THE GENETIC SYSTEM CONTROLLING HOMOTHALLISM IN SACCHAROMYCES YEASTS[1]

SATOSHI HARASHIMA, YASUHISA NOGI AND YASUJI OSHIMA

Department of Fermentation Technology, Osaka University, Yamadakami, Suita-shi, Osaka 565, Japan

Manuscript received December 28, 1973
Revised copy received March 28, 1974

ABSTRACT

There are four types of life cycles in *Saccharomyces cerevisiae* and its related species. A perfect homothallic life cycle (the Ho type) is observed in the classic D strain. Two other types show semi-homothallism; one of them shows a 2-homothallic diploid:2α heterothallic haploid segregation (the Hp type) and another, a 2-homothallic:2a segregation (the Hq type). In the segregants from these Ho, Hp, and Hq diploids, each homothallic segregant shows the same segregation pattern as its parental diploid. The fourth type has a heterothallic life cycle showing a 2a:2α segregation and the diploids are produced by the fusion of two haploid cells of opposite mating types. The diploids prepared by the crosses of α Hp (an α haploid segregant from the Hp diploid) to a Hq (an a haploid from the Hq diploid) segregated two types (Type I and II) of the Ho type homothallic clone among their meiotic segregants. Genetic analyses were performed to investigate this phenomenon and the genotypes of the Ho type homothallic clones of Type I and Type II. Results of these genetic analyses have been most adequately explained by postulating three kinds of homothallic genes, each consisting of a single pair of alleles, *HO/ho*, *HM*α*/hm*α, and *HMa/hma*, respectively. One of them, the *HM*α locus, was proved to be loosely linked (64 stranes) to the mating-type locus. A spore having the *HO hm*α *hma* genotype gives rise to an Ho type homothallic diploid (Type I), the same as in the case of the D strain which has the *HO HM*α *HMa* genotype (Type II). A spore having the a *HO hm*α *HMa* or α *HO HM*α *hma* genotype will produce an Hp or Hq type homothallic diploid culture, respectively. The other genotypes, a *HO HM*α *hma*, α *HO hm*α *HMa*, and the genotypes combined with the *ho* allele give a heterothallic character to the spore culture. A possible molecular hypothesis for the mating-type differentiation with the controlling elements produced by the *HM*α and *HMa* genes is proposed.

IN earlier papers (TAKANO and OSHIMA 1970b; OSHIMA and TAKANO 1971), it was reported that a pair of mating-type alleles, a and α on chromosome III, in *Saccharomyces cerevisiae* and its related species are interchangeable with each other due to the specific mutagenic action of the homothallic genes, HO_α and *HM*. The HO_α gene acts as a specific mutator for the α mating-type allele and changes α to a shortly after spore germination. On the other hand, conversion of the a mating type allele to α requires both the HO_α and *HM* genes. Allelism tests among homothallic genes and gene systems of different origin (WINGE and

[1] This study was supported in part by a grant from the Ministry of Education, Japan.

ROBERTS 1949; TAKAHASHI 1958) revealed that these genes or gene systems are included in the HO_{α} and *HM* gene system (TAKANO and OSHIMA 1970a). A homothallic strain having the HO_{α} *HM* genotype showed a perfect homothallic life cycle, i.e., all 4 spore cultures from each ascus gave rise to homothallic diploids (the Ho type of homothallism; SANTA MARIA and VIDAL 1970), while a homothallic culture originated from a spore of the α HO_{α} *hm* genotype showed a 2-homothallic:2*a* segregation in each ascus. Each homothallic segregant again showed a 2-homothallic:2*a* segregation (the Hq type of homothallism; SANTA MARIA and VIDAL 1970). In 1970, however, SANTA MARIA and VIDAL reported another type of meiotic life cycle which gave a 2-homothallic:2α segregation in each ascus in a strain of *Saccharomyces norbensis* (the Hp type of homothallism). This observation suggests an additional genetic factor(s) for controlling homothallism in Saccharomyces. This possibility was tested (OSHIMA and TAKANO 1972) by the genetic analysis of various hybrids prepared by crosses of *S. norbensis* strain SBY 2535, which was kindly supplied by DR. SANTA MARIA, with our strains having several different genotypes for the homothallic genes. Results of the analyses showed that the HO_{α} and *HM* gene system should be revised to a system consisting of *HO*, *HM*α, and *HMa* genes. The previous *HM* gene is now designated *HMa*. For the conversion of the *a* mating-type allele to α, both *HO* and *HMa* genes are required, the conversion of α to *a* requires the *HO* and *HM*α genes. A strain containing the *ho* allele is a stable heterothallic clone according to this system.

Two types of semi-homothallic life cycle designated as the Hp and Hq types of homothallism have been explained by this model as follows (OSHIMA and TAKANO 1972). The homothallic diploid clone showing the Hp type segregation has the *a HO hm*α *HMa*/α *HO hm*α *HM*a genotype for the homothallic genes. Upon self-sporulation, meiotic division occurs and each ascus contains two *a* and two α spores at haplophase. Since all these haploid spores are the same for homothallic genes, i.e., the *HO hm*α *HMa* genotype, the two *a* spores give rise to diploid cultures due to cell fusion subsequent to the conversion of the mating-type allele from *a* to α by the mutagenic action of the *HO* and *HMa* genes in some fraction of cells within a few generations after the spore germination. The latter two α spores, however, are able to produce stable haploid cultures because the *HO hm*α *HMa* genotype is not effective for the conversion of α to *a*. The homothallic diploid segregants will again show a 2-homothallic:2α segregation by the same mechanism. Similarly the Hq type strain has the *a HO HM*α *hma*/α *HO HM*α *hma* genotype. In this case, the two α spores formed in each ascus produce diploid cells by self-fertilization and two *a* spores give rise to stable haploid cultures by single-spore culture.

We also observed in the same report (OSHIMA and TAKANO 1972) that 15 of the 77 asci tested showed a 4-homothallic:0-heterothallic segregation in the crosses of α Hp (an α haploid segregant from the Hp type homothallic diploid) to *a* Hq (an *a* haploid from the Hq diploid). The expected genotype of this hybrid should be α *HO hm*α *HMa*/*a HO HM*α *hma*. Eight asci which showed enough potency of sporulation were selected from these 15 asci and subjected to further analysis. It was found that four homothallic spore cultures from each tetrad of these hybrids

did not always show a 2-Hp:2-Hq segregation. Three asci showed a 4-Ho:0-Hp:0-Hq segregation, four asci showed a 2-Ho:1-Hp:1-Hq segregation and the remaining ascus showed a 0-Ho:2-Hp:2-Hq segregation. These data suggest that the genetic activities of *HMα* and *HMa* are controlled by at least two duplicated loci, respectively, or there might exist some other mechanism which gives rise to homothallism in the culture. The present communication describes data which strongly support the idea that the 4-Ho:0-Hp:0-Hq and 2-Ho:1-Hp:1-Hq segregations from the α Hp to *a* Hq cross were not due to the independent duplicated genes for the *HMα* and *HMa* functions but were attributable to the homothallic phenotype (the Ho type) of the *HO hmα hma* clone or the *HMa* and *HMα* functions of the *hmα* and *hma* alleles, respectively.

MATERIALS AND METHODS

Organisms: The strains used in this paper are descendants from the above-mentioned crosses (OSHIMA and TAKANO 1972) between heterothallic haploid clones having the *a* HO_α *hm* (now designated *a HO HMα hma*) genotype and the haploid α clones originated from *Saccharomyces norbensis* SBY 2535 which was kindly supplied by DR. SANTA MARIA of the Instituto Nacional de Investigaciones Agronómicas, Madrid, Spain. According to the description by SANTA MARIA and VIDAL (1970), *S. norbensis* SBY 2535 is a spontaneous mutant derived from *S. norbensis* SBY 2314, and is distinguished from its original strain by the higher viability of ascospores at the dissection of asci. The original strain (SBY 2314) was diploid by its cell size (3.2–7.8 × 3.2–10 μm), cell shape (round or oval, single or in pairs), and the ability to sporulate without preceding cell fusion (SANTA MARIA 1963). We confirmed these facts with the strain SBY 2535 and observed that this strain is prototrophic on Burkholder's synthetic minimal medium. It sporulated well on sodium acetate sporulation medium (FOWELL 1952) and produced abundant four-spored asci. Results of tetrad dissection of asci showed virtually a 2-homothallic:2α segregation in the 29 asci tested so far except for 4 irregular asci (3 of which showed a 1-homothallic:3α segregation and the fourth, a 0-homothallic:4α segregation). Some of the homothallic segregants from SBY 2535 were subjected to further tetrad analyses and it was observed that these clones showed a 2-homothallic:2α segregation in every ascus. These observations were well in accord with the descriptions by SANTA MARIA and VIDAL (1970).

The Ho type homothallic strains carrying the *D* gene, C-1728b-4C and 1932-2A, allelic to the HO_α *HM* genotype, i.e., the *HO HMα HMa* genotype by the new theory (TAKANO and OSHIMA 1970a; OSHIMA and TAKANO 1972), were those strains which were kindly supplied by DR. T. TAKAHASHI of the Brewing Science Research Institute, Suita, Japan and DR. D. C. HAWTHORNE of the University of Washington, Seattle, U. S. A. Many other heterothallic haploid stocks for yeast genetics were also used as standards for the determination of mating types. Those strains selected from our stock cultures and the D strains were marked with several authentic auxotrophic genetic markers.

Techniques: The general techniques and media were described in a previous publication (TAKANO and OSHIMA 1967).

RESULTS

Genetic characterization of the Ho segregants derived from the hybrids of the α Hp to a *Hq cross:* In order to test the genetic behavior of the Ho segregants from the α Hp to *a* Hq cross, allelism tests were carried out among the four tetrad clones in each one of three asci, i.e., C-18–2, C-18–16, and C-24–15, in which all tetrad clones showed the Ho type of homothallism, as described in a previous paper (OSHIMA and TAKANO 1972) and briefly summarized in the introduction. In a typical experiment, four homothallic clones from a given ascus were sporu-

lated. Spores derived from each homothallic clone were isolated and crossed with spores from another clone of the same tetrad by spore-to-spore contact on a thin agar film of nutrient medium with the aid of a micromanipulator. Thus, six different hybrids were obtained by crossing the homothallic tetrad clones derived from the same ascus. Results of the tetrad analyses of these hybrids are summarized in Table 1. It is evident that the Ho clones in each of 3 original asci, C-18–2, C-18–16 and C-24–15, consisted of two different clones, respectively. The C-18–2A clone should have the same genotype as C-18–2D because the hybrid of this combination showed a 4-homothallic:0-heterothallic segregation in all of the 25 asci dissected. Similarly, the genotype of C-18–2B is the same as C-18–2C and differed from C-18–2A and C-18–2D. Though one ascus showed a 3-homothallic:1a:0α segregation, the genotype of C-18–16A must be the same as C-18–16C and different from C-18–16B and C-18–16D. It is also clear that C-24–15A and C-24–15B have the same genotype and which differs from that of C-24–15C and C-24–15D.

Next, we performed allelism tests among six homothallic clones from these three asci, C-18–2, C-18–16, and C-24–15, using two clones having the different genotypes in each respective ascus, as shown in Table 2. Results clearly showed that these clones were divisible into two classes. One, which we tentatively called Type I, consisted of C-18–2A, C-18–16B, and C-24–15C (which also included

TABLE 1

Tetrad segregations from hybrids obtained by intra-ascus crosses in asci C-18–2, C-18–16, and C-24–15

Ascus no.	Cross*	Segregation in asci (Homothallic†:a:α) 4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
C-18–2	A × B	3	6	1	6	4‡	1‡	3‡	1‡	0
	A × C	4	5	3	9	2‡	0	1	0	1
	A × D	25	0	0	0	0	0	0	0	0
	B × C	25	0	0	0	0	0	0	0	0
	B × D	7	4	4	10	0	0	0	0	0
	C × D	13	1	2	6	2‡	1‡	0	0	0
C-18–16	A × B	13	3	2	5	0	1‡	0	0	1
	A × C	24	1	0	0	0	0	0	0	0
	A × D	0	6	3	12	2‡	2‡	0	0	0
	B × C	8	2	2	10	0	0	0	0	3
	B × D	25	0	0	0	0	0	0	0	0
	C × D	11	2	3	7	1‡	0	0	0	1
C-24–15	A × B	25	0	0	0	0	0	0	0	0
	A × C	7	4	3	11	0	0	0	0	0
	A × D	6	3	6	7	1‡	0	1‡	0	1
	B × C	8	3	2	12	0	0	0	0	0
	B × D	11	4	3	7	0	0	0	0	0
	C × D	25	0	0	0	0	0	0	0	0

* Each cross was made by the spore-to-spore mating method.
† Three types of homothallism, Ho, Hp, and Hq, were included in this category.
‡ The asci belonging to the ascus types which could not be expected by hypothesis II were re-examined with the stock cultures. However, these asci marked with ‡ were not examined again, because they had been discarded at the time of re-examination.

TABLE 2

Tetrad segregations from hybrids obtained by inter-ascus crosses among the segregants of asci C-18-2, C-18-16, and C-24-15

Cross*		Segregation in asci (Homothallic†:a:α) 4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
C-18-2A	× C-18-16A	14	2	3	7	0	0	0	0	0
	× C-18-16B	25	0	0	0	0	0	0	0	0
	× C-24-15A	9	1	3	7	0	0	0	0	1
	× C-24-15C	27	0	0	0	0	0	0	0	0
C-18-2B	× C-18-16A	26	0	0	0	0	0	0	0	0
	× C-18-16B	6	3	0	10	0	0	0	0	0
	× C-24-15A	25	0	0	0	0	0	0	0	0
	× C-24-15C	11	5	4	5	0	0	0	0	0
C-18-16A	× C-24-15A	22	0	0	0	0	0	0	0	0
	× C-24-15C	8	3	3	2	0	0	0	0	0
C-18-16B	× C-24-15A	6	0	3	4	0	0	0	0	0
	× C-24-15C	25	0	0	0	0	0	0	0	0

* Each cross was made by the spore-to-spore mating method.
† Three types of homothallism, Ho, Hp, and Hq, were included in this category.

C-18-2D, C-18-16D, and C-24-15D, according to the data listed in Table 1). The other, Type II, consisted of C-18-2B, C-18-16A, and C-24-15A (C-18-2C, C-18-16C, and C-24-15B should also be included in this class).

Allelism tests were also performed with these Type I and Type II clones with the homothallic strain of the *D* gene. The *D* gene should be redesignated *HO HMα HMa*, as described above. Results of the tetrad analysis of hybrids prepared by spore-to-spore crosses between the D strains and the Type I or Type II clones are summarized in Table 3. The hybrids obtained by crosses of the D strains and the Type II clone showed a 4-homothallic:0-heterothallic segregation in all asci so far tested (41 asci), while the stable haploid clones of both mating types, *a* and *α*, were segregated from the hybrids prepared by crosses of the D strains and the Type I clone. These facts clearly indicated that the genotype of the Type II clone is the same as that of the D strain and that the Type I clone should bear another genotype which also gives the homothallic phenotype to the culture.

TABLE 3

Tetrad segregations from hybrids obtained by crossing the Type I and Type II class of the Ho strains to the homothallic strains carrying the D gene

Cross*	Segregation in asci (Homothallic†:a:α) 4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
1932-2A‡ × C-18-2A§	9	3	3	2	0	0	0	0	0
1932-2A × C-18-2B¶	25	0	0	0	0	0	0	0	0
C1728b-4C‡ × C-18-2A	34	19	12	13	0	0	0	0	3
C1728b-4C × C-18-2B	16	0	0	0	0	0	0	0	0

* Each cross was made by the spore-to-spore mating method.
† Three types of homothallism, Ho, Hp, and Hq, were included in this category.
‡ The homothallic strains carrying the *D* gene.
§ Type I class of the Ho strain.
¶ Type II class of the Ho strain.

Some of the Ho clones of Type I and Type II were backcrossed to the *α* Hp and the *a* Hq strains by spore-to-cell contact. These data are summarized in Table 4. It was observed that the same ascus types were segregated by tetrad analyses of the hybrids. If both Types of the Ho clones were backcrossed to the *α* Hp strain, hybrids never segregated the haploid clone of *a* mating type and contained two or fewer *α* clones in each ascus. On the other hand, hybrids prepared by crossing them with the *a* Hq strain did not segregate the haploid clone of *α* mating type and two or fewer *a* clones were observed in each ascus. Thus, virtually no difference was observed in the ascus types of segregants. However, the data listed in Table 4 showed some difference in the ratios of the ascus types with respect to their combination. This observation which suggests a linkage relationship of the mating-type locus to *HMα*, will be discussed later.

The observations described above could most probably be explained by the following alternatives. First, there are duplicated genes for the *HMα* and *HMa* functions, respectively, and the homothallic gene system consists of five independent loci, *HO, HMα1, HMα2, HMa1* and *HMa2* (hypothesis I). For example, the Ho type homothallic diploid of Type I should be homozygously marked with the *HO, HMα1, hmα2, HMa1,* and *hma2* genes. The Type II and also the D strains should be marked with the *HO, hmα1, HMα2, hma1,* and *HMa2* genes homozygously. The haploid *α* Hp and *a* Hq clones used in this study should have the *α HO hmα1 hmα2 HMa1 HMa2* and *a HO HMα1 HMα2 hma1 hma2* genotypes respectively. Another explanation (hypothesis II) is that each of the three kinds of homothallic genes consists of a single pair of alleles, i.e., *HO/ho, HMα/hmα* and *HMa/hma*, respectively. However, a spore having the *HO hmα hma* genotype would give rise to an Ho type homothallic culture. In this explanation, the Type I clone would be homozygous for the *HO, hmα,* and *hma* alleles while Type II and the D strains have the *HO HMα HMa* genotype at their haplophase nucleus. The haploid *α* Hp clone and the *a* Hq clone should have the *α HO hmα HMa* and *a HO HMα hma* genotype, respectively.

Test of the hypotheses: According to these alternative hypotheses, different segregation patterns would be expected for each hypothesis with respect to the type of ascus and to the type of homothallic clone in certain crosses. However, among the crosses between the Hp strain of *S. norbensis* SBY 2535 to various strains having different genotypes, i.e., ho_α *hm*, ho_α *HM*, HO_α *hm*, and HO_α *HM* (here we have followed the earlier symbols—TAKANO and OSHIMA 1970a), some of the combinations would be expected to segregate in the same or similar ways. The most significant differences in the segregation patterns would be expected in the crosses of *α* Hp to *a* Hq, particularly in the crosses mentioned in the previous paper (OSHIMA and TAKANO 1972) and in the introduction in which duplicated genes were suggested for *HMα* and *HMa* according to hypothesis I. Another combination, crosses between the Ho type clones of Type I and Type II, should also be useful for testing these hypotheses. The expected frequencies of the ascus types in these combinations, listed in Table 5, were calculated, assuming that the hypothetical homothallic genes and the mating-type locus were segregated independently of each other. The *HO hmα hma* genotype should give rise to the heterothallic phenotype according to hypothesis I and the Ho type of homo-

TABLE 4

Tetrad segregations from the hybrids prepared by crossing the Ho clones of Type I and Type II to the α Hp or a *Hq strains*

Cross*	Expected genotype†	Segregation in asci (Homothallic‡:a:α) 4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
α Hp × Type I	*α HO hmα HMa/a HO hmα hma*	5	0	22	0	0	3	0	0	0
α Hp × Type II	*α HO hmα HMa/a HO HMα HMa*	3	0	60	0	0	21	0	0	0
a Hq × Type I	*a HO HMα hma/α HO hmα hma*	3	39	0	0	26	0	0	0	0
a Hq × Type II	*a HO HMα hma/α HO HMα HMa*	17	78	0	0	31	0	0	0	0

* Each cross was made by the spore (of the Ho clone)-to-cell (of the α Hp or *a* Hq strain) mating method.
† Expected genotypes according to hypothesis II.
‡ Three types of homothallism, Ho, Hp, and Hq, were included in this category.

TABLE 5

The ascus types of the segregants derived from the diploids prepared by crossing the α Hp to a Hq strains and the Type I to Type II of the Ho type homothallic strains

Ascus type: Spore A	B	C	D	Observed*	Expected frequency†: Hypothesis I (α/a HO/HO hmα1/HMα1 hmα2/HMα2 HMa1/hma1 HMa2/hma2 or s‡/s HO/HO HMα1/hmα1 hmα2/HMα2 HMa1/hma1 hma2/HMa2)	Expected frequency†: Hypothesis II (α/a HO/HO hmα/HMα HMa/hma or s‡/s HO/HO hmα/HMα hma/HMa)
Ho	Ho	Ho	Ho	14/25	36	6
Ho	Ho	Ho	Hp	0	72	0
Ho	Ho	Ho	Hq	0	72	0
Ho	Ho	Hp	Hq	10/25	144	8
Ho	Ho	Hp	Hp	0	6	0
Ho	Ho	Hq	Hq	0	6	0
Ho	Hp	Hp	Hq	0	12	0
Ho	Hp	Hq	Hq	0	12	0
Hp	Hp	Hq	Hq	1/25	1	1
Ho	Ho	Ho	*a*	0	144	0
Ho	Ho	Hp	*a*	17/17	84	4
Ho	Ho	Hq	*a*	0	48	0
Ho	Hp	Hq	*a*	0	28	0
Ho	Ho	Ho	α	0	144	0
Ho	Ho	Hp	α	0	48	0
Ho	Ho	Hq	α	6/6	84	4
Ho	Hp	Hq	α	0	28	0
Ho	Ho	*a*	α	8/10	196	8
Ho	Hp	*a*	α	0	28	0
Ho	Hq	*a*	α	0	28	0
Hp	Hq	*a*	α	2/10	4	4
Ho	Ho	*a*	*a*	NT§	19	0
Ho	Ho	α	α	NT	19	0
Ho	*a*	*a*	α	—¶	16	0
Ho	*a*	α	α	—	16	0
a	*a*	α	α	19/735	1	1
Total					1296	36

* From the pooled tetrad clones of the diploids prepared by crosses between α Hp to *a* Hq or between the Ho clones of Type I and Type II, the tetrad clones showing 4-homothallic:0-heterothallic, 3-homothallic:1-heterothallic, and 2-homothallic:1*a*:1α segregations in which all the homothallic clones had high enough levels of sporulation were collected. Each homothallic diploid segregant was sporulated and the 4-spored asci were dissected. The type of the homothallism was decided by the segregation pattern of 10 to 20 asci in each homothallic clone. The results are indicated as the observed number of asci per total number of asci tested in each category.

† The expected frequency of each ascus type was calculated assuming that the hypothetical genes were unlinked to each other or with the mating-type locus.

‡ Mating by spore-to-spore fusion was employed for crossing between the Type I and Type II of the Ho strains.

§ Not tested.

¶ No proper tetrad was observed in this ascus type; for details, see text.

thallism according to hypothesis II. According to hypothesis II, 4-homothallic:0-heterothallic asci will be restricted to three types of asci, i.e., 4-Ho:0-Hp:0-Hq, 2-Ho:1-Hp:1-Hq, and 0-Ho:2-Hp:2-Hq, while nine ascus types would be expected from hypothesis I. In the other classes of asci showing the 3-homothallic:1*a* and 3-homothallic:1α segregations, four possible ascus types would be expected from hypothesis I and only one ascus type, 2-Ho:1-Hp:1*a* or 2-Ho:1-Hq:1α, respectively, would be expected from hypothesis II in each class. In asci showing the 2-homothallic:1*a*:1α segregation the ascus types would be expected to be 2-Ho:1*a*:1α or 1-Hp:1-Hq:1*a*:1α from hypothesis II. One would not expect to find asci showing 2-homothallic:2*a*:0α, 2-homothallic:0*a*:2α, 1-homothallic:2*a*:1α, or 1-homothallic:1*a*:2α segregations from hypothesis II, while the occurrence of these ascus types would be expected by hypothesis I, as shown in Table 5.

Hypotheses I and II were tested according to the above criteria. We were able to collect 25 asci showing a 4-homothallic:0-heterothallic segregation, 17 asci showing a 3-homothallic:1*a* segregation and 6 asci of a 3-homothallic:1α segregation (in which all the homothallic clones had high enough levels of sporulation for further analysis) from the pooled asci derived from hybrids prepared by the crosses of an α Hp × *a* Hq combination (217 asci) and from the diploids prepared by crossing between the Ho clones of Type I and Type II (518 asci). All the homothallic clones were sporulated and the four-spored asci were dissected. Results of the tetrad analyses, as listed in Table 5, indicated that all the clones in 14 of 25 asci showing a 4-homothallic:0-heterothallic segregation were identified as the Ho type homothallic strains; the other 10 asci showed a 2-Ho:1-Hp:1-Hq segregation and the remaining ascus consisted of two Hp and two Hq clones. We were not able to observe the other six ascus types which could be expected from hypothesis I. All 17 tested asci which showed a 3-homothallic:1*a* segregation fell into an ascus type of 2-Ho:1-Hp:1*a*. Similarly, all 6 asci showing a 3-homothallic:1α segregation were identified as a 2-Ho:1-Hq:1α type. Although, we tested only 10 asci which showed a 2-homothallic:1*a*:1α segregation, each one showed the ascus type either of a 2-Ho:1*a*:1α (8 asci) or a 1-Hp:1-Hq:1*a*:1α (2 asci) segregation.

On the other hand, we had observed a significant number of asci which showed the 2-homothallic:2*a*:0α, 2-homothallic:0*a*:2α, 1-homothallic:2*a*:1α, and 1-homothallic:1*a*:2α segregations from the hybrids prepared by α Hp to *a* Hq crosses or by crosses between the Ho clones of Type I and Type II, as shown in a previous paper (Table 1 of Oshima and Takano 1972) and also in Table 1 of this report. These ascus types would be expected from hypothesis I but never from hypothesis II. We tried to isolate the *HO hmα hma* clone from some of the asci showing a 1-homothallic:3-heterothallic segregation according to hypothesis I. For example, the haploid clones of *a* mating type from an ascus showing a 1-homothallic:2*a*:1α segregation should have one of the following four genotypes, i.e., *a HO HMα1 HMα2 hma1 hma2*, *a HO HMα1 hmα2 hma1 hma2*, *a HO hmα1 HMα2 hma1 hma2*, or *a HO hmα1 hmα2 hma1 hma2*. These four genotypes of the *a* clones can be classified by the genetic analyses of the hybrids prepared by crossing them to the α clone of the same asci which should have the *α HO hmα1 hmα2 HMa1 HMa2* genotype. Among those crosses, the diploids obtained by crossing the

a HO hmα1 hmα2 hma1 hma2 clone should segregate two *α* clones in each ascus while some of the *a* clones might convert to the homothallic diploids. If the cross were made with an *a* clone having either one of the other three genotypes, it should also be possible to isolate the *a HO hmα1 hmα2 hma1 hma2* clone by repeated backcrosses of the haploid *a* segregants to the *α HO hmα1 hmα2 HMa1 HMa2* haploid. These possibilities were attempted using the haploid clones from a tetrad showing a 1-homothallic:2*a*:1*α* segregation which occurred in the cross of C-18–2A × C-18–2C (Table 1). Another set of tetrad clones obtained by the additional dissections (those data were not included in Table 1) of the asci which originated from diploid of the C-18–16C × C-18–16D cross could also be analyzed. However, we observed no haploid clone showing the segregation patterns expected from hypothesis I as discussed in the above argument. Further pedigree analyses of all these haploids according to the above possibility always resulted in inconsistencies in their genotypes. It was also observed that some clones which previously had been classified as heterothallic haploids in tetrads of a 1-homothallic:3-heterothallic segregation in Table 1 were identified as diploids due to the recognition of their low but significant potencies of sporulation by careful re-examination of the stock cultures. These asci were classified as an ascus type of 2-homothallic:1*a*:1*α* and data listed in Table 1 were revised according to these observations. We did not re-examine the asci showing the 2-homothallic:2*a*, 2-homothallic:2*α* segregation or some of the asci with a 1-homothallic:3-heterothallic segregation which were observed in the previous study (Table 1 of OSHIMA and TAKANO 1972) and in Table 1 (marked with (‡)) of this report, because they had been discarded at the time of re-examination. These types of ascus were never observed in the latter experiments made by careful examination as shown in similar experiments listed in Tables 2 and 3. These results suggest that the occurrence of asci showing the 2-homothallic:2*a*:0*α*, 2-homothallic:0*a*:2*α* and 1-homothallic:3-heterothallic segregation could be attributable to errors in microscopic inspection or they might be irregular asci. In another report (TAKANO, KUSUMI and OSHIMA 1973), we were able to identify the conversion-insensitive *α* allele, *α-inc*, in *S. diastaticus*. If there occurred such a conversion-insensitiveness or a slow-converting mating-type allele in the present strains, this might also give tetrads showing such segregations which could not be expected from hypothesis II. In any case, we should now exclude the segregation data showing 1-homothallic:2*a*:1*α*, 1-homothallic:1*a*:2*α*, 2-homothallic:2*a*:0*α*, and 2-homothallic:0*a*:2*α* segregations as irregular asci. In addition, we observed only two genotypes, *HO HMα1 hmα2 HMa1 hma2* and *HO hmα1 HMα2 hma1 HMa2*, among 12 Ho type homothallic clones from 3 asci showing 4-homothallic:0-heterothallic segregation from the *α* Hp to *a* Hq crosses, while we could expect two other different genotypes, i.e., *HO HMα1 hmα2 hma1 HMa2* and *HO hmα1 HMα2 HMa1 hma2*, according to hypothesis I. All these observations excluded the idea proposing four independent loci for the *HMα1*, *HMα2*, *HMa1*, and *HMa2* genes and strongly supported hypothesis II.

In conclusion, all the observations of the genetic controlling system for homothallism in Saccharomyces can be explained with three kinds of homothallic genes, each of which consists of a single pair of alleles, *HO/ho*, *HMα/hmα*, and

HMa/hma, respectively. A spore having the *HO hmα hma* genotype will give rise to a homothallic diploid culture the same as the strain having the *HO HMα HMa* genotype. A spore having the *a HO hmα HMa* genotype will give rise to a homothallic diploid having the Hp type life cycle and the culture originating from a spore having the *α HO HMα hma* genotype will give a diploid culture showing the Hq type life cycle. The other genotypes, *a HO HMα hma*, *α HO hmα HMa*, and the *ho* allele combined with either of the alleles at *HMα* and *HMa* loci will give a heterothallic character to the culture. In a previous publication (TAKANO, KUSUMI and OSHIMA 1973), we had supposed that a spore having the combined genotype of the inactive alleles of *HMα* and *HMa*, i.e., the *hmα hma* genotype, would give rise to a stable heterothallic haploid culture. This prediction would be wrong and would contradict the present conclusion if a spore had the *HO* genotype.

Linkage relations between the HMα *and the mating-type loci:* Before interbreeding with *S. norbensis* SBY 2535, all of our strains had been marked with the *HMα* allele homogeneously. With these strains, no linkage relationship was detected in any gene pairs among the *HO*, *HMa*, and the mating-type loci (e.g., TAKANO and OSHIMA 1967). However, the data listed in Table 4 suggest some linkage between *HMα* and the mating-type locus. According to the present conclusion, the haploid *α* Hp clone has the *αHO hmα HMa* genotype and the Type II class of Ho is of the *HO HMα HMa* genotype. The genotype of the diploid hybrid prepared by crossing by cell-(*α*)-to-spore fusion would be *α HO hmα HMa/a HO HMα HMa*, because it was supposed that only the spore having the *a* mating type could contribute in mating with an *α* cell (OSHIMA and TAKANO 1971). Thus, this type of hybrid should be doubly heterozygous for both the *HMα* and the mating-type loci, as indicated in Table 4. Another combination, a cross between the haploid *a* Hq to the Type I class of the Ho strain should also be doubly heterozygous for both the *HMα* and the mating-type loci, while this combination has the *hma* allele homozygously. Pooled data of the tetrad distribution of this combination showed 47:6:99 in the ratio of the parental ditype:non-parental ditype:tetratype asci. These data indicate a loose linkage (approximately 64 stranes by the D_1 formula [SHULT and LINDEGREN 1956]) between the *HMα* and the mating-type loci on chromosome III.

DISCUSSION

For the explanation of the mating-type differentiation, we previously proposed the hypothesis (OSHIMA and TAKANO 1971; TAKANO, KUSUMI and OSHIMA 1973) that the elementary structure of the mating-type locus for both the *a* and *α* alleles is essentially the same. The association of some kind of controlling element with this locus would cause the differentiation of two mating-type alleles. The mating-type locus on chromosome III would act as an affinity site for a controlling element. The *HMα* and *HMa* genes produce the specific controlling element; the association of an *HMα*-element with the mating-type locus would form the *a* mating-type allele and the association of an *HMa*-element with the mating-type locus would give rise to the *α* mating-type allele.

To expand this hypothesis to cover the present observations, we should make a revision of the molecular mechanism of the mating-type differentiation, or another speculation on the genetic function of the *hmα* and *hma* alleles. It seems not impossible, but difficult, to explain the molecular mechanism in Ho homothallism caused by the *HO hmα hma* genotype by assuming the presence of inactive alleles of the *HMα* and *HMa* genes. On the other hand, another idea has been suggested by NAUMOV and TOLSTORUKOV (1973) from a genetic analysis of homothallic strains, including a similar Hp strain originated from SANTA MARIA. According to their suggestion, the *hmα* allele has the same function as the *HMa* gene and *hma* has the same function as the *HMα* allele. We have recognized that their idea is well in accord with all of our observations and is also attractive to explain the molecular mechanism described above without any modification. However, whether the *hmα* and *hma* genes exert their function positively or whether they have no positive function for the conversion of the mating-type alleles is not yet clear; in other words, whether the Ho type of homothallism in the *HO hmα hma* clone is caused by the same mechanism or by another occurring in the *HO HMα HMa* clone remains for further study.

LITERATURE CITED

FOWELL, R. R., 1952 Sodium acetate agar as a sporulation medium for yeast. Nature **170**: 578.

NAUMOV, G. I. and I. I. TOLSTORUKOV, 1973 Comparative genetics of yeast. X. Reidentification of mutators of mating types in *Saccharomyces*. Genetika **9**: 82–91.

OSHIMA, Y. and I. TAKANO, 1971 Mating types in Saccharomyces: their convertibility and homothallism. Genetics **67**: 327–335. ——, 1972 Genetic controlling system for homothallism and a novel method for breeding triploid cells in *Saccharomyces*. pp. 847–852. In: *Fermentation Technology Today: Proc. IVth Intern. Ferment. Symp.* Edited by G. TERUI. Soc. Ferment. Technol., Osaka.

SANTA MARIA, J., 1963 New melibiose-utilizing yeasts, isolated from "alpechin." Antonie van Leeuwenhoek **29**: 329–343.

SANTA MARIA, J. and D. VIDAL, 1970 Segregación anormal del "mating type" en Saccharomyces. I. N. Investigaciones Agronómicas (Madrid) **30**: 1–21.

SHULT, E. E. and C. C. LINDEGREN, 1956 Mapping methods in tetrad analysis. 1. Provisional arrangement and ordering of loci preliminary to map construction by analysis of tetrad distribution. Genetica **28**: 165–176.

TAKAHASHI, T., 1958 Complementary genes controlling homothallism in Saccharomyces. Genetics **43**: 705–714.

TAKANO, I. and Y. OSHIMA, 1967 An allele specific and a complementary determinant controlling homothallism in *Saccharomyces oviformis*. Genetics **57**: 875–885. ——, 1970a Allelism tests among various homothallism-controlling genes and gene systems in Saccharomyces. Genetics **64**: 229–238. ——, 1970b Mutational nature of an allele-specific conversion of the mating type by the homothallic gene HO_{α} in Saccharomyces. Genetics **65**: 421–427.

TAKANO, I., T. KUSUMI and Y. OSHIMA, 1973 An α mating-type allele insensitive to the mutagenic action of the homothallic gene system in *Saccharomyces diastaticus*. Molec. Gen. Genet. **126**: 19–28.

WINGE, Ö. and C. ROBERTS, 1949 A gene for diploidization in yeast. Compt. Rend. Trav. Lab. Carlsberg Sér. Physiol. **24**: 341–346.

Corresponding editor: R. E. ESPOSITO