PRECISE MAPPING OF THE HOMOTHALLISM GENES HML AND HMR IN SACCHAROMYCES CEREVISIAE

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ABSTRACT

The HML and HMR loci carry unexpressed copies of MATa and MATα information, and a replica of that information is transposed to MAT during mating-type interchange in Saccharomyces yeasts. A negative control mechanism keeps silent the information located at the HML and HMR loci. We mapped these loci by constructing strains in which these loci are expressed. In these strains, the mating type of the segregants is dependent upon the allele at HML and HMR. This novel approach is independent of their switching function. HML is located on the left arm of chromosome III distal to his4 by about 26.8 centimorgans (cM). HMR maps on the right arm of the same chromosome distal to thr4 by about 39.8 cM and proximal to MAL2 by about 1.0 cM. The results allow the exact placement of these loci and are in accord with the observations made by HARASHIMA and OSHIMA (1976).

INTERCONVERSION of Saccharomyces cerevisiae mating types occurs by transposition of a replica of the HML or HMR locus, which contains unexpressed information of MATa or MATα, to MAT. The MAT locus maps about 20 cM from the centromere on the right arm of chromosome III (MORTIMER and HAWTHORNE 1969). A replica of the silent genes replaces the information at MAT and is thereby expressed (HICKS, STRATHERN and HERSKOWITZ 1977; OSHIMA and TEKANO 1971; KLAR and FOGEL 1979; HICKS et al. 1979; BLAIR, KUSHNER and HERSKOWITZ 1979; KUSHNER, BLAIR and HERSKOWITZ 1979; KLAR 1980a; STRATHERN et al. 1979; KLAR, FOGEL and MACLEOD 1979; RINE et al. 1979; HABER and GEORGE 1979; HICKS, STRATHERN and KLAR 1979; NASMYTH and TATCHELL 1980). The HML and HMR loci are defined on the basis of their role in switching. Both loci exist as alternate alleles; HML as HMLa or HMLα, and similarly, HMR as HMRα or HMRα (HARASHIMA, NOGI and OSHIMA 1974; NAUMOV and TOLSTORUKOV 1973; KLAR and FOGEL 1977). Mapping these loci is complicated because of the complex correspondence of genotype to phenotype due to mating-type interchange and because these loci exist in co-dominant and equivalent allelic forms. Because of these difficulties, a standard 2:2 segregation for their functions cannot be obtained in each tetrad. These difficulties led HARASHIMA and OSHIMA (1976) to develop a complex algebraic procedure for their mapping. Their method failed to locate the exact position of these loci, and there was ambiguity for the placement of HMR with
respect to the MAL2 locus. We describe here a simple procedure that does not require the switching function of these loci and that allowed us to map them precisely.

The information at HML and HMR is hypothesized to be kept unexpressed by a negative control, as exemplified by the MAR1 (Mating-type Regulator) locus (Klar, Fogel and MacLeod 1979; Rine et al. 1979; Haber and George 1979). In mar1 mutants, the silent information situated at HML and HMR is expressed. Therefore, with an appropriate arrangement of the mutant and the wild-type alleles at the HML, HMR, MAT and MAR1 loci, it should be possible to map mating type directly to the normally silent loci. Here, we demonstrate that a HMLA/HMLa mata/mata hmra/hmra mar1/mar1 strain yields 2α:2α meiotic products in each tetrad. The mating type in this strain maps distal to his4. Similarly, in another strain, the HMR locus was mapped.

MATERIALS AND METHODS

Strains: All strains of Saccharomyces cerevisiae used are listed in Table 1.

Media and techniques: All media for growth and sporulation and techniques for micro-manipulation and tetrad analysis have been described (Mortimer and Hawthorne 1969).

Strain construction: Construction of strains needed for this study required the development of procedures for sporulation of diploids, which are usually unable to do so. Diploid MATa/MATa cells heterozygous at the mating-type locus are unable to mate, but can undergo meiosis and sporulation. In contrast, MATa/MATa and MATa/MATa diploid cells express their respective mating types and are unable to sporulate (Roman and Sands 1953). Clearly, both MATa and MATa functions are needed for sporulation. Because of this requirement, MATa/mata diploid cells are incapable of meiosis and sporulation, and these cells express an α mating phenotype because the recessive mata allele does not contribute to the diploid phenotype (Kassin and Simchen 1976; Klar, Fogel and Radin 1979). Sporulation of MATa/mata diploids (α phenotype) was achieved by mating them to MATa kar1 (karyogamy defective) haploid cells on nonselective rich medium for about 5 hr. The kar1 cells mate readily, but the zygotic nuclei fail to fuse (Conde and Fink 1976), resulting in heterokaryotic cells that contain a diploid α nucleus and a haploid a nucleus. The zygotic cells are capable of sporulation when they are inoculated into sporulation medium. The diploid genome undergoes a typical meiosis to yield 4 spores, and the haploid genome yields 2 haploid spores. Similarly, MATa/mata or MATa/MATa cells may be induced to sporulate by crossing with the MATa kar1 cells. This procedure has been detailed elsewhere (Klar 1980b).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Genotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>K95</td>
<td>α</td>
<td>HMLα mata hmra mar1 ho met13 trp1-1 lys1-1</td>
</tr>
<tr>
<td>K96</td>
<td>a</td>
<td>HMLα mata hmra mar1 ho his4 leu2 thr4 met13 trp1-1 lys1-1</td>
</tr>
<tr>
<td>K97</td>
<td>α</td>
<td>hmra mata HMRa mar1 ho ade6 ade8 ura1 mal2</td>
</tr>
<tr>
<td>K98</td>
<td>a</td>
<td>hmra mata HMRa mar1 ho his4 leu2 thr4 MAL2 lys1-1 met13</td>
</tr>
</tbody>
</table>

* The genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (Plishee et al. 1976), except that a new terminology, defined earlier (Hicks et al. 1979), for the homothallism genes is used. The mutant alleles of the homothallism loci are assigned small letters, for example, hmra, hmnil, etc.
Another technique deserves a few comments. The hybrid clones constructed by fusions between \textit{HML} \textit{mata hmra mar1} (a phenotype, due to the expression of \textit{HMLa} as established in this paper; \textit{hmra} is a mutant form of \textit{HMRa}) and \textit{MATa MARI} cells express an \textit{a} phenotype and are incapable of meiosis and sporulation. (Isolation and characterization of the \textit{MAT}, \textit{HML} and \textit{HMR} mutations has been described: KLAR, FOGEL and MACLEOD 1979; KLAR and FOGEL 1979; KLAR 1980a.) This result is obtained because \textit{MARI} is dominant over \textit{mar1}; therefore, the \textit{HMLa} information is kept silent in these hybrids by the \textit{MARI} gene function. The hybrids express an \textit{a} phenotype and are incapable of meiosis and sporulation, presumably due to the lack of \textit{a} function. However, if the zygotic cells constructed by mating these strains on YEPD medium for about 5 hr are directly transferred to the sporulation medium, abundant asci are observed within 48 hr. The mating mix was directly transferred to the sporulation medium. Apparently, the \textit{01} function expressed in the \textit{HML mata hmra mar1} cells is sufficient to permit diploid zygotic cells to sporulate. Presumably, the \textit{a} product is diluted during subsequent growth of the zygotic cells as they become incapable of sporulation. Similarly, zygotic cells constructed by mating strain \textit{HML mata hmra mar1} (\textit{a} phenotype) with \textit{MATa MARI} were successfully induced to sporulate.

A combination of these two procedures allowed us to manipulate and construct strains with mutations in the \textit{MARI}, \textit{HML}, \textit{HMR} and \textit{MAT} loci.

**RESULTS**

\textit{The HML locus maps on the left arm of chromosome III}: Strain K95, possessing the genotype \textit{HMLa mata hmra mar1}, expresses an \textit{a} phenotype. Strain K96, with genotype \textit{HMLa mata hmra mar1}, expresses an \textit{a} phenotype. The hybrids constructed by mating the cells of K95 and K96 were observed to be sterile and were capable of sporulation. When analyzed by tetrad analysis, each tetrad contained 2\textit{a}:2\textit{a} segregants, a result similar to that of the wild-type strains. But, unlike the wild-type strains, the mating type maps distal to \textit{his4} by about 26.8 cM and is located farther away from \textit{leu2} (Table 2). Since \textit{his4} and \textit{leu2} are located on the left arm of chromosome \textit{III} (MORTIMER and HAWTHORNE 1969), \textit{HML} locus maps on that arm. We presume that in this cross \textit{HMLa} acts as \textit{MATa} and \textit{HMLa} acts as \textit{MATa}. The mutant information at \textit{MAT} and \textit{HMR} does not contribute to the cell phenotype.

\textit{The HMR locus maps on the right arm of chromosome III}: Similar to the experiment discussed above, the \textit{HMR} locus was mapped by mapping mating type directly to that locus. Cells from strain K97 (\textit{hmla mata HMRa mar1}, \textit{a} pheno-

**TABLE 2**

\textit{Mapping mating type to the HML locus; analysis of tetrads produced by a K95 (HMLa mata hmra mar1)} × \textit{K96 (HMLa mata hmra mar1)} hybrid

<table>
<thead>
<tr>
<th>Marker pairs</th>
<th>Ascus type and numbers</th>
<th>Map distance (cM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mat and \textit{his4}</td>
<td>PD 55</td>
<td>NPD 1</td>
</tr>
<tr>
<td>Mat and \textit{leu2}</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>Mat and \textit{thr4}</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>\textit{his4} and \textit{leu2}</td>
<td>80</td>
<td>0</td>
</tr>
</tbody>
</table>

* Map distances were calculated from PERKINS' (1949) formula. PD, NPD and TT correspond to Parental ditype, Nonparental ditype and Tetratype tetrads, respectively. Mat refers to the mating-type phenotype. Variations in the numbers of tetrads reported for each pair of markers result from occasional aberrant segregations as a result of gene conversion.
type) were hybridized to cells of K98 (HMLa, mata, HMRa, marl, a phenotype). The hybrids were sterile and capable of sporulation, both phenotypes being identical to those of MATa/MATa cells. When analyzed by tetrad analysis, the K97 × K98 hybrids produced 2a:2a segregants in each tetrad and the mating type mapped distal to thr4 (39.8 cM) and within 1.0 map unit of MAL2 (Table 3). Apparently, in this hybrid, HMRa acted as MATa and HMRa acted as MATa. Further, segregation of thr4 in two tetratype tetrads between the gene pair, HMR and MAL2, unambiguously established the gene order as thr4–HMR–MAL2.

DISCUSSION

Mapping of the HML and HMR loci is made difficult by the complex correlation of the genotype and phenotype because of the mating-type interchange and because of the linkage of MAT with the HML and the HMR loci (Harashima and Oshima 1976). We have described a novel procedure that does not require the switching function. Exact placement of the loci on chromosome III was achieved by releasing the loci in question from a negative control. Thus, it was possible to map mating type directly to these loci. Our results, summarized in Figure 1, are in accord with those obtained by Harashima and Oshima (1976). However, their value for the map distance between HML and his4 is twice that we found. Furthermore, our results unambiguously place HMR proximal to MAL2 by about 1.0 cM.

Placement of the HML and the HMR loci is consistent with the interpretation of chromosome III rearrangements that are selected as rare matings of cells of the same mating type. These events fuse MAT to HMR (the so-called Hawthorne deletion) or to HML (the so-called ring chromosome) and thereby activate the information residing there (Hawthorne 1963; Strathern et al. 1979; Klar 1980a; Strathern et al. 1980). The Hawthorne deletion extends from MAT and terminates proximal to MAL2 on the right arm of chromosome III. Thus, our placement of HMR proximal to MAL2 is in accord with the interpretation of the Hawthorne deletion, as this event leaves the MAL2 locus intact. Similarly, the location of the HML locus on the left arm of chromosome III distal to his4 is consistent with the interpretation of the ring chromosome that results from fusion of MAT to HMR (Strathern et al. 1980). These results

<table>
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<tr>
<th>Marker pairs</th>
<th>Ascus type and numbers</th>
<th>Map distance (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mat and MAL2</td>
<td>PD: 105, NPD: 0, TT: 2</td>
<td>1.0</td>
</tr>
<tr>
<td>Mat and thr4</td>
<td>PD: 42, NPD: 4, TT: 62</td>
<td>39.8</td>
</tr>
<tr>
<td>Mat and leu2</td>
<td>PD: 14, NPD: 11, TT: 72</td>
<td>unlinked</td>
</tr>
<tr>
<td>thr4 and leu2</td>
<td>PD: 36, NPD: 6, TT: 67</td>
<td>47.7</td>
</tr>
</tbody>
</table>
lend additional support to the model that yeast has functional mating information at \textit{HML} and \textit{HMR}. Recently, we have demonstrated that the information at \textit{HML} and \textit{HMR} is kept unexpressed by a negative transcriptional control, since \textit{mar1} mutant strains produce messages corresponding to these otherwise silent loci (unpublished results).

We have successfully used the approach described here to map and test the phenotypic properties of \textit{HML} and \textit{HMR} mutants. Further, it was possible to correlate their phenotypic properties with those of the mating-type alleles obtained by switches (Klar 1980a). This technique should also be useful for the mapping and the analysis of cryptic mating-type loci that may not be able to provide the switching function.

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\section*{LITERATURE CITED}


Haber, J. E. and J. P. George, 1979 A mutation that permits the expression of normally silent copies of mating type information in \textit{Saccharomyces cerevisiae}. Genetics 93: 13–25.


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