MATING TYPES IN SACCHAROMYCES: THEIR CONVERTIBILITY AND HOMOTHALLISM

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GENETIC systems controlling homothallism *versus* heterothallism in Saccharomyces yeasts have been reported by several authors (WINGE and ROBERTS 1949; TAKAHASHI 1958; HERMAN and ROMAN 1966; TAKANO and OSHIMA 1967). Allelism tests indicated that all the homothallism gene systems in *S. cerevisiae* and its related species are the same (TAKANO and OSHIMA 1970a). The controlling system in these strains consists of two unlinked 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 (TAKANO and OSHIMA 1970b). A cell converted from α to *a* can make a zygote with its neighboring α cell. An *a* allele converted from α, designated a', is as stable as normal *a*, and responds like a normal *a* to HO_α. Thus the α HO_α *hm* clone is homothallic, and after meiosis it segregates 2 homothallic : 2*a* in every ascus (TAKANO and OSHIMA 1967, 1970a, 1970b). The *HM* gene has no effect alone, but when combined with HO_α, it converts *a* to homothallism (TAKANO and OSHIMA 1967).

This paper presents data demonstrating that the combination of two complementary genes, HO_α and *HM*, brings about the conversion of an *a* allele to α. An α mating-type allele, α', derived from the *a* or a' allele is stable and cannot be distinguished from normal α either in its ability to form zygotes with *a* cells or in its response to HO_α.

MATERIALS AND METHODS

Organisms: The strains of Saccharomyces breeding stocks employed in this study were derived from progeny shown in pedigrees reported in previous studies (TAKANO and OSHIMA 1967, 1970a, 1970b). These strains were marked with several standard genetic markers including the mating-type-linked markers *thr4*, *leu2*, and *his4* on chromosome III (HAWTHORNE and MORTIMER 1968).

Techniques: The general techniques and media were described in a previous publication (TAKANO and OSHIMA 1967). Designation of the standard genetic markers follows the recommendations of the nomenclature committee in yeast genetics (VON BORSTEL 1969).

RESULTS

Construction of spores with the a (*or* a′) HO_α HM *genotype:* The method by which we obtained homothallic spores having the *a* (or a') HO_α *HM* genotype is shown in Figure 1. Crosses were made between heterothallic haploid strains of

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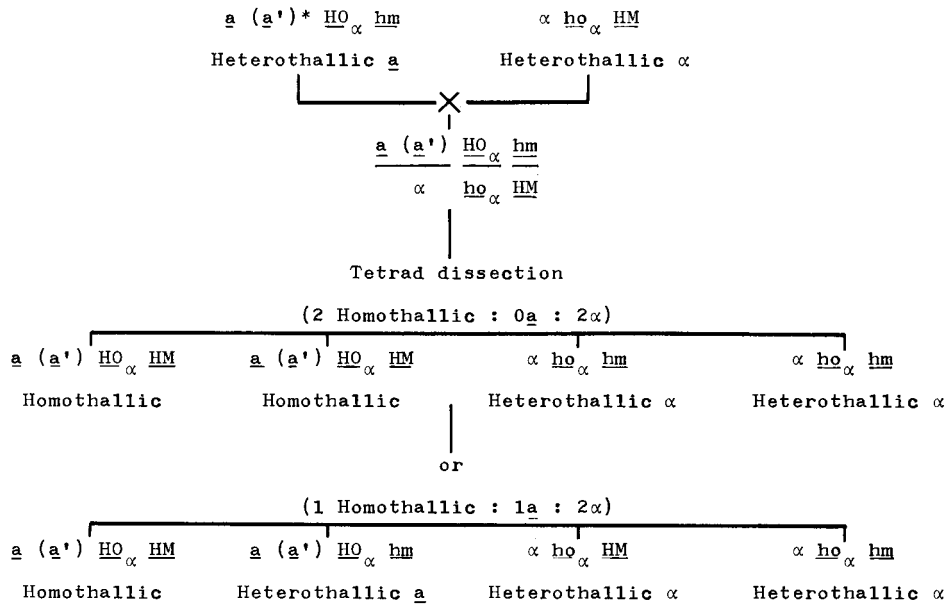

FIGURE 1.—Scheme to illustrate the method of breeding an *a* (or *a'*) HO_α *HM* spore culture. * Alternative mating-type allele in similar crosses. The designation *a'* is used to signify an *a* mating-type allele produced from the α mating-type allele by the action of HO_α.

the *a* (or *a'*) *HOα hm* and *α hoα HM* genotypes containing appropriate genetic markers by a mass mating procedure (LINDEGREN and LINDEGREN 1943) in a few ml of glucose nutrient medium. Resultant hybrid cells in the mass mating culture were sporulated and four-spored asci were dissected. From these diploids, we expect six different segregation types in asci (TAKANO and OSHIMA 1970a). We selected homothallic clones from asci showing the 2 homothallic : 2α or the 1 homothallic : 1*a* : 2α segregation in an ascus. The genotype of homothallic spores from these two ascus types is expected to be *a* (or *a'*) HO_α *HM* as shown in Figure 1. Spore cultures selected in this way gave stable homothallic diploid clones. Upon sporulation these homothallic clones segregated four homothallic spores in each ascus and each of these daughter spores upon germination produced asci in which all four spores showed homothallism again. The four spores derived from a homothallic strain contain identical genetic markers.

Direct spore-to-cell mating experiment between homothallic and heterothallic clones: Zygote formations were always recognized in the *a* (or *a'*) HO_α *HM* spore cultures between the mother and daughter or granddaughter cells within a few generations after spore germination (TAKANO and OSHIMA 1967). There are a number of possible mechanisms by which an ascospore could give rise to a diploid clone. (1) The formation of the zygote was preceded by the conversion of the mating-type allele from *a* to α in a certain fraction of cells by the specific mutagenic action of two complementary genes HO_α and *HM*. A similar process has been recognized in the α HO_α *hm* spore culture. In this case, zygote formation is preceded by the conversion of the α mating-type allele to *a'* by the action of the

HO$_\alpha$ gene (TAKANO and OSHIMA 1970b). (2) The zygote was formed by fusion of two *a* cells by the direct function, without the mutagenic action, of two complementary genes *HO*$_\alpha$ and *HM*. In this case, the genotype at the mating-type locus of homothallic diploid cells should be homozygous for an *a* (or *a'*) allele.

In order to test above alternatives, six different types of crosses were made between homothallic clones originated from *a* (or *a'*) *HO*$_\alpha$ *HM* spores and heterothallic *ho*$_\alpha$ *hm* clones with various mating-type alleles. Most of these crosses were made between each particular homothallic clone and various heterothallic clones with different mating types. All crosses were heterozygous for several genetic markers including the mating-type-linked markers *thr4*, *leu2*, and *his4* on chromosome III. The zygote was formed by direct spore-to-cell fusion using a spore from a homothallic clone and a haploid vegetative cell from a heterothallic clone. Four-spored asci of a self-sporulated homothallic clone were dissected. In a typical experiment each of the four spores was placed on a nutrient agar film separately and was crossed with a heterothallic haploid cell of mating type *a* by spore-to-cell contact. These matings were carried out under a microscope with the aid of a micromanipulator. Results of the direct spore-to-cell mating experiments are listed in Table 1 as numbers of spores which directly fused with the haploid tester in each ascus. In addition to the present experiments, results of similar direct spore-to-cell crosses between a homothallic spore and a heterothallic haploid cell were collected from previous experiment. It is evident that each ascus contains not more than two spores which could fuse with a vegetative cell of a particular heterothallic haploid clone. Regardless of the mating type of the heterothallic tester, no more than two of the four homothallic spores were able to mate (Table 1). This evidence supports the contention that prior to germination there are 2*a* and 2α spores in the tetrads derived from homothallic strains.

Tetrad analyses of the hybrids between homothallic and heterothallic clones: A reasonable interpretation of the finding that two of four homothallic spores mate with an *a* heterothallic strain is that these two homothallic spores are mating type α (or α'). To test this possibility, hybrids produced by the foregoing direct spore-to-cell fusion were sporulated and the resulting four-spored asci were dissected. Many α segregants were obtained from every hybrid, even from the crosses where the α mating-type allele had never been involved, as shown in Table 2. The segregation pattern of the mating-type traits and the thallism for each cross indicated that the mating-type locus of the hybrids was heterozygous for *a* and α and that *HO*$_\alpha$ and *HM* had no linkage with the mating-type locus and with each other (TAKANO and OSHIMA 1970a). Standard genetic markers showed essentially the normal 2 : 2 segregation in these crosses. All the heterothallic segregants from these six crosses were tested for their combination of the mating-type traits and the three linked markers *thr4*, *leu2*, and *his4*. Results presented in Table 3 were scored as two classes, parental or recombinant, assuming that each homothallic spore behaved as either an *a* or α cell at the time of cell fusion, complementary to the mating type of its heterothallic haploid partner. In general, the frequencies of parentals and recombinants for gene-pairs of mating types and each genetic marker except the mating-type linked markers were almost equal. Re-

TABLE 1

Direct spore-to-cell mating experiments between homothallic clones originated from a (or a') HO_α HM *spores and heterothallic* ho_α hm *clones with various mating-type alleles*

Combination	Numbers of spores which directly fused with haploid tester in each ascus				
	4	3	2	1	0
a HO_α *HM** × *a* ho_α *hm*	0	0	1	2	1
× *a'* ho_α *hm*	0	0	3	2	1
× *α* ho_α *hm*	0	0	1	1	0
a' HO_α *HM** × *a* ho_α *hm*	0	0	2	4	0
× *a'* ho_α *hm*	0	0	1	2	3
× *α* ho_α *hm*	0	0	2	1	3
			Pooled data		
α HO_α *hm** × *a* ho_α *hm*	0	0	1	12	4
× *a'* ho_α *hm*	0	0	1	4	1
× *α* ho_α *hm*	0	0	6	8	10
HO_α *HM*† × *a* ho_α *hm*	0	0	2	5	2
× *a'* ho_α *hm*	0	0	3	2	0
× *α* ho_α *hm*	0	0	3	2	2
$\frac{a\ ho_\alpha\ hm}{\alpha\ ho_\alpha\ \mathrm{hm}}$ × *a* ho_α *hm*	0	0	3	1	0
× *α* ho_α *hm*	0	0	3	1	0
$\frac{a'\ ho_\alpha\ hm}{\alpha\ ho_\alpha\ \mathrm{hm}}$ × *a* ho_α *hm*	0	0	2	2	0
× *α* ho_α *hm*	0	0	3	0	1

Four-spored asci of self-sporulated homothallic clones or heterothallic diploids were dissected. Each tetrad spore was placed on a nutrient agar film separately and it was crossed with a haploid vegetative cell from a heterothallic clone by direct spore-to-cell contact under a microscope. Pooled data collected from other experiments.

* Homothallic diploid clone originated from the spore with the *a* (or *a'*) HO_α *HM* or *α* HO_α *hm* genotype.

† Genotypes for the mating-type locus of these homothallic diploid clones are not clear.

combination frequencies between the mating-type traits and the mating-type-linked markers, however, showed specific ratios similar to those of standard $a \times \alpha$ heterothallic crosses as shown in Table 3.

Comparative mapping of α and α' traits: An α mating-type character derived from the *a* or *a'* allele by contribution of HO_α and *HM*, here designated as α', was phenotypically stable and could not be distinguished from normal α either in its ability to mate with *a* cells and or in its sensitivity to the specific activity of HO_α. The α' character must be controlled by a stable gene located at the mating-type locus on chromosome III and it must be allelic with the *a* or *a'* allele because a regular $2a : 2\alpha$ segregation was observed in each ascus from heterothallic *a* (or α') $\times$ α' crosses. This was confirmed by statistical comparison of pooled tetrad distributions between the heterothallic $a' \times \alpha$ and $\alpha' \times \alpha'$ crosses for each gene-pair of the mating-type traits and the mating-type-linked markers. Tetrad distributions, i.e., the frequencies of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) tetrads in a doubly heterozygous mating, obtained for each of three

TABLE 2

Accumulated tetrad segregations from hybrids between homothallic clones originated from a (*or* a′) HO_α HM *spores and heterothallic* ho_α hm *clones with various mating-type alleles*

Combination	Segregation in asci (Homothallic : a : α) 2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2	3:0:1	3:1:0	1:3:0	0:3:1
a HO_α HM* × a ho_α hm	12	5	1	14	4	2	0	0	0	0
× a' ho_α hm	18	9	3	19	12	1	0	0	0	0
× α ho_α hm	13	2	2	11	4	1	0	1†	0	1†
a' HO_α HM* × a ho_α hm	21	12	1	21	6	2	1†	0	2†	0
× a' ho_α hm	7	10	0	15	4	0	0	0	1†	1†
× α ho_α hm	13	3	1	9	3	0	0	0	0	0
Expected ratio‡	12	6	1	12	4	1	0	0	0	0

Crosses were made by direct spore-to-cell fusion between spores from self-sporulated homothallic diploid clones and heterothallic haploid ho_α hm clones with various mating types (see footnote of Table 1).

* Homothallic diploid clone originated from the spore with the a (or a') HO_α HM genotype.

† These asci were considered to be aberrant.

‡ Expected phenotypic ratio was calculated inferring that HO_α and HM have no linkage with the mating-type locus and with each other.

gene-pairs: α'–*thr4*, α'–*leu2*, and α'–*his4* in $a' \times \alpha'$ crosses, are fairly homogeneous for each cross, and the pooled data are:

Pair of genes	PD NPD T
α'–*thr4*	47 : 0 : 19
α'–*leu2*	121 : 0 : 78
α'–*his4*	77 : 0 : 71

These data are comparable with those derived from similar gene pairs in $a' \times \alpha$ crosses: 383:5:165 (PD:NPD:T) ratio for α–*thr4* ($\chi^2 = 0.619$, $.75 > P > .50$); 333:6:249 for α–*leu2* ($\chi^2 = 2.833$, $.25 > P > .10$); and 242:13:273 for α–*his4* ($\chi^2 = 4.900$, $.10 > P > .5$).

DISCUSSION

These results support the following model of homothallism in yeast. Homothallic clones derived from an a (or a') HO_α HM spore give rise to diploids heterozygous for the mating-type alleles. Upon sporulation this diploid segregates $2a : 2\alpha$ spores in each ascus, since each ascus contains two or less spores which can make a zygote with a haploid vegetative clone (Table 1). If an α spore from the homothallic HO_α HM diploid is mated directly with an a heterothallic cell (a ho_α hm), the α state is fixed. This diploid, presumed to be $\frac{\alpha}{a}\frac{HO_\alpha}{ho_\alpha}\frac{HM}{hm}$, should segregate stable α clones (α ho_α) after meiotic sporulation. This prediction is supported by the results shown in Table 2. In the absence of the spore-to-cell mating it must be supposed that shortly after germination of the spore, the genes

TABLE 3

Recombination frequencies (in percent) between mating type and linked nutritional markers in crosses between homothallic clones originated from a (*or* a′) HO_α HM *spores and heterothallic* ho_α hm *clones with various mating-type alleles*

Combination	Heterothallic segregant with α									Heterothallic segregant with a								
	thr4			*leu2*			*his4*			*thr4*			*leu2*			*his4*		
	P	R	% R/(P+R)	P	R	% R/(P+R)	P	R	% R/(P+R)	P	R	% R/(P+R)	P	R	% R/(P+R)	P	R	% R/(P+R)
a HO_α *HM** × *a* ho_α *hm*	28	12	30	32	8	20	25	15	38	43	15	26	49	9	16	43	15	26
× *a′* ho_α *hm*	44	3	6	48	21	30	44	25	36	54	5	8	74	14	16	66	22	25
× *α* ho_α *hm*	..	..	..	32	7	18	27	12	31	..	..	..	40	9	18	33	16	33
a′ HO_α *HM** × *a* ho_α *hm*	..	..	..	54	7	11	30	5	14	..	..	..	89	14	14	45	8	15
× *a′* ho_α *hm*	24	7	23	27	4	13	27	4	13	57	10	15	53	14	21	51	16	24
× *α* ho_α *hm*	22	8	27	26	4	13	29	1	3	32	8	20	34	6	15	30	10	25
Control†																		
a ho_α *hm* × *α* ho_α *hm*	503	107	18	586	144	20	483	171	26									
a′ ho_α *hm* × *α* ho_α *hm*	352	62	15	319	87	21	270	118	30									

All the heterothallic segregants listed in Table 2 were scored as two classes, parental (P) and recombinant (R), assuming that each spore from the homothallic clone, upon direct spore-to-cell fusion, behaved as either an *a* or *α* cell complementary to the mating type of its heterothallic haploid partner.

* Homothallic diploid clone originated from the spore with the *a* (or *a′*) $HO_\alpha HM$ genotype.

† Data collected from previous experiments (TAKANO and OSHIMA 1970b). Those data for the *a* mating-type segregants were essentially the same as the data for the *α* segregants.

for homothallism cause conversion of the mating-type allele in a certain fraction of cells. The *a* and α cells in the spore culture mate and form a diploid. Thus, the meiotic products of $\frac{a}{\alpha}\frac{HO_\alpha}{HO_\alpha}\frac{HM}{HM}$ diploids are four homothallic clones.

A number of other possibilities will be considered regarding the origin of an α mating-type allele from the crosses between homothallic clones originated from *a* (or *a'*) HO_α *HM* spores and heterothallic *a* (or *a'*) ho_α *hm* clones. For example, it might be supposed that an α allele could be formed by mutation from *a* to α in the heterothallic parent. This possibility can be excluded by the linkage data listed in Table 3. Another possibility is that the *a* allele is contributed to the homothallic spore, but the direct spore-to-cell mating event involves a mutation of this *a* to α in the haploid homothallic spore. This possibility, however, seems unlikely, since not more than two spores in each four-spored ascus of homothallic clones could make a zygote directly with a heterothallic haploid cell (Table 1).

The facts presented here together with those from the previous paper (TAKANO and OSHIMA 1970b) indicate that the *a* and α mating-type alleles in Saccharomyces are interchangeable with each other by mutagenic action of the homothallism genes. Conversion from an *a* to an α allele requires both HO_α and *HM*, while only HO_α is required for conversion from α to *a*. The mutagenic effects of the homothallism genes on the mating-type locus are strictly locus specific (TAKANO and OSHIMA 1970b). Conversion of one mating-type allele to the other is performed with extraordinarily high frequency, probably 100%, within a few generations subsequent to spore germination. These observations cannot be explained by analogy with the mutator *mutT* in *Escherichia coli* (YANOFSKY, COX and HORN 1966; COX and YANOFSKY 1967) or analogy with the mutator gene in *Salmonella typhimurium* strain LT7 (KIRCHNER 1960; KIRCHNER and RUDDEN 1966). The activity of these bacterial mutators is not locus specific and their effects are not really comparable to the homothallism gene system. Our observations, however, do show some similarities to the specific transposable genic controllers of mutation in maize both in their locus-specific action and in their mode of modulation. MCCLINTOCK (1956) has called these genic units "controlling elements." Similar genic elements have been suggested in both eukaryotes such as Drosophila (GREEN 1967, 1969a, 1969b) and in prokaryotes such as Salmonella (DAWSON and SMITH-KEARY 1963).

To explain the foregoing observations, a hypothesis can be proposed that the elementary structure of the mating-type locus for both *a* and α is essentially the same. The association of some kind of controlling element with this locus would cause differentiation of two mating-type alleles. The mating-type locus on chromosome III would act as an affinity site for a controlling element. The mapping data (TAKANO and OSHIMA 1970b) indicated a slight difference (approximately 5 stranes) of the mapping position between *a* and *a'*, which suggests that the mating-type locus or the α mating-type allele occupies a rather wide region of chromosome III and that *a* and *a'* are different points of modification in this

locus. One of the two homothallism genes, HO_α or *HM*, could be concerned with the production of a controlling element; the other gene could control the association of the controlling element with its affinity site. Thus the mutagenic event at the mating-type locus appears to consist of several steps. In HO_α *HM* cells, the genic event may go to completion, whereas the event is interrupted at some step in haploid cells of HO_α *hm*, ho_α *HM*, and ho_α *hm* genotypes. Activities of the homothallism genes would be blocked as soon as heterozygosity of the mating-type alleles is established by zygote formation between cells with complementary mating types. The mechanism of repression is not yet clear.

The controlling elements in maize undergo transposition from one chromosomal location to another within the genome (McCLINTOCK 1956). In the homothallism gene system in Saccharomyces, however, the only affinity site for the controlling element so far tested (TAKANO and OSHIMA 1970b) is the mating-type locus.

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SUMMARY

This study demonstrated that the combination of two unlinked homothallism genes, HO_α and *HM*, brings about conversion of the *a* mating-type allele to α. Crosses were made between homothallic clones originated from *a* (or *a'*) HO_α *HM* spores and heterothallic ho_α *hm* clones with various mating-type alleles by the direct spore-to-cell mating method. The mating experiments suggested that each ascus consisted of $2a : 2\alpha$ spores. Many α clones segregated from every cross in tetrad analyses, even from the crosses where the α mating-type allele had never been involved. Recombination frequencies of the heterothallic segregants for mating type and markers linked to mating type obviously support the idea that each spore from the homothallic clone has either an *a* or α mating type complementary to its heterothallic haploid partner at the time of direct spore-to-cell fusion. An α mating-type trait, i.e., α', derived from the *a* or *a'* allele by the contribution of HO_α and *HM*, is proved to be controlled by an allele at the mating-type locus and could not be distinguished from normal α in its ability to form zygotes with *a* cells and in its response to HO_α.

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