a** *HML*α genotype will give rise to a haploid clone of **a** mating type, while the same defect in the α *HO HMR***a** *HML*α genotype will give rise to homothallism. On the other hand, a defect of the *HMR***a** gene function in the α *HO HMR***a** *HML*α genotype will give rise to an α mating-type haploid clone. If such mutations as *HML*α to *hml*α and *HMR***a** to *hmr***a** occur in perfect homothallic spores, type III and type IV of segregations (Table 4) will be expected in crosses of the mutants with the *ho HMR***a** *HML*α and *HO HMR***a** *HML*α standards, respectively. Of the 48 mutants tested, 11 showed type III segregation, and 14 showed type IV segregation. For example (Table 6), diploid hybrids J-14 and J-33, prepared by crossing two mutants, 1–19 and 3–3, with the *ho HMR***a** *HML*α strain, showed similar segregation, which is comparable to type III segregation in the similar diploid hybrid listed in Table 4. When the same mutants (1–19 and 3–3) were crossed with the *HO HMR***a** *HML*α strain, the diploid hybrids (J-53 and J-65) gave three ascus types, 2 homothallic:2**a**, 3 homothallic:1**a** and 4 homothallic:0 heterothallic segregations, as expected from a mutant carrying the *HO HMR***a** *hml*α genotype. On the other hand, diploid strains J-34 and J-39, obtained by crossing two mutants, 3–4 and 3–12, with the *ho HMR***a** *HML*α strain, and the other two diploid strains, J-62 and J-64, obtained by crossing with the *HO HMR***a** *HML*α strain, showed segregation patterns similar to the type IV segregation expected from *HMR***a** to *hmr***a** mutants.

Mutation of the mating-type locus insensitive to the homothallic genes: If a mutation insensitive to the function of the homothallic genes occurs at the mating-type locus in a spore of *HO HMR***a** *HML*α genotype, the spore will give rise to a haploid cell culture having **a** or α mating type. An α mating-type allele that is inconvertible to **a** (α-*inc*) has been found in a strain of *Saccharomyces diastaticus* (TAKANO, KUSUMI and OSHIMA 1973), and it was demonstrated that the inconvertibility is due to the insensitivity of the mating-type allele itself to the action

of the homothallic genes (TAKANO and ARIMA 1979). Six of the 48 mutants tested were thought to be this type of mutation. Two of them (3–7 and 5–22) showed essentially the same behavior as a strain carrying the *α-inc* allele, and the other four (2–5, 3–5, 3–8 and 4–28) were thought to carry an inconvertible **a** mating-type allele (designated **a**-*inc*; Table 5). Typical segregations observed in asci from diploids J-22 and J-56 and from the other type of diploid strains, J-37 and J-63, which were observed by crossing two mutants, 2–6 (**a**-*inc*) and 3–7 (*α-inc*), with the *ho HMR***a** *HMLα* and *HO HMR***a** *HMLα* standards, respectively, are shown in Table 6. These segregation patterns are comparable to type V and type VI segregations in Table 4, respectively. Three other mutants, 3–5, 3–8 and 4–28, having **a** mating type, and another mutant, 5–22, having *α* mating type, showed essentially the same segregation as observed with mutants 2–6 and 3–7, respectively. Thus, it could be concluded that the six mutants are due to a mutation at the **a** or *α* mating-type allele. Temperature dependency for the phenotypes was not observed in these two classes of mutations. A diploid hybrid prepared by the 2–6 (**a**-*inc*) × 3–7 (*α-inc*) cross always showed 2**a**:2*α* segregation on tetrad analysis, and a diploid hybrid between 2–6 (**a**-*inc*) and N-90–16C having the *α-inc HO HMR***a** *HMLα* genotype (TAKANO, KUSUMI and OSHIMA 1973) also showed 2**a**:2*α* segregation in all asci tested (data not shown).

Mutation blocking the expression of mating type: Of the 48 mutants tested, three (2–9, 6–26 and 5–17) showed a novel segregation pattern that was not comparable with any of the tetrad segregation types in Table 4 when they were crossed with the *ho HMR***a** *HMLα* and *HO HMR***a** *HMLα* strains. One mutant (2–9) showed temperature dependency for mating ability, *i.e.*, cells grown at 35° showed the normal mating potency of *α* mating type, while cells grown at 25° showed no mating potency with either the **a** or *α* standard strain and showed self-sporulation ability. The two other mutants, 6–26 and 5–17, showed, respectively, *α* and **a** mating type at both 25° and 35°. A self-sporulating clone that was obtained by cultivation of mutant 2–9 at 25° gave four *α* segregants in all asci tested when the single-spore cultures were incubated at 35°, but showed a 4 homothallic:0 heterothallic segregation in all asci when incubated at 25°. The diploid hybrid obtained by the mutant 2–9 × **a** *HO HMR***a** *HMLα* cross segregated 2 homothallic:2*α* at 35° and 4 homothallic:0 heterothallic at 25° in all asci tested (data not shown). When diploid J-25, obtained by the 2–9 × **a** *ho HMR***a** *HMLα* cross, was subjected to tetrad dissection and the spores were grown at 35°, a complicated segregation pattern was observed in the mating behavior, while a simple segregation (2 homothallic:2 heterothallic) was observed when the spore cultures were subcloned at 25° (Table 7). The segregants showing neither mating nor sporulation ability at 35° are designated Ste in Table 7. These Ste segregants showed **a** mating type when they were subcloned at 25°. Approximately half of the segregants (18 of 42) showing *α* mating type at 35° were changed to diploid cells (homothallism) showing sporulation ability, but no mating potency, when they were subcloned at 25°. After the change from *α* to homothallism, stable diploid colonies resulted, *i.e.*, the mating potency was not restored by temperature shift from 25° to 35° or by subculture at 35°. All segregants show-

TABLE 7

Segregations of mating behavior in asci of a hybrid, J-25, prepared by crossing a temperature-dependent mutant, 2–9, with the ho HMR**a** HMLα *strain*

Mating behavior of segregants in asci at 35°				at 25°				Number of asci	
A	B	C	D	A	B	C	D	Observed	Expected*
α	α	α	α	α	α	Hom	Hom†	1	0.7
α	α	α	Hom	α	α	Hom	Hom	2	2.7
a	α	α	α	**a**	α	Hom	Hom	0	2.7
a	**a**	α	α	**a**	**a**	Hom	Hom	1	0.7
Ste‡	α	α	Hom	**a**	α	Hom	Hom	3	5.3
a	α	α	Hom	**a**	α	Hom	Hom	8	5.3
α	α	Hom	Hom	α	α	Hom	Hom	0	0.7
a	Ste	α	Hom	**a**	**a**	Hom	Hom	1	2.7
Ste	α	Hom	Hom	**a**	α	Hom	Hom	7	2.7
Ste	Ste	Hom	Hom	**a**	**a**	Hom	Hom	1	0.7

* Expected frequency of each ascus type was calculated by supposing that a temperature-dependent mutation inhibiting the expression of **a** mating type at 35° occurred at a gene unlinked to any homothallic locus or to the mating-type locus.
† Hom indicates a segregant that is nonmating but able to sporulate.
‡ Ste indicates a sterile segregant that is nonmating and unable to sporulate.

ing **a** mating ability at 35° remained heterothallic with normal **a** mating type at 25°, *i.e.*, changes from **a** to α and **a** to homothallism were not observed upon a temperature shift from 35° to 25°. All the auxotrophic genetic traits marked heterozygously showed a 2+:2− segregation in each ascus at both temperatures (data not shown). These observations could be explained by supposing that mutant 2–9 had a temperature-sensitive mutation that inhibits the expression of **a** mating type specifically at the restrictive temperature (35°) and that the mutant locus is unlinked to the mating-type locus. That is, a cell carrying the mutation cannot express the **a** mating type at 35°, even if it has the normal **a** mating-type allele, but can express it at 25°. These observations suggest that, in a clone carrying the mutation, both mating-type coversions, α to **a** and **a** to α, can occur normally by the action of the homothallic genes at both temperatures, although the **a** mating type cannot be expressed at 35°. Therefore, the mutant clones generated from spores of **a** or α *HO HMR***a** *HML*α genotype or expected to be a mixed culture of unexpressed **a** and normal α mating-type cells, and the mixed culture should show α mating type at 35°. Since the **a** mating type is expressed at 25°, the sterile **a** cells in the mixed culture should give rise to nonmating diploid clones at the permissive temperature through the formation of zygotes with normal α mating-type cells. In fact, we observed many zygotes in the mutant cultures within a few hours after the temperature was shifted from 35° to 25°. We tentatively designated this mutation *mex1* (expression for mating type), but did not ascertain dominance or recessiveness with respect to the wild-type counterpart.

Two other mutants in mutant class VII (Table 5), 6–26 (α) and 5–17 (**a**), showed similar behavior in the expression of **a** and α mating types, respectively.

However, their mutations were not temperature dependent. A diploid strain obtained by the 6–26 × **a** *ho HMRa HMLα* cross gave similar segregation patterns to diploid J-25 dissected at 35° (Table 7), and a diploid strain obtained by crossing with *HO HMRa HMLα* showed a 2 homothallic:2*α* segregation in each ascus (data not shown). This mutation was also inferred to have a mutation inhibiting the expression of **a** mating type and was tentatively called *mex2*. The *mex2* mutant locus was thought to be different from the *mex1* locus since normal **a** mating-type segregants were recovered in tetrads of a diploid hybrid obtained by an **a** *ho HMRa HMLα mex1* × *α ho HMRa HMLα mex2* cross when the spore-cultures were incubated at 35° (data not shown). Segregations in asci of diploid J-169 obtained by a 5–17 × *α ho HMRa HMLα* cross were complex, as shown in Table 8. However, the complicated distributions were explained by assuming that the mutant carried a mutation that specifically inhibited the expression of *α* mating type and that the mutant locus was unlinked to the mating-type locus. The sterile segregants (designated Ste in Table 8) probably have the *ho HMRa HMLα* genotype for homothallism since, if they had the *HO* allele, they would show **a** mating type (even if the **a** to *α* conversion was affected by the homothallic genes, the *α* mating type could not be expressed). A diploid strain constructed by forced mating between one of the sterile segregants and the **a** *ho HMRa HMLα* strain gave three ascus types: 2**a**:2*α* (2 asci), 2**a**:1*α*:1 sterile (11 asci) and 2**a**:2 sterile (1 ascus). A diploid hybrid obtained by the mutant 5–17 × *α HO HMRa HMLα* cross showed a 2 homothallic:2**a** segregation in all asci tested (data not shown). These observations support the assumption that mutant 5–17 possesses a mutation inhibiting the expression of *α* mating type. This mutation was tentatively called *mex3*, although complementation tests with the *mex1* and *mex2* mutants were not carried out.

TABLE 8

Segregations of mating behavior in asci of a hybrid, I-169, obtained by mutant 5–17 × ho HMRa HML*α cross*

Segregation in asci				Number of asci	
A	B	C	D	Observed	Expected*
a	**a**	**a**	**a**	1	0.7
a	**a**	**a**	*α*	2	2.7
a	**a**	**a**	Hom†	3	2.7
a	**a**	*α*	*α*	0	0.7
a	**a**	Ste†	Hom	6	5.3
a	**a**	*α*	Hom	5	5.3
a	**a**	Hom	Hom	0	0.7
a	*α*	Ste	Hom	2	2.7
a	Ste	Hom	Hom	3	2.7
Ste	Ste	Hom	Hom	2	0.7

* Expected frequency of each ascus type was calculated by assuming that a mutation inhibiting the expression of *α* mating type occurred at a gene unlinked to any homothallic locus or to the mating-type locus.

† See footnotes † and ‡ of Table 7.

Genetic analysis of temperature-dependent mutants: We isolated 11 temperature-dependent mutants (Table 3), one of which was designated *mex1*, as described above. Seven of the remaining 10 temperature-sensitive mutants showed sporulation ability when they were subcloned at 25°. The sporogenous clones obtained from the mutants of **a** mating type gave four **a** segregants in their asci and those from the mutant of α mating type gave four α segregants in their asci when the spore cultures were incubated at 35°, while all the sporogenous clones showed a 4 homothallic:0 heterothallic segregation in asci when the spore-cultures were incubated at 25°. Diploid hybrids obtained at 35° by crossing the temperature-sensitive **a** and α mutants with a spore of *HO HMR***a** *HML*α genotype gave, respectively, 2 homothallic:2**a** and 2 homothallic:2α segregations in asci when single spores were grown at 35°, while both hybrids showed a 4 homothallic:0 heterothallic segregation at 25°. These observations suggest that these mutants might belong to the mutant classes III, IV or VII listed in Table 5 and not to classes I, II, V and VI. These mutants were further analyzed by crosses with the *ho HMR***a** *HML*α standard strain, but they could not be characterized since the observed segregation patterns for mating behaviors in asci were too complicated.

DISCUSSION

It is established that the *HO* allele, which mediates both the α to **a** and **a** to α conversions in combination with the *HMR***a** (or *HML***a**) and *HML*α (or *HMR*α) alleles, is dominant to its counterpart *ho* allele (HOPPER and HALL 1975b; HICKS, STRATHERN and HERSKOWITZ 1977; TAKANO and ARIMA 1979). Therefore, the *HO* allele may be a structural gene for a product having possitive function in the mating-type conversions. If this is true, temperature-sensitive mutants of the *HO* gene should be isolatable. Ten of the 48 mutants tested were due to an *HO* to *ho* mutation, but no temperature-sensitive *HO* mutant has so far been observed in the present study. Temperature dependency of mating behavior was observed in mutant 2–9, in which the expression of **a** mating type was specifically inhibited at 35° (Tables 5 and 7). Genetically, this mutation, designated *mex1*, seems to be a temperature-sensitive sterile mutant, though it is unlike the temperature-sensitive sterile mutants *ste4* and *ste5* described by MACKAY and MANNEY (1974a, b), in that it is strictly specific to the **a** mating type, while *ste4* and *ste5* mutants are not specific. The *mex1* mutation is not linked to the mating-type locus or to any genetic traits so far tested. In the *mex1* mutant (2–9; Table 5), mating-type conversion occurs normally by the action of the homothallic genes even at a restrictive temperature (35°), although the **a** mating-type cell formed from an α cell is sterile at 35°. Since the **a** mating type of this mutant is expressed at permissive temperature (25°) and the **a** cell forms a zygote with an α cell to produce an **a**/α cell, the mutant should be useful for studying the timing or regulation of the switch of mating type during vegetative growth of a homothallic cell. Two other mating-type-specific sterile mutants, tentatively called *mex2* for mutant 6–26 and *mex3* for mutant 5–17, did not show temperature dependency and neither was linked to the mating-type locus.

Mating-type-specific sterile mutations unlinked to the mating-type locus, *ste2* and *ste3*, have been reported by MACKAY and MANNEY (1974a, b). However, we have not tested whether the *mex1*, *mex2* or *mex3* correspond to these mutations.

Three mutants, 4–35, 5–4 and 4–27, were thought to be caused by a new mutation, designated *csm* (controlling switch of mating type; Table 5), which blocks the *HO* gene function, but is not linked to the *HO* locus. Diploid strains obtained by crossing the mutants with the *HO HMR***a** *HML*α strain segregated as if the mutants carried the *ho* allele, but diploid strains obtained by crossing with the *ho HMR***a** *HML*α strain showed a different segregation from a similar diploid with the *HO* to *ho* mutant (Table 6). This finding suggests that a new gene (*CSM*) controlling the *HO* gene function exists in the homothallic system. It has been reported that a mutation unlinked to any homothallic loci, *cmt* (control of mating type), causes mating-type conversion without the function of the *HO* allele (HOPPER and HALL 1975a). Therefore, it is likely that at least three different genes, *HO*, *CSM* and *cmt*, participate in mating-type conversion with the *HMR***a**/*HMR*α and *HML*α/*HML***a** genes. It may be interesting to study mating-type conversion in a mutant carrying both *csm* and *cmt* mutations. Although the data were not shown, results were obtained suggesting that the *csm* mutant might be correlated with the meiotic processes. The mutation may affect excision/recombination of DNA, as observed in radiation-sensitive mutants, *rad51* and *rad52*, which are thought to affect mitotic recombination (MORI and NAKAI 1968; GAME and MORTIMER 1974).

The most interesting mutants isolated in this study were those of *HML*α and *HMR***a** genes, which were observed at higher frequency than other mutants (Table 5). The real frequency might be much higher (theoretically twice) than the observed one, since mutation at the *HML*α gene cannot be detected by cultivation of spores having the α *HO HMR***a** *HML*α, and mutation at the *HMR***a** gene cannot be detected from **a** *HO HMR***a** *HML*α spores (those spores should show homothallism). For these two classes of mutants (mutant classes III and IV; Table 5), two alternative possibilities can be envisaged. The first possibility is that the *HML*α allele is switched to the *HML***a** allele for mutant class III and that the *HMR***a** allele is switched to the *HMR*α allele for mutant class IV, *i.e.*, the original allele of a given *HML* or *HMR* locus is mutated to the other functional allele. The second possibility is a defective mutation at the *HML*α locus for mutant class III and at the *HML***a** locus for mutant class IV, *i.e.*, the functional allele is mutated to a nonfunctional allele. These possibilities cannot be distinguished by the segregation patterns observed in dissection of hybrids between the mutant and the *ho HMR***a** *HML*α or *HO HMR***a** *HML*α standard strain (Table 4), since both types of mutation at the *HML*α or the *HMR***a** gene give the same segregation in these hybrids. The two types of mutations in mutant classes III and IV (Table 5) are discussed in the following paper.

Six mutants in classes V and VI (designated **a**-*inc* and α-*inc*; Table 5) are thought to be caused by a genetic event at the mating-type locus. Inconvertibility of an α mating-type (α-*inc*) allele has been observed in a strain of *S. diastaticus* (TAKANO, KUSUMI and OSHIMA 1973) and an inconvertible **a** mating-

type allele, which might correspond to the **a**-*inc*, was suggested by HABER (personal communication). The mechanism of the inconvertibility of the mating-type allele of the mutants isolated in this study was not tested, though it has been demonstrated that an *α-inc* allele found in *S. diastaticus* is able to turn on the homothallic genes, but is insensitive to the function of the homothallic genes (TAKANO and ARIMA 1979). According to the controlling element model (OSHIMA and TAKANO 1971; HARASHIMA, NOGI and OSHIMA 1974) or the cassette model (HICKS and HERSKOWITZ 1977), these mutants might be caused by alteration of the conformation of the element inserted into the mating-type locus, which decreases its sensitivity to the switching action of the homothallic genes.

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