DUPLICATED GENES PRODUCING TRANSPOSABLE CONTROLLING ELEMENTS FOR THE MATING-TYPE DIFFERENTIATION IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Mutation of the two homothallic genes, *HMLα/HMLa* and *HMRa/HMRα*, in homothallic strains of *Saccharomyces cerevisiae* was studied. Of 11 mutants of the *HMLα* gene, eight were due to a phenotypic mutation from *HMLα* to *HMLa*, *i.e.*, a mutation causing a change in function of the original *HML* allele to that of the other *HML* allele (functional mutation), and three were due to a defective mutation at the *HMLα* gene, *i.e.*, a mutation causing a nonfunctional allele (nonfunctional mutation). All 14 mutants of the *HMRa* gene, on the other hand, were due to a phenotypic mutation from *HMRa* to *HMRα* *i.e.*, a functional mutation. Phenotypic reverse mutations, *i.e.*, *HMLa* to *HMLα* and *HMRα* to *HMRa*, were also observed in the cultivation of EMS (ethyl methanesulfonate) treated spores having the *HO HMRα HMLa* genotype. Mutation from heterothallic cells to homothallism was observed in a nonfunctional mutant of the *HMLα* gene, by mutagenesis with EMS, but not in the functional mutants of the *HMLα* and *HMRa* genes or in the authentic strains having the *α HO HMRα HMLα* (*α Hp*) and **a** *HO HMRa HMLa* (**a** *Hq*) genotypes. These observations suggest that the functional mutation is not caused by the direct mutation from a homothallic allele to the opposite, but by replacement of a transposable genic element produced from a homothallic locus with a region of a different homothallic locus. These observations also support the controlling-element model and the cassette model, which have been proposed to explain the mating-type differentiation by the homothallic genes.

IN a homothallic strain of Saccharomyces, the interconversion of mating-type alleles occurs at extremely high frequency during vegetative growth of spores or cells (TAKANO and OSHIMA 1967; HICKS and HERSKOWITZ 1976), while in a heterothallic strain the conversion of mating-type allele occurs rarely, with the frequency of normal mutation (HICKS and HERSKOWITZ 1977). It is well established that the mating-type interconversions are promoted by three homothallic loci, *HO*, *HMR* and *HML*. In combination with the *HO* allele, the *HMRa* and *HMLa* alleles specify the *α* to **a** conversion, and the *HMLα* and *HMRa* alleles specify the **a** to *α* conversion.

As for the molecular mechanism of the mating-type differentiation by the

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homothallic genes, HARASHIMA, NOGI and OSHIMA (1974) have revised the controlling-element model that was previously proposed by OSHIMA and TAKANO (1971) and TAKANO, KUSUMI and OSHIMA (1973). Recently, HICKS and HERSKOWITZ (1977) proposed the cassette model, which seems basically similar to the controlling-element model. According to these models, mating-type interconversions are caused by the association with or insertion into the mating-type locus of products from the *HMR* and *HML* loci, mediated by the action of the *HO* allele. Since the α to **a** conversion is caused by the *HMR***a** and *HML***a** alleles and the **a** to α conversion by the *HML*α and *HMR*α alleles, these models also suggest that the *HMR***a** and *HML***a** alleles produce transposable elements having **a** mating-type information. Similarly, the *HML*α and *HMR*α alleles would produce transposable elements having α information. In recent years, various transposable genic elements have been reported in prokaryotic and eukaryotic cells. In prokaryotic cells, for example, the insertion of DNA fragments of R-factor into bacteriophage (BERG *et al.* 1975), of the mutator phage, Mu, into *E. coli* (HIRSH, STARLINGER and BRACHET 1972; HOWE and BADE 1975) are well known. In maize, transposable elements appear to be involved in the expression of specific genetic information (FINCHAM and SASTRY 1974). In *Drosophila melanogaster*, a controlling element at a location of one chromosome is spontaneously transposed to a new site on another chromosome (GREEN 1969). In *Saccharomyces cerevisiae*, transposition of an ochre (UAA) suppressor gene to two different sites has been demonstrated (LATEN *et al.* 1976). Transposable controlling elements and their transposition from one site to another may occur widely in both eukaryotic and prokaryotic cells.

In this study, genetic analyses were made of mutants of the *HML*α and the *HMR***a** genes, which were isolated in the previous work (OSHIMA and TAKANO 1980), and of mutants of the *HML***a** and *HMR*α genes. The results suggested that a functional *HM* (*HML* or *HMR*) allele is mutated to the alternative functional allele by transposition of a genic element produced from an *HM* locus into the other *HM* locus.

MATERIALS AND METHODS

Strains: All the standard homothallic and heterothallic strains listed in Table 1 were selected from our genetic stock cultures. Mutant strains of the *HML*α and *HMR***a** genes were selected from mutants isolated by ethyl methanesulfonate mutagenesis of ascospores of the *HO HMR***a** *HML*α homothallic strain, T-1851-2D (OSHIMA and TAKANO 1980). Mutant strains of the *HML***a** and *HMR*α genes were isolated from single spore cultures of a perfect homothallic strain, C-18-16B, by the procedures reported (OSHIMA and TAKANO 1980), except that colonies having mating potency were detected at 30° instead of 35°.

Media: Media for vegetative growth of strains, sporulation and auxotrophic-trait determination are described in the foregoing paper (OSHIMA and TAKANO 1980).

Detection of mutation from heterothallism to homothallism: Mutation from heterothallism to homothallism was detected by sporulation ability of cells. Cells of **a** or α mating type were shaken in YPD medium at 30° for 16 to 18 hr and were treated with EMS by the procedures employed for isolation of mutants from homothallism to heterothallism (OSHIMA and TAKANO 1980). The treated cells were collected on a membrane filter of 0.45 μm pore-size and washed twice with 5% sodium thiosulfate. The washed cells were suspended in sterilized water to give

TABLE 1

Standard strains for homothallism used in this study

Strain no.	Genotype*	Remarks
T-1171-5D	**a** *HO HMR***a** *HML*α *lys2 thr4* / α *HO HMR***a** *HML*α *lys2 thr4*	Type II *Ho*† homothallic diploid
C-18-16D	**a** *HO HMR*α *HML***a** *lys2 his4 trp1* / α *HO HMR*α *HML***a** *lys2 his4 trp1*	Type I *Ho*† homothallic diploid
S-14-9C	**a** *HO HMR*α *HML*α *lys2 his4 leu2* / α *HO HMR*α *HML*α *lys2 his4 leu2*	*Hp*† type of homothallic diploid
S-14-9C-1A	α *HO HMR*α *HML*α *lys2 his4 leu2*	*Hp* type of heterothallic haploid
T-1023-23B	**a** *HO HMR***a** *HML***a** *ade1 lys2 his4 leu2 trp1 arg4* / α *HO HMR***a** *HML***a** *ade1 lys2 his4 leu2 trp1 arg4*	*Hq*† type of homothallic diploid
T-1023-23B-1A	**a** *HO HMR***a** *HML***a** *ade1 lys2 his4 leu2 trp1 arg4*	*Hq* type of heterothallic haploid
J-1-2B	**a** *ho HMR***a** *HML*α *ura3*	Standard **a** mating type
J-1-5A	α *ho HMR***a** *HML*α *ura3*	Standard α mating type

* Revised nomenclature is employed for the homothallci genes (ARIMA and TAKANO 1979).

† These symbols have been given to homothallic strains depending on the segregation of mating types in asci (HARASHIMA, NOGI and OSHIMA 1974). Symbols *Hp* and *Hq* are also assigned to heterothallic segregants obtained from the *Hp* and *Hq* types of homothallic diploids, respectively.

a concentration of approximately 10^8 cells per ml, and 0.2 ml portions of the suspension were spread on YPD agar plates. The plates were incubated at 30° for 16 hr and were replicated on the sporulation agar plates. After 3 days of incubation at 30°, the cells on the sporulation medium were collected and observed microscopically to confirm ascus formation. Frequency of heterothallic to homothallic mutation was scored by dividing the number of asci by that of nonsporulating cells.

Genetic methods: The procedures for mating-type determination, hybridization, sporulation and tetrad dissection were described in the previous paper (OSHIMA and TAKANO 1980).

RESULTS AND DISCUSSION

Genetic analysis on mutants of the HML*α and* HMR**a** *genes:* In an earlier study (OSHIMA and TAKANO 1980), we isolated mutants of the *HMLα* and *HMR***a** genes showing altered functions from the originals. Two alternative mechanisms were proposed for the mutations: (1) the functional *HM* (*HML* or *HMR*) allele is mutated to the alternative functional allele, *i.e.*, functional mutation from *HMLα* to *HML***a** or *HMR***a** to *HMRα*, and (2) the functional allele is mutated to a nonfunctional allele, i.e., nonfunctional mutation from *HMLα* to *hmlα* or *HMR***a** to *hmr***a**, where *hmlα* and *hmr***a** indicate mutation of the *HMLα* and *HMR***a** to the respective nonfunctional alleles. If the former type of mutation occurred, the mutant from the *HO HMR***a** *HMLα* spore would have a genotype equivalent to **a** *HO HMR***a** *HML***a** or *α HO HMRα HMLα*. If the latter possibility occurred, the mutant would have the **a** *HO HMR***a** *hmlα* or *α HO hmr***a** *HMLα* genotype. These two possible mutations could not be distinguished by tetrad analysis of diploid hybrids prepared by crossing the mutants with the *HO HMR***a** *HMLα* and *ho HMR***a** *HMLα* standard strains, as described in the preceding paper (OSHIMA and TAKANO 1980), since the *HO HMR***a** *HML***a** and *HO HMR***a** *hmlα* genotypes and the *HO HMRα HMLα* and *HO hmr***a** *HMLα* genotypes, respectively, give rise to the same patterns of segregation for mating types and homothallism in asci of the hybrids.

To distinguish these two possibilities, we constructed diploid hybrids between the two mutants (one having the mutation at *HMLα* and the other at *HMR***a**), and the hybrids were subjected to tetrad analysis. Four different types of combinations can be expected for the configuration of the homothallic genes in the hybrids. These four types of hybrids will show different segregation patterns for mating type and homothallism in asci (Table 2). Since the *HMR* and *HML* loci are very loosely linked to each other and to the mating-type locus on chromosome III (HARASHIMA and OSHIMA 1976), and *HO* segregates independently, frequencies of each ascus type were calculated by assuming that the homothallic loci and the mating-type locus segregate independently. Fifteen diploid hybrids were constructed between the 11 mutants of the *HMLα* gene and 14 *HMR***a** mutants, and these were dissected after sporulation. All the diploid hybrids showed high spore viability. The observed segregation patterns for mating type and homothallism (Table 3) were compared with the expected ones listed in Table 2. Although the distribution of ascus types differed slightly among the diploid strains, 12 of the 15 diploid hybrids showed segregation patterns similar to those of the type 1 hybrid listed in Table 2, with one ascus showing an unex-

TABLE 2

Theoretical segregations of mating types and homothallism in asci of hybrids obtained by four possible combinations of crosses between the mutants of the HML*α and* HMR**a** *genes**

Hybrids Type	Supposed genotype	Ascus-type I: **a** **a** *α* *α*	II: **a** **a** *α* hom	III: **a** **a** hom hom	IV: **a** *α* *α* hom	V: **a** *α* hom hom	VI: **a** hom hom hom	VII: *α* *α* hom hom	VIII: *α* hom hom hom	IX: hom† hom hom hom
1	$\frac{\mathbf{a}\ HO\ HMR\mathbf{a}\ HML\mathbf{a}}{\alpha\ HO\ HMR\alpha\ HML\alpha}$	1	0	0	0	12	4	0	4	15
2	$\frac{\mathbf{a}\ HO\ HMR\mathbf{a}\ hml\alpha}{\alpha\ HO\ hmr\mathbf{a}\ HML\alpha}$	1	4	1	4	16	4	1	4	1
3	$\frac{\mathbf{a}\ HO\ HMR\mathbf{a}\ hml\alpha}{\alpha\ HO\ HMR\alpha\ HML\alpha}$	1	0	0	4	12	0	1	12	6
4	$\frac{\mathbf{a}\ HO\ HMR\mathbf{a}\ HML\mathbf{a}}{\alpha\ HO\ hmr\mathbf{a}\ HML\alpha}$	1	4	1	0	12	12	0	0	6

* Theoretical frequencies of each ascus type were calculated by assuming that the mating-type locus and the homothallic loci segregated independently.
† Indicates homothallism.

TABLE 3

Segregation data observed in various combinations of crosses between HML*α and* HMR**a** *mutants*

Hybrid no.	Cross *HML*α mutant	Cross *HMR***a** mutant	Asci tested	Ascus type I: **a** **a** *α* *α*	II: **a** **a** *α* hom	III: **a** **a** hom hom	IV: **a** *α* *α* hom	V: **a** *α* hom hom	VI: **a** hom hom hom	VII: *α* *α* hom hom	VIII: *α* hom hom hom	IX: hom* hom hom hom	Expected hybrid type*
J-111	1–19 ×	3–4	32	1	0	0	0	10	10	0	5	6	1
J-115	1–11 ×	3–1	19	1	0	0	0	8	4	0	2	5	1
J-116	3–3 ×	3–12	42	1	0	0	4	15	0	2	17	3	3
J-118	3–2 ×	2–11	39	1	0	0	0	16	5	0	7	10	1
J-119	2–8 ×	1–8	24	1	0	0	0	10	2	0	3	8	1
J-221	4–18 ×	5–28	37	4	0	0	5	13	0	6	9	0	3
J-222	4–3 ×	6–33	28	1	0	0	0	8	5	0	3	11	1
J-224	5–9 ×	5–20	30	1	0	0	0	12	4	0	2	11	1
J-225	5–19 ×	5–16	24	0	0	0	1†	14	0	0	2	7	1
J-226	5–9 ×	5–12	26	2	0	0	0	8	7	0	4	5	1
J-227	1–19 ×	5–20	19	0	0	0	0	8	2	0	1	8	1
J-228	1–19 ×	6–9	32	2	0	0	1†	10	10	0	4	5	1
J-229	1–19 ×	6–28	20	0	0	0	0	9	1	0	1	9	1
J-230	1–19 ×	4–4	20	1	0	0	0	7	2	0	3	7	1
J-234	5–6 ×	3–4	17	0	1†	0	4	3	0	3	3	1	3
J-4‡	*α Hp* ×	**a** *Hq*	25	2	1†	0	0	8	3	0	2	9	1

* See Table 2.
† These asci were considered to be aberrant.
‡ Diploid hybrid between the standard *α Hp* (S–14–9C–1A) and **a** *Hq* (T–1023–23B–1A) strains.

pected 1 homothallic:1**a**:2α segregation in two hybrids, J-225 and J-228. The remaining three hybrids, J-116, J-221 and J-234, showed segregations similar to each other, but different from those of the other 12 hybrids. These three showed the segregation of the type 3 hybrid (Table 2) with a slight difference and with an unexpected ascus (ascus type II) from the type 3 hybrid, J-234. The most significant difference between these two classes of segregation was seen in ascus types IV, VI and VII. These results indicate that of the 11 mutants of the *HML*α gene, three (3–3, 4–18 and 5–6) are due to a nonfunctional mutation at the *HML*α locus, while all other mutants of the *HML*α and *HMR***a** genes are caused by a mutation of the original allele to the alternative functional allele. Thus, eight of the 11 *HML*α mutants tested were suspected to have an **a** *HO HMR***a** *HML***a** genotype and three to have an **a** *HO HMR***a** *hml*α genotype. All 14 *HMR***a** mutants tested, on the other hand, were suspected to have an α *HO HMR*α *HML*α genotype.

Evidence for the functional mutation of the HMLα *and* HMR**a** *genes:* To confirm the mutation of *HML*α and *HMR***a** to the alternative functional alleles, meiotic segregants obtained from the diploid hybrids showing the segregation of the type 1 hybrid (Table 2) were subjected to further genetic analysis. If a mutation to the alternative functional allele, namely, *HML*α to *HML***a** and *HMR***a** to *HMR*α occurred, all but three of the hybrids (J-116, J-221 and J-234) listed in Table 3 should have the genotype of the type 1 hybrid, *HO/HO HMR***a**/*HMR*α *HML***a**/*HML*α. It is known that both the **a** or α *HO HMR***a** *HML*α and **a** or α *HO HMR*α *HML***a** genotypes give rise to perfect homothallism of the *Ho* type, while the **a** *HO HMR*α *HML*α or α *HO HMR***a** *HML***a** genotype gives rise to a semi-homothallism of the *Hp* or *Hq* type (HARASHIMA, NOGI and OSHIMA 1974). Therefore, some of the asci showing ascus type IX in the type 1 hybrid (Table 2) should yield four perfect homothallic segregants (*Ho* type of homothallism) in which two clones in each tetrad are derived from the *HO HMR***a** *HML*α genotype and the other two from the *HO HMR*α *HML***a** genotype, while such asci should not be observed in the other three types of hybrid, types 2, 3 and 4, listed in Table 2.

To test this inference, 10 asci showing ascus type IX were selected from those hybrids that showed type 1 hybrid segregation, and the four homothallic segregants in each ascus were dissected after sporulation. All segregants from six of the selected ten asci showed the *Ho* type of homothallism, *i.e.*, 4 homothallic:0 heterothallic segregation in asci. Four segregants in each tetrad of the other four selected asci gave two of the *Ho* type, one the *Hp* and one the *Hq* type of homothallism. These results support the idea that the diploid hybrids in question have the genotype of the type 1 hybrid.

As described above, the asci yielding four *Ho* type of homothallic segregants should contain two spores of the *HO HMR***a** *HML*α genotype and two of the *HO HMR*α *HML***a** genotype. To examine this possibility, one of the asci of ascus type IX obtained from hybrid J-111 (Table 3) was analyzed further. Four different combinations of crosses were made by spore-to-spore mating among the four *Ho* type of homothallic segregants from an ascus of hybrid J-111, and the

four resultant hybrids were dissected after sporulation (Table 4). Two of the four hybrids, J-111-4A × J-111-4D and J-111-4B × J-111-4C, yielded only an ascus type showing a 4 homothallic:0 heterothallic segregation, and the other two hybrids, J-111-4A × J-111-4C and J-111-4B × J-111-4D, gave rise to various ascus types. Then, diploid strains were constructed by crossing J-111-4A and J-111-4B with the standard homothallic strain, J-1171-5D, having the *HO HMR***a** *HML*α genotype, by spore-to-spore mating. The resultant diploid strains were studied with respect to their segregation, and it was found that J-111-4A has the *HO HMR*α *HML***a** genotype and that J-111-4B has the *HO HMR***a** *HML*α genotype. It could also be concluded that J-111-4C has the *HO HMR***a** *HML*α genotype and that J-111-4D has the *HO HMR*α *HML***a** genotype. These observations clearly indicate that hybrid J-111 (1–19 × 3–4; Table 3) had the *HO/HO HMR*α/*HMR***a** *HML*α/*HML***a** genotype. In other words, mutant 1–19 was caused by a mutation from *HML*α to *HML***a** and mutant 3–4 by a mutation from *HMR***a** to *HML*α. Thus, we can conclude that a functional allele can mutate to the other functional allele in the homothallism gene system.

Functional mutations of the HML**a** *and* HMRα *loci:* In the foregoing experiments, we demonstrated the possibility of the mutation of the *HML*α and *HMR***a** genes to their respective opposite alleles. To examine the possibility of the reverse mutation, *i.e.*, *HML***a** to *HML*α and *HMR*α to *HMR***a**, we attempted to isolate heterothallic clones from ascospores having the *HO HMR*α *HML***a** genotype, basically by the same procedure as employed in the previous study (OSHIMA and TAKANO 1980). In this experiment, mutants were derived from the parental strain C-18-16B (Table 1). Colonies showing **a** or α mating potency were observed with almost the same frequency as in the previous experiment with the *HO HMR***a** *HML*α parental strain; they were tested for their mutations by segregation of mating types and homothallism in asci of diploid hybrids obtained by crossing with the standard strains having *ho HMR*α *HML***a** and *HO HMR*α *HML***a** genotypes. Although various types of mutations in the homothallism and

TABLE 4

Genetic analysis of four homothallic segregants in an ascus type IX from a hybrid J-111

Cross*	Segregation in asci					Asci tested
	a **a** α α	**a** α hom hom	**a** hom hom hom	α hom hom hom	hom† hom hom hom	
J-111-4A × J-111-4C	1	4	3	0	8	16
J-111-4A × J-111-4D	0	0	0	0	21	21
J-111-4B × J-111-4C	0	0	0	0	20	20
J-111-4B × J-111-4D	0	10	2	2	5	19
J-111-4A × T-1171-5D	0	8	2	1	9	20
J-111-4B × T-1171-5D	0	0	0	0	13	13

* All crosses were made by spore-to-spore mating. J-111-4A, -4B, -4C and -4D are segregants in an ascus type IX from hybrid J-111 (Table 3). T-1171-5D is a standard strain of type II *Ho* homothallism (Table 1).

† Indicates homothallism.

mating systems were observed among the colonies showing mating potency, we selected only the mutants of the *HML***a** and *HMR*α genes in this experiment. Three of the 10 colonies showing α mating type were thought to be due to a mutation at the *HML***a** gene and two of the 10 colonies showing **a** mating type were thought to be a mutation at the *HMR*α gene. The results of genetic analysis on an *HML***a** mutant, 16B-1, and an *HMR*α mutant, 16B-20, are shown in Table 5. A hybrid, J-602, obtained by mating a cell of 16B-1 and a spore of the *HO HMR*α *HML*α (S-14-9C; Table 1) genotype, showed a 2 homothallic:2α segregation (*Hp* type of segregation) in all asci. A nonfunctional mutation of the *HML***a** gene, *i.e.*, an *HML***a** to *hml***a** mutation, would be unlikely, since no segregants showing **a** mating type were observed in asci of hybrid J-542 (16B-1 × *HO HMR***a** *HML*α; Table 5). If 16B-1 had carried a nonfunctional allele of *HML***a**, the hybrid (J-542) would have yielded segregants of **a** mating type with the *HO HMR***a** *hml***a** genotype. This suggested that 16B-1 has the *HO HMR*α *HML*α genotype and carries a mutation from *HML***a** to *HML*α. Segregation patterns from two other hybrids, J-521 (16B-1 × *HO HMR*α *HML***a**) and J-601 (16B-1 × *HO HMR***a** *HML***a**), are compatible with the idea of the functional mutation at the *HML***a** gene. Similarly, mutant 16B-20 was suspected of having the *HO HMR***a** *HML***a** genotype, since hybrid J-606 (16B-20 × *HO HMR***a** *HML***a**; Table 5) showed a 2 homothallic:2**a** (*Hq* type) segregation and hybrid J-552 (16B-20 × *HO HMR***a** *HML*α) did not yield heterothallic segregants of α mating type in asci tested so far. Thus, it can be concluded that a functional mutation of the *HMR*α gene, i.e., an *HMR*α to *HMR***a** mutation, occurred in 16B-20. Results suggesting a nonfunctional mutation at the *HML***a** and *HMR*α genes, *i.e.*, *HML***a** to *hml***a** and *HMR*α to *hmr*α mutations, were not observed in the mutants isolated from the *HO HMR*α *HML***a** strain, C-18-16B.

Proposed models for the functional mutation of the homothallic genes: To explain the molecular mechanism of mating-type interconversion by the homothallic genes, several models have been proposed (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). In the controlling-element model and the cassette model, the mating-type differentiation is caused by association with or insertion into the mating-type locus of the products (controlling elements or cassettes) from the *HMR***a**/*HMR*α and *HML*α/*HML***a** loci, mediated by the function of the *HO* allele. This argument suggests that the two homothallic loci, *HMR***a**/*HMR*α and *HML*α/*HML***a**, may have a common structure, since products of both loci can associate with the mating-type locus. This speculation leads to two alternative models, shown in Figure 1, that can explain the present observations of mutation to the opposite allele in the two homothallic loci. The first possibility is that the mutation from the *HML*α to the *HML***a** allele, or from the *HMR***a** to the *HMR*α allele, and *vice versa*, in both loci is caused by such mutational events as a base change or a flame shift in the DNA sequence at the mutant loci. The second possibility is that the controlling element or the cassette, for example, from the *HMR***a** locus is replaced by a segment of the *HML*α locus. In other

TABLE 5

Segregation data of mating types and homothallism in asci of hybrids obtained by crossing mutants of the HML**a** *and* HMR*α* *genes with the standard strains for homothallism*

Hybrid no.	Cross: Mutant*	Standard	Asci tested	Ascus type I: **a** **a** *α* *α*	II: **a** **a** *α* hom	III: **a** **a** hom hom	IV: **a** *α* *α* hom	V: **a** *α* hom hom	VI: **a** hom hom hom	VII: *α* *α* hom hom	VIII: *α* hom hom hom	IX: hom† hom hom hom	Supposed genotype of mutant
J-521	16B-1 ×	*HO HMRα HML***a**	21	0	0	0	0	0	0	6	10	5	*HO HMRα HMLα*
J-542	16B-1 ×	*HO HMR***a** *HMLα*	26	0	0	0	0	0	0	5	20	1	*HO HMRα HMLα*
J-601	16B-1 ×	*HO HMR***a** *HML***a**	34	1	0	0	0	13	7	0	3	10	*HO HMRα HMLα*
J-602	16B-1 ×	*HO HMRα HMLα*	16	0	0	0	0	0	0	16	0	0	*HO HMRα HMLα*
J-540	16B-20 ×	*HO HMRα HML***a**	20	0	0	6	0	0	13	0	0	1	*HO HMR***a** *HML***a**
J-552	16B-20 ×	*HO HMR***a** *HMLα*	20	0	0	6	0	0	14	0	0	0	*HO HMR***a** *HML***a**
J-606	16B-20 ×	*HO HMR***a** *HML***a**	13	0	0	13	0	0	0	0	0	0	*HO HMR***a** *HML***a**
J-605	16B-20 ×	*HO HMRα HMLα*	16	1	0	0	0	3	2	0	7	3	*HO HMR***a** *HML***a**
J-607‡	16B-1 ×	16-20	18	0	0	0	0	11	0	0	3	4	

* 16B-1 showing *α* mating type and 16B-20 showing **a** mating type are mutants of the *HML***a** and *HMRα* genes, respectively. These mutants were obtained by cultivation of EMS-treated ascospores from a perfect homothallic strain C-18-16B having the *HO HMRα HML***a** genotype.

† Indicates homothallism.

‡ Hybrid between the *HML***a** (16B-1) and *HMRα* (16B-20) mutants.

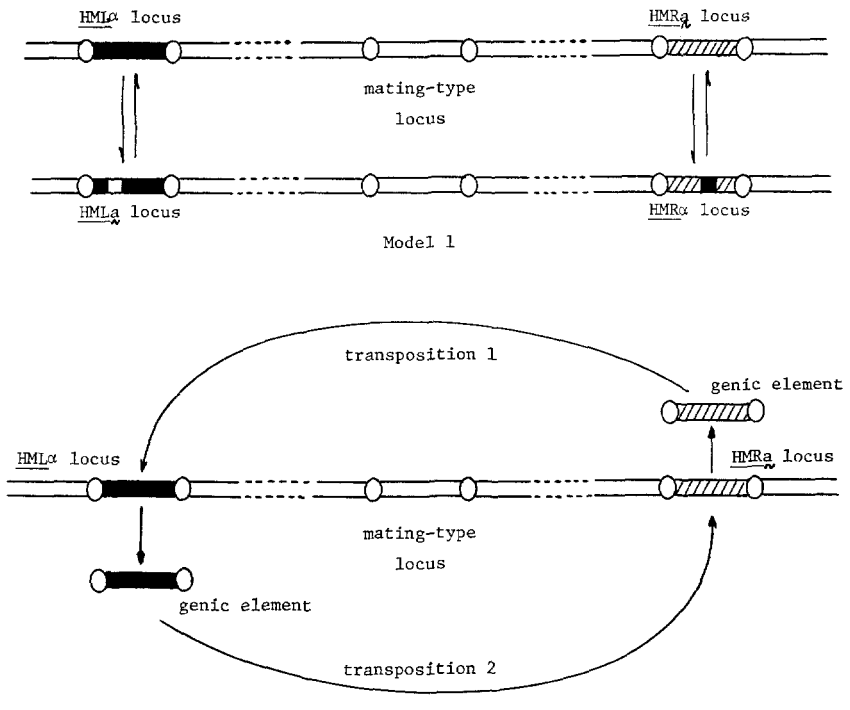

FIGURE 1.—Two possible models for functional mutations of the *HMLα/HMLa* and *HMRa/HMRα* genes. Model 1 indicates the direct mutation of a homothallic gene to the opposite functional allele. Model 2 indicates transposition of a genic element produced by a homothallic locus to another homothallic locus. Transposition 1 causes a phenotypic change of *HMLα* to *HMLa* and transposition 2 causes a phenotypic change to *HMRa* to *HMLα*.

words, a specific transposable genic element is produced, for example, by the *HMRa* locus that can replace a DNA segment at the *HMLα* locus. It is not easy to determine which mechanism operates by mapping the mutant loci or by linkage analysis, since we have not identified any genetic trait carried by the *HM* (*HML* and *HMR*) loci or closely linked to the loci.

However, these models (Figure 1) were tested by the following protocol: In the first model, perfect homothallic diploid strains of two *Ho* types, *HO/HO HMRα/HMRα HMLa/HMLa* and *HO/HO HMRa/HMRa HMLα/HMLα*, would be expected from a heterothallic haploid cell of *α Hp* or **a** *Hq* (*α HO HMRα HMLα* or **a** *HO HMRa HMLa*) by the direct mutation of *HMLα* allele to *HMLa*, *HMRa* allele to *HMRα*, or *vice versa*. In the second model, on the other hand, such homothallic diploid cells would not be expected if the controlling elements model or the cassette model is assumed since an *α Hp* strain of the *HO HMRα HMLα* genotype would not have an element or cassette for **a** mating type. Similarly, the **a** *Hq* strain has no element or cassette for *α* mating type. To test these possibilities, an *α Hp* and an **a** *Hq* strain, S-14-9C-1A and T-1023-23B-1A, were

subjected to mutagenesis with EMS. In addition to these authentic *Hp* and *Hq* strains, two mutants, 1–19 and 3–4, which were supposed to have the alternative functional alleles from the original *HMLα* and *HMR***a** alleles, respectively, and one mutant, 3–3, which was supposed to have a nonfunctional allele of *HMLα*, were subjected to the EMS mutagenesis. Frequencies of occurrence of homothallic cells were scored by counting asci among the heterothallic cells after cultivation or sporulation medium (Table 6). No asci were observed from the two functional mutants, 1–19 and 3–4, or from the authentic *α Hp* and **a** *Hq* strains, while a few asci were detected in the nonfunctional mutant, 3–3, at a frequency of approximately 10^{-3}. These results indicate that the second model in Figure 1 is more likely, *i.e.*, specific transposable genic elements participate in homothallism-controlling system of Saccharomyces yeasts.

Since the *HMLα* and *HMRα* alleles cause the **a** to *α* conversion and the *HMR***a** and *HML***a** alleles cause the *α* to **a** conversion in combination with the *HO* allele, it is possible to suppose that the mating-type locus has a specific site for all the products of these homothallic genes. This argument suggests that in the functional mutants, 1–19 and 3–4, the product from an *HM* locus could be inserted into the other *HM* locus instead of into the mating-type locus. The possibility of the second model (Figure 1) strongly suggests that some regions of the genic elements and the mating-type locus have genetic material of similar structure, possibly the DNA sequences. In the nonfunctional mutant, 3–3, some alteration of the base sequence would occur at the *HMLα* locus, and the normal transposable element might not be produced or an abnormal element might be produced. In fact, a diploid strain obtained by forced mating of the nonfunctional mutant, 3–3, and the **a** *ho HMR***a** *HML***a** standard strain gave sterile segregants of *α* mating type in tetrads (data will be described elsewhere). This observation suggests that the mutant allele (*hmlα*) in this mutant might produce a defective genic element that could associate with the mating-type locus but not give a normal function for *α* mating type. Thus, our observations strongly suggest that specific transposable elements control the differentiation of mating-type alleles in Saccharomyces yeasts. The controlling elements may be DNA fragments. It is hard

TABLE 6

*Frequency of mutation to homothallism in various heterothallic strains treated with EMS**

No.	Strain† Genotype	No. of cells tested	No. of asci observed	Frequency
S-14-9C-1A	*α HO HMRα HMLα*	1.5×10^5	0	$< 6.7 \times 10^{-6}$
T-1023-23B-1A	**a** *HO HMR***a** *HML***a**	1.0×10^5	0	$< 1.0 \times 10^{-5}$
1–19	**a** *HO HMR***a** *HML***a**	1.3×10^5	0	$< 7.7 \times 10^{-6}$
3–4	*α HO HMRα HMLα*	1.2×10^5	0	$< 8.3 \times 10^{-6}$
3–3	**a** *HO HMR***a** *hmlα*	3.6×10^4	44	1.4×10^{-3}

* The mutations were detected by sporulation ability of cells among the heterothallic strains.
† S-14-9C-1A and T-1023-23B-1A are authentic strains. Strains 1–19 and 3–4 are functional mutants of the *HMLα* and *HMR***a** genes, respectively, and strain 3–3 is a nonfunctional mutant of the *HMLα* gene. Genotypes expected from the observed segregations listed in Table 3 were given to these three mutants.

to explain our observations by the flip-flop model proposed by HOLLIDAY and PUGH (1975) and BROWN (1976).

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