EVIDENCE FOR CO-DOMINANCE OF THE HOMOTHALLIC GENES, *HMα/hmα* AND *HM***a**/*hm***a**, *IN SACCHAROMYCES YEASTS*

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Manuscript received February 5, 1979

ABSTRACT

To investigate the dominance and recessiveness of the homothallism genes, *HMα/hmα* and *HM***a**/*hm***a**, for mating-type conversion, we constructed hybrids with various configurations of the homothallic genes by fusion of protoplasts prepared from haploid strains having identical mating types. Eight different combinations of the homothallic genes were tested for their function by observing the mating and sporulation abilities of the fusion products. With few exceptions, nonmating and sporogenous fusion products were obtained from the following combinations: *α HO hmα HM***a** + *α ho hmα hm***a**, *α HO hmα HM***a** + *α ho HMα hm***a**, *α HO hmα HM***a** + *α ho HMα HM***a**, **a** *HO HMα hm***a** + **a** *ho hmα hm***a**, **a** *HO HMα hm***a** + **a** *ho hmα HM***a** and **a** *HO HMα hm***a** + **a** *ho HMα HM***a**. All the fusion products from the *α HO hmα HM***a** + *α ho hmα HM***a** and **a** *HO HMα hm***a** + **a** *ho HMα hm***a** combinations showed mating types identical to those of the respective haploid strains. These results clearly support the co-dominance of the *HMα/hmα* and *HM***a**/*hm***a** alleles and indicate that the *hmα* allele has the same function as the *HM***a** allele and that the *hm***a** allele has the same function as the *HMα* allele.

MATING-TYPE specificity in *Saccharomyces cerevisiae* and related species is controlled by a pair of alleles, **a** (*MAT***a**) and *α* (*MATα*), on chromosome III. In a heterothallic strain, the mating types are stable, although rare changes occur. In a homothallic strain, on the other hand, the two mating types are mutually interchangeable by the function of the homothallic genes, *HO*, *HMα*, and *HM***a** (HARASHIMA, NOGI and OSHIMA 1974). In general, the function of the *HO* gene is required for both *α* to **a** and **a** to *α* conversions in the homothallic system, although such mutations as *cmt* (change of mating type; HOPPER and HALL 1975a) are able to change mating types without the function of the *HO* allele. It has been demonstrated that specific conversions, *α* to **a** and **a** to *α*, are caused, respectively, by the *HMα* and *HM***a** genes in combination with the *HO* allele (HARASHIMA, NOGI and OSHIMA 1974) and that the *HO* allele is dominant to its counterpart, *ho* (HOPPER and HALL 1975b; HICKS and HERSKOWITZ 1977). The question has been posed whether the alternate alleles of *HMα* and *HM***a**, *i.e.*, *hmα* and *hm***a**, participate in the conversion. NAUMOV and TOLSTORUKOV (1973) have suggested that the *hmα* allele has the same function as *HM***a**, and that the *hm***a** allele has the same function as *HMα*. This idea is supported by the fact

that the *HO hmα hm***a** genotype shows a perfect homothallic life cycle, as does the *HO HMα HM***a** genotype (HARASHIMA, NOGI and OSHIMA 1974), but the evidence is not conclusive. KLAR and FOGEL (1977) found that the α/α cells generated from a diploid strain of the **a**/α *HO/HO hmα/hmα hm***a**/*HM***a** genotype by reciprocal mitotic recombination were switched to **a**/α or **a**/**a** and suggested the equivalence of the *hm***a** and *HMα* alleles. Their result was not conclusive without knowing the result in a diploid of *hmα/HMα hm***a**/*hm***a** genotype. HARASHIMA and OSHIMA (1978) proposed the co-dominance of the *HMα*/*hmα* and *HM***a**/*hm***a** genes from a statistical analysis of segregation patterns of mating types and homothallism in meiotic segregants from tetraploids having various configurations of the homothallic genes. However, no direct evidence of the co-dominance of the homothallic genes, *HMα/hmα* and *HM***a**/*hm***a**, has been presented.

The function of the homothallic genes is blocked when the heterozygosity of the mating-type alleles is established (TAKANO and OSHIMA 1970; HICKS, STRATHERN and HERSKOWITZ 1977). Therefore, it is necessary to construct diploid cells homozygous for the mating-type alleles to investigate the dominance and functional relations among the homothallic genes. Such a diploid clone can be obtained in meiotic segregants from an appropriately marked tetraploid cell or from an **a**/α diploid cell by mitotic recombination at a region between the mating-type locus and the centromere. However, those procedures are not easy, since (1) methods for the estimation of theoretical frequencies of phenotypes are not yet established in the segregants from the tetraploid, and (2) the *HMα* and *HM***a** loci, located on chromosome *III*, are linked to the mating-type locus (HARASHIMA and OSHIMA 1976). This difficulty can be overcome by using the technique of protoplast fusion, since fusion of two protoplasts prepared from cells having an identical mating-type allele causes the fusion of nuclei and gives rise to a diploid nucleus in Saccharomyces yeasts (GUNGE and TAMARU 1978; TAKANO and ARIMA 1979).

In this study, we investigated the relations between the mating-type conversion and the configuration of the homothallic genes by constructing diploid cells homozygous for mating type by means of protoplast fusion. The results demonstrated that the alternate alleles of *HMα* and *HM***a**, i.e., *hmα* and *hm***a**, are both co-dominant. The *hmα* allele has the equivalent function of *HM***a**, and the *hm***a** allele has the equivalent function of *HMα*.

MATERIALS AND METHODS

Yeast strains: Strains used are listed in Table 1. Two heterothallic diploid strains, T-1071-8-U1 and T-1071-8-U2, which were derived from a heterothallic diploid strain, T-1071, (**a**/α *ho/ho HMα/HMα hm***a**/*hm***a** *ade1*/+ *lys2/lys2 his4*/+ *leu2*/+ *thr4*/+ *trp1*/+ *ura3*/+ *arg4*/+) by mitotic recombination (TAKANO *et al.* 1977), were selected as the α/α and **a**/**a** standards, respectively. Terminology for genetic symbols follows the recommendations of the Nomenclature Committee for Yeast Genetics (PLISCHKE *et al.* 1976) except for the mating types and the homothallic genes.

Media: The nutrient (YPD) medium contained 20 g of glucose, 20 g of polypeptone (Daigo Eiyo and Co. Ltd., Japan) and 10 g of yeast extract (Daigo Eiyo and Co. Ltd.)

TABLE 1

List of strains used

Strain no.	Mating type	Homothallic gene	Genetic markers	Source and Reference
C-437-10B-1B	α	*HO hmα HM***a**	*leu2 thr4*	Y. OSHIMA
DR-129-1A	α	*ho hmα hm***a**	*his4 arg4*	Y. OSHIMA
S-14-9C-1A	α	*HO hmα HM***a**	*lys2 his4 leu2*	Y. OSHIMA
T-1068-13B	α	*ho HMα hm***a**	*ade1 lys2 thr4 trp1 ura3*	This laboratory
T-1087-3C	α	*ho HMα HM***a**	*ade1 lys2 trp1 arg4*	This laboratory
C-328-2D	α	*ho hmα HM***a**	*arg4 lys7*	Y. OSHIMA
DR-129-3A	**a**	*ho hmα hm***a**	*his4*	Y. OSHIMA
T-1049-32B	**a**	*HO HMα hm***a**	*ade1 lys2 trp1 ura3*	This laboratory
C-16-53D	**a**	*ho hmα HM***a**	*lys2 his4 leu2 ura3*	Y. OSHIMA
T-1059-18C	**a**	*HO HMα hm***a**	*lys2 thr4 trp1 ura3*	This laboratory
T-1058-3C	**a**	*HO HMα hm***a**	*lys2 his4 leu2 ura3 arg4*	This laboratory
T-1087-19C	**a**	*ho HMα HM***a**	*lys2 thr4 trp1 arg4*	This laboratory
T-1074-38D	**a**	*ho HMα hm***a**	*lys2 his4 leu2 trp1 met2*	This laboratory
T-1071-8-U1	α / α	*ho HMα hm***a** / *ho HMα hm***a**	*ade1 lys2 his4 leu2 thr4 trp1 ura3 arg4* / + *lys2* + + *thr4* + + +	TAKANO *et al.* (1977)
T-1071-8-U2	**a** / **a**	*ho HMα hm***a** / *ho HMα hm***a**	*ade1 lys2 his4 leu2 thr4 trp1 ura3 arg4* / + *lys2* + + + + + +	TAKANO *et al.* (1977)

per liter of tap water. Minimal medium consisted of 6.7 g of Difco Yeast Nitrogen base without amino acids and 20 g of glucose per liter of deionized water. For testing auxotrophic genetic markers, minimal agar medium was supplemented with appropriate amounts of amino acids and/or nucleic-acid bases. For regeneration of protoplasts to vegetative cells and isolation of fusion products between two different haploid protoplasts, minimal agar medium containing 0.6 M KCl (MMK) was used with or without addition of appropriate nutrients. MMK medium supplemented with appropriate amounts of all the nutrients required for the growth of both protoplasts (MMAK) was used in examining the regeneration frequency of the protoplasts. Amino acids and nucleic acid bases for nutrients were purchased from Wako Pure Chemical Industries Ltd., Japan. Sporulation medium contained 5 g of anhydrous potassium acetate and 20 g of agar per liter of tap water.

Protoplast formation: For preparation of protoplasts, the procedure described by KANEKO, KITAMURA and YAMAMOTO (1973) was adopted. Cells grown in YPD medium for 17 hr at 30° with shaking were washed twice with sterilized water and suspended in 0.067 M phosphate buffer (pH 7.5) containing 10 mM 2-mercaptoethanol, 0.6 M KCl and three units of Zymolyase-5000 (Kirin Brew. Co. Ltd., Japan) per ml of the reaction mixture. The concentration of cells was adjusted to approximately 2×10^7 cells per ml. The suspension was gently shaken for four hr at 30° to complete conversion of cells to protoplasts. The protoplasts were collected by centrifugation at 1,500 × g for ten min, washed with and suspended in a 0.6 M KCl solution to give final concentration of approximately 2×10^7 protoplasts per ml.

Protoplast fusion: For fusion of protoplasts, the procedure of SIPICZKI and FERENCZY (1977) was employed with a minor modification. One ml portions of each protoplast suspension prepared from two different strains were mixed and centrifuged at 1,500 × g for ten min. Supernatant was discarded and the pellet was immediately suspended in 2 ml of 30% polyethylene glycol-6000 (Wako Pure Chem. Indust. Ltd.) solution containing 50 mM $CaCl_2$. The suspension was incubated for 15 min at 20°, then 0.1 ml portions were taken, appropriately diluted with 0.6 M KCl, and spread on selective MMK agar plates supplemented or not with appropriate nutrients and on MMAK plates. The plates were covered with 10 ml of the same media and were incubated for a week at 30°. Colonies appearing on the selective plates were transferred onto YPD agar as fusion products between the two haploid strains. The frequency of fusion of protoplasts was calculated by comparing the number of colonies appearing on the selective plates with that on the MMAK plates.

Genetic methods: The procedures for determination of mating types, sporulation and tetrad dissection have been described in a previous paper (TAKANO *et al.* 1977). Hybrids between fusion products showing the mating potency and the **a**/**a** or α/α standard strain were obtained by single-cell isolation by micromanipulation from mass mating culture.

RESULTS

Mating and sporulation abilities of fusion products: To investigate the relations between the mating-type conversion and the genotypes of homothallism genes, we constructed eight different classes of fusion products by using protoplasts prepared from different haploid strains having identical mating-type alleles but different genotypes for the homothallic genes (Table 2). Fusion products between two different strains were detected on selective media with the aid of the complementary auxotrophic genetic markers. Frequency of protoplast fusion between two different strains varied from 10^{-4} to 10^{-3} per regenerated protoplasts depending upon the combination of strains. No prototrophic colonies appeared on cultivation of approximately 1×10^7 protoplasts of each strain independently on the same plates used for the selection of the fusion

TABLE 2

Mating ability of the fusion products obtained from eight different combinations

No.	Combination Strain	Genotype	Number of fusion products Nonmater	**a**	*α*
1	C-437-10B-1B	*α HO hmα HM***a**	100	0	0
		+			
	DR-129-1A	*α ho hmα hm***a**			
2	S-14-9C-1A	*α HO hmα HM***a**	100	0	0
		+			
	T-1068-13B	*α ho HMα hm***a**			
3	S-14-9C-1A	*α HO hmα HM***a**	100	0	0
		+			
	T-1087-3C	*α ho HMα HM***a**			
4	S-14-9C-1A	*α HO hmα HM***a**	0	0	100
		+			
	C-328-2D	*α ho hmα HM***a**			
5	T-1049-32B	**a** *HO HMα hm***a**	94	3 + 3*	0
		+			
	DR-129-3A	**a** *ho hmα hm***a**			
6	T-1059-18C	**a** *HO HMα hm***a**	99	0	1*
		+			
	C-16-53D	**a** *ho hmα HM***a**			
7	T-1058-3C	**a** *HO HMα hm***a**	100	0	0
		+			
	T-1087-19C	**a** *ho HMα HM***a**			
8	T-1049-32B	**a** *HO HMα hm***a**	0	100	0
		+			
	T-1074-38D	**a** *ho HMα hm***a**			

*These isolates showed **a** or *α* mating potency and sporulation ability.

products. One hundred prototrophic colonies were randomly isolated for each combination and their mating and sporulation abilities were tested (Table 2).

All the isolates of the *α HO hmα HM***a** + *α ho hmα hm***a**, *α HO hmα HM***a** + *α ho HMα hm***a**, *α HO hmα HM***a** + *α ho HMα HM***a** and **a** *HO HMα hm***a** + **a** *ho HMα HM***a** combinations (combinations 1, 2, 3 and 7, respectively) showed good sporulation and no mating potency with either the **a** or *α* standard. Among the isolates of the **a** *HO HMα hm***a** + **a** *ho hmα hm***a** and **a** *HO HMα hm***a** + **a** *ho hmα HM***a** combinations (combinations 5 and 6), the great majority (94 of 100 isolates of combination 5 and 99 of 100 of combination 6) showed no mating potency and sporulation ability. All the fusion products of the *α HO hmα HM***a** + *α ho hmα HM***a** combination (combination 4) showed *α* mating type and no sporulation ability, and all of the **a** *HO HMα hm***a** + **a** *ho HMα hm***a** combination (combination 8) showed **a** mating type and no sporulation ability. Of com-

bination 5, six isolates showed **a** mating type and three of them had both the mating potency and sporulation ability. One isolate of combination 6 showed both α mating type and sporulation ability.

The appearance of the nonmating and sporogenous fusion products of combinations 1, 2, 3, 5, 6 and 7 conflicts with the finding of VAN SOLINGEN and VAN DER PLAAT (1977) and GUNGE and TAMARU (1978) that fusion products showed stable mating potency of the mating type identical with that of the original haploid strains. Our observations strongly suggest that one or both of the mating-type alleles were converted to the opposite alleles in the fusion products by the function of the homothallic genes in all combinations except 4 and 8. In the fusion products in which mating-type conversion occurred, cells heterozygous for the mating-type alleles would be produced during regeneration or subsequent vegetative growth of the fused protoplasts. Since each combination has the *HO/ho* configuration, the observations clearly support the dominance of the *HO* allele over the *ho* allele in the fusion product of two different protoplasts. The appearance of nonmating fusion products of combinations 2, 3, 6 and 7 can be explained by the idea that if diploid cells homozygous for mating-type alleles carried at least one pair of *HM*α and *HM***a** alleles, both α to **a** and **a** to α conversions would be possible. Although 6 of the 100 isolates of combination 5 showed **a** mating type, the fact that the combinations of α *HO hm*α *HM***a** + α *ho hm*α *hm***a** and **a** *HO HM*α *hm***a** + **a** *ho hm*α *hm***a** (combinations 1 and 5) gave rise to nonmater products indicates that the *hm*α*/hm*α *HM***a***/hm***a** configuration is effective for the α to **a** conversion and the *HM*α*/hm*α *hm***a***/hm***a** configuration for the **a** to α conversion. These observations clearly support the idea that the *hm***a** allele has the same function as the *HM*α allele and that the *hm*α allele has the same function as the *HM***a** allele, *i.e.*, the alternate alleles of *HM*α and *HM***a**, *hm*α and *hm***a**, respectively, are both co-dominant. The observation that all the fusion products of combinations 4 and 8 showed, respectively, α and **a** mating type, together with no sporulation ability, clearly indicates that the *hm*α*/hm*α *HM***a***/HM***a** genotype is not effective for the α to **a** conversion and that the *HM*α*/HM*α *hm***a***/hm***a** genotype is not effective for the **a** to α conversion.

Genetic analysis of nonmating and sporogenous fusion products: To verify the above arguments and confirm the configuration of the auxotrophic genetic markers in the fusion products, two isolates from each of the six combinations (combinations 1, 2, 3, 5, 6 and 7) showing sporulation ability and no mating potency were randomly selected and subjected to tetrad dissection after sporulation. The auxotrophic markers expected to be marked heterozygously were normally segregated (2+:2−) in tetrads of all 12 nonmater isolates selected (data not shown). This result strongly suggests that these isolates are diploids. Tetrad segregations of mating types and homothallism in asci of the isolates are presented in Table 3. Both **a** and α segregants were observed in asci of all the isolates dissected, although sample sizes were small and the segregation patterns slightly differed even in the same combinations of the homothallic genes. However, the segregation patterns are compatible with the idea that these fusion

TABLE 3

Segregations of mating types and homothallism in asci of nonmating sporogenous fusion products

Combination no.*	Expected genotype for homothallic genes	Fusion product†	Segregation in asci (homothallic : **a** : α) 2:2:0	2:0:2	2:1:1	1:2:1	1:1:2	0:2:2
1	$\frac{HO}{ho}\ \frac{hm\alpha}{hm\alpha}\ \frac{HM\mathbf{a}}{hm\mathbf{a}}$	A	0	2	5	2	0	0
		B	0	1	2	0	0	3
2	$\frac{HO}{ho}\ \frac{hm\alpha}{HM\alpha}\ \frac{HM\mathbf{a}}{hm\mathbf{a}}$	A	1	1	5	2	3	2
		B	0	0	3	2	5	1
3	$\frac{HO}{ho}\ \frac{hm\alpha}{HM\alpha}\ \frac{HM\mathbf{a}}{HM\mathbf{a}}$	A	0	0	2	1	5	2
		B	1	1	3	2	1	0
5	$\frac{HO}{ho}\ \frac{HM\alpha}{hm\alpha}\ \frac{hm\mathbf{a}}{hm\mathbf{a}}$	A	1	0	1	3	0	1
		B	0	0	1	4	1	2
6	$\frac{HO}{ho}\ \frac{HM\alpha}{hm\alpha}\ \frac{hm\mathbf{a}}{HM\mathbf{a}}$	A	0	0	2	1	1	1
		B	0	0	0	2	2	3
7	$\frac{HO}{ho}\ \frac{HM\alpha}{HM\alpha}\ \frac{hm\mathbf{a}}{HM\mathbf{a}}$	A	3	0	7	2	0	0
		B	0	0	6	3	0	2

*See Table 2.

†Two fusion products, A and B, were selected randomly from each combination and dissected after sporulation.

products carried the expected genotypes for the homothallic genes, as presented in Table 3, and the **a**/α configuration for the mating-type locus. These observations clearly indicate that the conversion of α/α or **a**/**a** to **a**/α occurred in the diploid fusion products by the function of the homothallic genes.

Most of the fusion products showing no mating potency were of diploid cell size, but some of them were larger (cell sizes were calculated by measuring along the long and short axes of cells grown in YPD medium). The fusion products with larger cell sizes might be produced by tetraploidization through the

α/α to **a**/**a** or **a**/**a** to α/α conversion by the action of the homothallic genes, as observed by TAKANO *et al.* (1977) and KLAR and FOGEL (1977), or by the fusion of three or more protoplasts, as suggested from the other experiments in which multiple fusion of protoplasts was verified (data will be described elsewhere). Thus, the appearance of nonmating fusion products having larger cell size does not negate the conclusion drawn from the presented study.

Genetic analysis of fusion products having mating potency: To test the configuration of the auxotrophic markers of the fusion products of the α *HO hm*α *HM***a** + α *ho hm*α *HM***a** combination (combination 4; Table 2) and the **a** *HO HM*α *hm***a** + **a** *ho HM*α *hm***a** combination (combination 8; Table 2), we selected two fusion products of each of these combinations. All the fusion products of these combinations showed α (combination 4) and **a** (combination 8) mating potency in the 100 isolates of each so far tested. The four selected isolates were crossed with the appropriate standard diploid strain homozygous for mating-type allele, T-1071-8-U2 (**a**/**a**) or T-1071-8-U1 (α/α), and the hybrids were subjected to tetrad dissection (Table 4). Segregation patterns of the auxotrophic genetic markers in asci of the hybrids were closely similar to those from tetraploid strains (ROMAN, PHILLIPS and SANDS 1955), and the genetic markers for the simplex (+/−/−/−) configuration, *i.e.*, *lys2* for hybrid I and II and *trp1* for hybrid III and IV, showed 2+:2− segregations, with few exceptions. These observations suggest that the mater fusion products are diploid, originated from fusion of a single pair of protoplasts of two different haploid strains and retain the original mating potency of the parental haploid strains. This further suggests that the *HO/ho hm*α*/hm*α *HM***a***/HM***a** genotype is not effective for α/α to **a**/α (or to **a**/**a**) conversion and that *HO/ho HM*α*/HM*α *hm***a***/hm***a** is not effective for **a**/**a** to α/α (or to α/α) conversion. This suggestion is in accord with the expected gene function in a haploid cell (HARASHIMA, NOGI and OSHIMA 1974).

Six of the 100 isolates of combination 5 showed **a** mating type, and three of them showed sporulation ability (Table 2). The three products having both the mating and sporulation abilities might be attributable to a mixed population of **a**/**a** (which might contribute to mating potency, but not to sporulation) and **a**/α cells (which might contribute to sporulation, but not to mating potency). In fact, eight of the 12 single-cell isolates from one of the fusion products gave rise to colonies showing **a** mating type and sporulation ability, and the remaining four gave rise to nonmaters capable of sporulation. The mixed population might arise through the delay of the **a** to α conversion. The appearance of the other three **a** mating-type products of combination 5 that were incapable of sporulation might be due to inhibition of the **a** to α conversion. Tetrad analysis of hybrids between these three **a** mating-type products and the α/α diploid strain (T-1071-8-U1) suggested that the products are diploids homozygous for the **a** mating-type allele, although the exact genotype for the homothallic genes could not be predicted since the phenotypic segregations of mating types were complicated (data not shown). One exceptional fusion product of the **a** *HO HM*α *hm***a** + **a** *ho hm*α *HM***a** combination (combination 6; Table 2), which showed the α

TABLE 4

Segregations of auxotrophic genetic markers in asci of hybrids between fusion products showing mating ability and the standard diploid strains homozygous for mating types*

Genetic marker	Isolate from combination 4 (α) × T-1071-8-U2 (**a**/**a**)								Isolate from combination 8 (**a**) × T-1071-8-U1 (α/α)							
	Hybrid I				Hybrid II				Hybrid III				Hybrid IV			
	4:0†	3:1	2:2	1:3	4:0	3:1	2:2	1:3	4:0	3:1	2:2	1:3	4:0	3:1	2:2	1:3
Ade	13	0	0	0	9	0	0	0	8	1	3	0	8	3	5	0
Lys	0	0	13	0	0	0	7	2	all asci showed 0+ : 4—							
His	5	8	0	0	3	4	2	0	3	5	4	0	8	7	1	0
Leu	6	4	3	0	4	2	3	0	5	2	5	0	11	1	4	0
Thr	13	0	0	0	9	0	0	0	10	2	0	0	12	4	0	0
Trp	12	1	0	0	8	1	0	0	0	0	11	1	0	0	16	0
Ura	12	1	0	0	9	0	0	0	9	2	1	0	6	6	4	0
Arg	9	4	0	0	6	3	0	0	12	0	0	0	16	0	0	0
Met	13	0	0	0	9	0	0	0	12	0	0	0	16	0	0	0

*Hybrids were obtained by mass mating between a fusion product of combination 4 or 8 (Table 2) and the respective standard diploid strain, T-1071-8-U2 (**a**/**a**) or T-1071-8-U1 (α/α). If the mater products were produced by fusion of a single pair of two different protoplasts, from the genotypes presented in Table 1, hybrids I and II would be expected to have the triplex (+/+/+/—) configuration for *ade1*, *thr4*, *trp1*, *ura3* and *lys7* alleles, the duplex (+/+/—/—) configuration for *his4*, *leu2* and *arg4* alleles, and the simplex (+/—/—/—) configuration for a *lys2* allele. Hybrids III and IV would be expected to have the triplex configuration for *arg4* and *met2* alleles, the duplex configuration for *ade1*, *his4*, *leu2*, *thr4* and *ura3* alleles, and the simplex configuration for a *trp1* allele.

†The ratio indicates + : — segregation of auxotrophic markers in asci.

mating type and sporulation ability, gave less than two viable colonies from asci and all the viable colonies showed α mating type (data not shown). These observations suggest that missing or aberrant pairing of chromosome III and of the other chromosomes might happen infrequently during the regeneration or subsequent vegetative growth of the fused protoplasts, as observed in higher plants (MALIGA *et al.* 1978). The event may give unexpected phenotypes to the fusion products. However, the exact mechanism is not known.

DISCUSSION

Using the technique of protoplast fusion, we observed that in the presence of the *HO* allele the *HM*α/*hm*α *hm***a**/*hm***a** genotype is effective for the **a** to α conversion and the *hm*α/*hm*α *HM***a**/*hm***a** genotype is effective for the α to **a** conversion. Since the *HM*α/*HM*α *hm***a**/*hm***a** genotype is not effective for the **a** to α conversion and the *hm*α/*hm*α *HM***a**/*HM***a** genotype is not effective for the α to **a** conversion, these observations demonstrate that the *hm*α allele has a positive function in the **a** to α conversion in combination with the *HO* allele, as does the *HM***a** allele, and that the *hm***a** allele has a positive function in the α to **a** conversion, as does the *HM*α allele. These conclusions support the suggestion of

NAUMOV and TOLSTORUKOV (1973) that two different loci, *HTH1* and *HTH2*, which would correspond to the two *HM* genes (*HMα* and *HM***a**), have the same efficiency for the mating-type conversion, and also support the equivalence of function of the *HMα* and *hm***a** loci described by KLAR and FOGEL (1977). The same results were obtained by the statistical analysis of segregation patterns of mating types and homothallism in tetraploid cells having various combinations of the homothallic genes (HARASHIMA and OSHIMA 1978). Thus, we can conclude that the *HMα* and *hm***a** alleles and the *HM***a** and *hmα* alleles have, respectively, the same functions in the mating-type conversion. In other words, this conclusion indicates that two different loci of the homothallic genes mutually have the same function and each alternate allele of the two loci has the opposite function. This argument is compatible with the fact that the alternate alleles, **a** and *α*, at the mating-type locus are co-dominant and have the opposite mating functions.

For the molecular mechanism for the mating-type differentiation, HARASHIMA, NOGI and OSHIMA (1974) proposed a revision of the controlling-element model postulated by OSHIMA and TAKANO (1971). According to the model, *HMα*, *HM***a** and their alternate alleles, *hmα* and *hm***a**, produce specific controlling elements analogous to the specific transposable genic controllers in maize (MCCLINTOCK 1956) and the controlling elements specifically associate with the mating-type locus. The association and elimination of the elements are controlled by the *HO* gene and cause the differentiation of the **a** and *α* mating-type alleles. Recently, HICKS and HERSKOWITZ (1977) proposed the cassette model, which seems similar to the controlling-element model. According to the cassette model, *HMα* (and *hm***a**) and *HM***a** (and *hmα*) alleles produce a silent copy (cassette; controlling element) and having **a** and *α* functions, respectively, and recombination or insertion of the copy at the mating-type locus mediated by the *HO* gene causes the mating-type conversion. Our result on the co-dominance and equivalence of function of the homothallic genes can be explained by either the controlling element model or the cassette model, *i.e.*, the *HMα* and *hm***a** alleles and the *HM***a** and *hmα* alleles would, respectively, produce essentially the same controlling elements or cassettes.

Since the co-dominance and equivalence of function of the homothallic genes have been proved in this study and the other workers (KLAR and FOGEL 1977; HARASHIMA and OSHIMA 1978), the nomenclature of the *HMα* and *HM***a** genes should be revised. This problem was informally discussed by several yeast geneticists at the 9th International Conference on Yeast Genetics and Molecular Biology, held at University of Rochester in 1978. It has been indicated that the *HMα* is mapped on the right arm and the *HM***a** on the left arm of chromosome *III* (HARASHIMA and OSHIMA 1976) and it was argued that the *HMα* and *hm***a** alleles and the *HM***a** and *hmα* alleles have the concealed ability to give **a** and *α* mating types, respectively, as described above. With these facts, it was proposed that *HMα* and *hm***a** be revised to *HMR***a** (homothallic locus on right arm, **a** ability) and *HML***a** (homothallic locus on left arm, **a** ability), respectively. Similarly, *HM***a** and *hmα* would be designated *HMLα* (homothallic locus on left

arm, α ability) and *HMR*α (homothallic locus on right arm, α ability), respectively. For example, the *HM*α/*hm*α *HM***a**/*hm***a** genotype in the previous nomenclature would be read *HMR***a**/*HMR*α *HML*α/*HML***a**. The nomenclature of *HO*/*ho* is effective as it is (dominant allele: *HO*, recessive allele: *ho*).

We thank SHIRO SENOH, Director of the Central Research Institute of Suntory Ltd., for permission to publish this paper. We especially thank YASUJI OSHIMA for discussions during the preparation of the manuscript and for strains, and KUMIKO MATSUBARA for her excellent technical assistance.

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Corresponding editor: F. SHERMAN