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, HMLa/HMLa and HMRa/HMRa, in homothallic strains of Saccharomyces cerevisiae was studied. Of 11 mutants of the HMLa gene, eight were due to a phenotypic mutation from HMLa 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 HMLa gene, i.e., a mutation causing a non-functional allele (nonfunctional mutation). All 14 mutants of the HMRa gene, on the other hand, were due to a phenotypic mutation from HMRa to HMRa i.e., a functional mutation. Phenotypic reverse mutations, i.e., HMLa to HMLa and HMRa to HMRa, were also observed in the cultivation of EMS (ethyl methanesulfonate) treated spores having the HO HMRa HMLa genotype. Mutation from heterothallic cells to homothallism was observed in a nonfunctional mutant of the HMLa gene, by mutagenesis with EMS, but not in the functional mutants of the HMLa and HMRa genes or in the authentic strains having the \( a \) HO HMRa HMLa (\( a \) 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 \( a \) to \( a \) conversion, and the HMLa and HMRa alleles specify the \( a \) to \( \alpha \) 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 HMRα and HMLα alleles and the α to a conversion by the HMLα and HMRα alleles, these models also suggest that the HMRα and HMLα alleles produce transposable elements having a mating-type information. Similarly, the HMLα and HMRα alleles would produce transposable elements having a 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 HMLα and the HMRα genes, which were isolated in the previous work (Oshima and Takano 1980), and of mutants of the HMLα and HMRα 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 HMLα and HMRα genes were selected from mutants isolated by ethyl methanesulfonate mutagenesis of ascospores of the HO HMRα HMLα homothallic strain, T-1851-2D (Oshima and Takano 1980). Mutant strains of the HMLα and HMRα 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 a 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>
<thead>
<tr>
<th>Strain no.</th>
<th>Genotype*</th>
<th>Remarks</th>
</tr>
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<tr>
<td>T-1171-5D</td>
<td>a HO HMRa HMLa lys2 thr4</td>
<td>Type II H0† homothallic diploid</td>
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<td></td>
<td>a HO HMRa HMLa lys2 thr4</td>
<td></td>
</tr>
<tr>
<td>C-18-16D</td>
<td>a HO HMRa HMLa lys2 his4 trp1</td>
<td>Type I H0† homothallic diploid</td>
</tr>
<tr>
<td></td>
<td>a HO HMRa HMLa lys2 his4 trp1</td>
<td></td>
</tr>
<tr>
<td>S-14-9C</td>
<td>a HO HMRa HMLa lys2 his4 leu2</td>
<td>Hp† type of homothallic diploid</td>
</tr>
<tr>
<td></td>
<td>a HO HMRa HMLa lys2 his4 leu2</td>
<td></td>
</tr>
<tr>
<td>S-14-9C-1A</td>
<td>a HO HMRa HMLa lys2 his4 leu2</td>
<td>Hp type of heterothallic haploid</td>
</tr>
<tr>
<td>T-1023-23B</td>
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<td>Hp† type of homothallic diploid</td>
</tr>
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<tr>
<td>T-1023-23B-1A</td>
<td>a HO HMRa HMLa ade1 lys2 his4 leu2 trp1 arg4</td>
<td>Hp type of heterothallic haploid</td>
</tr>
<tr>
<td>J-1-2B</td>
<td>a ho HMRa HMLa ura3</td>
<td>Standard α mating type</td>
</tr>
<tr>
<td>J-1-5A</td>
<td>a ho HMRa HMLa ura3</td>
<td>Standard α mating type</td>
</tr>
</tbody>
</table>

* Revised nomenclature is employed for the homothallic genes (Arima and Takano 1979).
† These symbols have been given to homothallic strains depending on the segregation of mating types in asci (Harashima, Noz 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 asus formation. Frequency of heterothallic to homothallic mutation was scored by dividing the number of ascis by that of non-sporulating 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 HMLα and HMRα genes: In an earlier study (OSHIMA and TAKANO 1980), we isolated mutants of the HMLα and HMRα 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 HMLα or HMRα to HMRα, and (2) the functional allele is mutated to a nonfunctional allele, i.e., nonfunctional mutation from HMLα to hmlα or HMRα to hmra, where hmlα and hmra indicate mutation of the HMLα and HMRα to the respective nonfunctional alleles. If the former type of mutation occurred, the mutant from the HO HMRα HMLα spore would have a genotype equivalent to a HO HMRα HMLα or a HO HMRα HMLα. If the latter possibility occurred, the mutant would have the a HO HMRα hmlα or a HO hmra HMLα genotype. These two possible mutations could not be distinguished by tetrad analysis of diploid hybrids prepared by crossing the mutants with the HO HMRα HMLα and ho HMRα HMLα standard strains, as described in the preceding paper (OSHIMA and TAKANO 1980), since the HO HMRα HMLα and HO HMRα hmlα genotypes and the HO HMRα HMLα and HO hmra HMLα genotypes, respectively, give rise to the same patterns of segregation for mating types and homothallism in ascis 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 HMRα), 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 ascis (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 HMRα 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 HMLa and HMRa genes

<table>
<thead>
<tr>
<th>Hybrids Type</th>
<th>Supposed genotype</th>
<th>Acus-type</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
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<td>a</td>
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<td>hom</td>
<td>a</td>
<td>hom</td>
<td>hom</td>
<td>hom</td>
<td>hom</td>
<td>hom</td>
</tr>
<tr>
<td>1</td>
<td>a HO HMRa HMLa</td>
<td>a HO HMRa HMLa</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>a HO HMRa hmla</td>
<td>a HO hmla HMLa</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>16</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>a HO HMRa hmla</td>
<td>a HO HMLa HMLa</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>12</td>
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<td>4</td>
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<td>4</td>
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<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
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</table>

* 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 HMLa and HMRa mutants

<table>
<thead>
<tr>
<th>Hybrid no.</th>
<th>Crosses</th>
<th>Ascus</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>Expected hybrid type*</th>
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<tr>
<td>J-111</td>
<td>1-19 X 3-4</td>
<td>32</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>J-115</td>
<td>1-11 X 3-1</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>J-116</td>
<td>3-3 X 3-12</td>
<td>42</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>15</td>
<td>0</td>
<td>2</td>
<td>17</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>J-118</td>
<td>3-2 X 2-11</td>
<td>39</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>J-119</td>
<td>2-8 X 1-8</td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>J-221</td>
<td>4-18 X 5-28</td>
<td>37</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>13</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>J-222</td>
<td>4-3 X 6-33</td>
<td>28</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>J-224</td>
<td>5-9 X 5-20</td>
<td>30</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>J-225</td>
<td>5-19 X 5-16</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>J-226</td>
<td>5-9 X 5-12</td>
<td>26</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>J-227</td>
<td>1-19 X 5-20</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>J-228</td>
<td>1-19 X 6-9</td>
<td>32</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>J-229</td>
<td>1-19 X 6-28</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>J-230</td>
<td>1-19 X 4-4</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>0</td>
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<td>7</td>
<td>1</td>
</tr>
<tr>
<td>J-234</td>
<td>5-6 X 3-4</td>
<td>17</td>
<td>0</td>
<td>1+</td>
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<tr>
<td>J-45</td>
<td>a Hp x a Hq</td>
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<td>2</td>
<td>1+</td>
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<td>8</td>
<td>3</td>
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<td>9</td>
<td>1</td>
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</table>

* See Table 2.
† These asci were considered to be aberrant.
‡ Diploid hybrid between the standard a Hp (S-14-9C-1A) and a Hq (T-1023-23B-1A) strains.
pected 1 homothallic:1α: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 HMLα gene, three (3–3, 4–18 and 5–6) are due to a nonfunctional mutation at the HMLα locus, while all other mutants of the HMLα and HMRα genes are caused by a mutation of the original allele to the alternative functional allele. Thus, eight of the 11 HMLα mutants tested were suspected to have an α HO HMRα HMLα genotype and three to have an α HO HMRα hmlα genotype. All 14 HMRα mutants tested, on the other hand, were suspected to have an α HO HMRα HMLα genotype.

Evidence for the functional mutation of the HMLα and HMRα genes: To confirm the mutation of HMLα and HMRα 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, HMLα to HMLα and HMRα to HMRα 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 HMRα HMLα/HMLα. It is known that both the α or α HO HMRα HMLα and α or α HO HMRα HMLα genotypes give rise to perfect homothallism of the Ho type, while the α HO HMRα HMLα or α HO HMRα HMLα 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 HMRα HMLα genotype and the other two from the HO HMRα HMLα genotype, while such ascus 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 ascus. 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 ascus yielding four Ho type of homothallic segregants should contain two spores of the HO HMRα HMLα genotype and two of the HO HMRα HMLα genotype. To examine this possibility, one of the ascus 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 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 HMRa HMLa 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 HMRa HMLa genotype and that J-111-4B has the HO HMRa HMLa genotype. It could also be concluded that J-111-4C has the HO HMRa HMLa genotype and that J-111-4D has the HO HMRa HMLa genotype. These observations clearly indicate that hybrid J-111 (1-19 × 3-4; Table 3) had the HO HMRa HMLa/HMRa HMLa genotype. In other words, mutant 1-19 was caused by a mutation from HMLa to HMLa and mutant 3-4 by a mutation from HMRa to HMLa. Thus, we can conclude that a functional allele can mutate to the other functional allele in the homothallism gene system.

Functional mutations of the HMLa and HMRa loci: In the foregoing experiments, we demonstrated the possibility of the mutation of the HMLa and HMRa genes to their respective opposite alleles. To examine the possibility of the reverse mutation, i.e., HMLa to HMLa and HMRa to HMRa, we attempted to isolate heterothallic clones from ascospores having the HO HMRa HMLa 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 HMRa HMLa 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 HMRa HMLa and HO HMRa HMLa genotypes. Although various types of mutations in the homothallism and

<table>
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<th>Cross*</th>
<th>Segregation in asci</th>
<th>Asci tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>J-111-4A × J-111-4C</td>
<td>a a hom hom</td>
<td>16</td>
</tr>
<tr>
<td>J-111-4A × J-111-4D</td>
<td>0 0 0 0</td>
<td>21</td>
</tr>
<tr>
<td>J-111-4B × J-111-4C</td>
<td>0 0 0 0</td>
<td>20</td>
</tr>
<tr>
<td>J-111-4B × J-111-4D</td>
<td>0 10 2 2</td>
<td>19</td>
</tr>
<tr>
<td>J-111-4A × T-1171-5D</td>
<td>0 8 2 1</td>
<td>20</td>
</tr>
<tr>
<td>J-111-4B × T-1171-5D</td>
<td>0 0 0 0</td>
<td>13</td>
</tr>
</tbody>
</table>

* 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.

TABLE 4
Genetic analysis of four homothallic segregants in an ascus type IX from a hybrid J-111
mating systems were observed among the colonies showing mating potency, we selected only the mutants of the HMLa and HMRa genes in this experiment. Three of the 10 colonies showing \( \alpha \) mating type were thought to be due to a mutation at the HMLa gene and two of the 10 colonies showing \( \alpha \) mating type were thought to be a mutation at the HMRa gene. The results of genetic analysis on an HMLa mutant, 16B-1, and an HMRa 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 \( \text{Ho} \text{HMRa} \text{HMLa} \) (S-14-9C; Table 1) genotype, showed a 2 homothallic:2\( \alpha \) segregation (\( \text{Hp} \) type of segregation) in all asci. A nonfunctional mutation of the HMLa gene, i.e., an HMLa to hmla mutation, would be unlikely, since no segregants showing \( \alpha \) mating type were observed in asci of hybrid 5-542 (16B-1 \( \times \) \( \text{Ho} \text{HMRa} \text{HMLa} \); Table 5). If 16B-1 had carried a nonfunctional allele of HMLa, the hybrid (J-542) would have yielded segregants of a mating type with the \( \text{Ho} \text{HMRa} \text{hmla} \) genotype. This suggested that 16B-1 has the \( \text{Ho} \text{HMRa} \text{HMLa} \) genotype and carries a mutation from HMLa to HMLa. Segregation patterns from two other hybrids, J-521 (16B-1 \( \times \) \( \text{Ho} \text{HMRa} \text{HMLa} \)) and J-601 (16B-1 \( \times \) \( \text{Ho} \text{HMRa} \text{HMLa} \)), are compatible with the idea of the functional mutation at the HMLa gene. Similarly, mutant 16B-20 was suspected of having the \( \text{Ho} \text{HMRa} \text{HMLa} \) genotype, since hybrid J-606 (16B-20 \( \times \) \( \text{Ho} \text{HMRa} \) HMLa; Table 5) showed a 2 homothallic:2\( \alpha \) (\( \text{Hg} \) type) segregation and hybrid J-552 (16B-20 \( \times \) \( \text{Ho} \text{HMRa} \text{HMLa} \)) did not yield heterothallic segregants of \( \alpha \) mating type in asci tested so far. Thus, it can be concluded that a functional mutation of the HMRa gene, i.e., an HMRa to HMRa mutation, occurred in 16B-20. Results suggesting a nonfunctional mutation at the HMLa and HMRa genes, i.e., HMLa to hmla and HMRa to hmrat mutations, were not observed in the mutants isolated from the \( \text{Ho} \text{HMRa} \text{HMLa} \) 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 HMRa/HMRa and HMLa/HMLa loci, mediated by the function of the Ho allele. This argument suggests that the two homothallic loci, HMRa/HMRa and HMLa/HMLa, 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 HMLa to the HMLa allele, or from the HMRa to the HMRa allele, and vice versa, in both loci is caused by such mutational events as a base change or a frame shift in the DNA sequence at the mutant loci. The second possibility is that the controlling element or the cassette, for example, from the HMRa locus is replaced by a segment of the HMLa locus. In other
### TABLE 5

**Segregation data of mating types and homothallism in asci of hybrids obtained by crossing mutants of the HMLα and HMRα genes with the standard strains for homothallism**

<table>
<thead>
<tr>
<th>Hybrid no.</th>
<th>Mutant*</th>
<th>Cross</th>
<th>Standard</th>
<th>Asci tested</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>Supposed genotype of mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>J-521</td>
<td>16B-1</td>
<td>× HO HMRα HMLα</td>
<td>21</td>
<td>α a a a a a a a hom hom hom hom hom</td>
<td>6</td>
<td>10</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
</tr>
<tr>
<td>J-542</td>
<td>16B-1</td>
<td>× HO HMRα HMLα</td>
<td>26</td>
<td>α a a a a a a hom hom hom hom hom</td>
<td>5</td>
<td>20</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
</tr>
<tr>
<td>J-541</td>
<td>16B-1</td>
<td>× HO HMRα HMLα</td>
<td>34</td>
<td>1 0 0 0 0 0 13 7 0 3 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-540</td>
<td>16B-1</td>
<td>× HO HMRα HMLα</td>
<td>16</td>
<td>0 0 0 0 0 0 0 16 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-540</td>
<td>16B-20</td>
<td>× HO HMRα HMLα</td>
<td>20</td>
<td>0 0 6 0 0 13 0 0 0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-552</td>
<td>16B-20</td>
<td>× HO HMRα HMLα</td>
<td>20</td>
<td>0 0 6 0 0 14 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-606</td>
<td>16B-20</td>
<td>× HO HMRα HMLα</td>
<td>13</td>
<td>0 0 13 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-605</td>
<td>16B-20</td>
<td>× HO HMRα HMLα</td>
<td>16</td>
<td>1 0 0 0 0 3 2 0 7 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-607†</td>
<td>16B-1</td>
<td>× 16-20</td>
<td>18</td>
<td>0 0 0 0 11 0 0 3 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HO HMRα HMLα</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

† Indicates homothallism.

‡ Hybrid between the HMLα (16B-1) and HMRα (16B-20) mutants.
words, a specific transposable genic element is produced, for example, by the HMRa locus that can replace a DNA segment at the HMLa 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 HMRa/HMRa HMLa/HMLa and HO/HO HMRa/HMRa HMLa/HMLa, would be expected from a heterothallic haploid cell of a Hp or a Hq (a HO HMRa HMLa or a HO HMRa HMLa) by the direct mutation of HMLa allele to HMLa, HMRa allele to HMRa, 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 a Hp strain of the HO HMRa HMLa genotype would not have an element or cassette for a mating type. Similarly, the a Hq strain has no element or cassette for a mating type. To test these possibilities, an a 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 \( HMLa \) and \( HMRa \) alleles, respectively, and one mutant, 3–3, which was supposed to have a nonfunctional allele of \( HMLa \), 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 \( \alpha Hp \) and \( a Hq \) strains, while a few asci were detected in the nonfunctional mutant, 3–3, at a frequency of approximately \( 10^{-5} \). 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 \( HMLa \) and \( HMRa \) alleles cause the \( \alpha \) to \( a \) conversion and the \( HMRa \) and \( HMLa \) alleles cause the \( a \) to \( \alpha \) 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 \( HMLa \) 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 \( HMRa HMLa \) standard strain gave sterile segregants of \( \alpha \) mating type in tetrads (data will be described elsewhere). This observation suggests that the mutant allele (\( hmla \)) in this mutant might produce a defective genic element that could associate with the mating-type locus but not give a normal function for \( \alpha \) 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Strain†</th>
<th>Genotype</th>
<th>No. of cells tested</th>
<th>No. of asci observed</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-14-9C-1A</td>
<td>( a ) HO ( HMRa ) ( HMLa )</td>
<td>( 1.5 \times 10^5 )</td>
<td>0</td>
<td>( &lt; 6.7 \times 10^{-6} )</td>
<td></td>
</tr>
<tr>
<td>T-1023-23B-1A</td>
<td>( a ) HO ( HMRa ) ( HMLa )</td>
<td>( 1.0 \times 10^5 )</td>
<td>0</td>
<td>( &lt; 1.0 \times 10^{-5} )</td>
<td></td>
</tr>
<tr>
<td>1-19</td>
<td>( a ) HO ( HMRa ) ( HMLa )</td>
<td>( 1.3 \times 10^5 )</td>
<td>0</td>
<td>( &lt; 7.7 \times 10^{-6} )</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>( a ) HO ( HMRa ) ( HMLa )</td>
<td>( 1.2 \times 10^5 )</td>
<td>0</td>
<td>( &lt; 8.3 \times 10^{-6} )</td>
<td></td>
</tr>
<tr>
<td>3-3</td>
<td>( a ) HO ( HMRa ) ( hmla )</td>
<td>( 3.6 \times 10^4 )</td>
<td>44</td>
<td>( 1.4 \times 10^{-3} )</td>
<td></td>
</tr>
</tbody>
</table>

* 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 \( HMLa \) and \( HMRa \) genes, respectively, and strain 3–3 is a nonfunctional mutant of the \( HMLa \) 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).

LITERATURE CITED

Arima, K. and I. Takano, 1979 Evidence for co-dominance of the homothallic genes, \( HMa/hma \) and \( HMa/hma \), in Saccharomyces yeasts. Genetics 93: 1–12.


