# 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 *MAT***a** and *MAT*$\alpha$ 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 *MAT***a** or *MAT*$\alpha$, 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 *HML***a** or *HML*$\alpha$, and similarly, *HMR* as *HMR***a** or *HMR*$\alpha$ (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* (*Ma*ting-type *R*egulator) 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 *HML*$\alpha$/*HML***a** *mat***a**/*mat***a** *hmr***a**/*hmr***a** *mar1*/*mar1* strain yields 2$\alpha$:2**a** 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 micromanipulation 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 *MAT***a**/*MAT*$\alpha$ cells heterozygous at the mating-type locus are unable to mate, but can undergo meiosis and sporulation. In contrast, *MAT***a**/*MAT***a** and *MAT*$\alpha$/*MAT*$\alpha$ diploid cells express their respective mating types and are unable to sporulate (ROMAN and SANDS 1953). Clearly, both *MAT***a** and *MAT*$\alpha$ functions are needed for sporulation. Because of this requirement, *MAT*$\alpha$/*mat***a** diploid cells are inacapable of meiosis and sporulation, and these cells express an $\alpha$ mating phenotype because the recessive *mat***a** allele does not contribute to the diploid phenotype (KASSIR and SIMCHEN 1976; KLAR, FOGEL and RADIN 1979). Sporulation of *MAT*$\alpha$/*mat***a** diploids ($\alpha$ phenotype) was achieved by mating them to *MAT***a** *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 $\alpha$ 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, *MAT***a**/*mat***a** or *MAT***a**/*MAT***a** cells may be induced to sporulate by crossing with the *MAT*$\alpha$ *kar1* cells. This procedure has been detailed elsewhere (KLAR 1980b).

TABLE 1

*Strain list*

| Strain | Phenotype | Genotype* |
|---|---|---|
| K95 | $\alpha$ | *HML*$\alpha$ *mat***a** *hmr***a** *mar1 ho met13 trp1-1 lys1-1* |
| K96 | **a** | *HML***a** *mat***a** *hmr***a** *mar1 ho his4 leu2 thr4 met13 trp1-1 lys1-1* |
| K97 | $\alpha$ | *hml***a** *mat***a** *HMR*$\alpha$ *mar1 ho ade6 ade8 ura1 mal2* |
| K98 | **a** | *hml***a** *mat***a** *HMR***a** *mar1 ho his4 leu2 thr4 MAL2 lys1-1 met13* |

* The genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (PLISCHKE *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, *hmr***a**, *hml***a**, etc.

Another technique deserves a few comments. The hybrid clones constructed by fusions between *HMLα mat***a** *hmr***a** *mar1* (α phenotype, due to the expression of *HMLα* as established in this paper; *hmra* is a mutant form of *HMR***a**) and *MAT***a** *MAR1* cells express an **a** phenotype and are incapable of meiosis and sporulation. (Isolation and characterization of the *MAT*, *HML* and *HMR* mutations has been described: KLAR, FOGEL and MACLEOD 1979; KLAR and FOGEL 1979; KLAR 1980a.) This result is obtained because *MAR1* is dominant over *mar1*; therefore, the HMLα information is kept silent in these hybrids by the *MAR1* gene function. The hybrids express an **a** phenotype and are incapable of meiosis and sporulation, presumably due to the lack of α 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 α function expressed in the *HMLα mata hmra mar1* cells is sufficient to permit diploid zygotic cells to sporulate. Presumably, the α product is diluted during subsequent growth of the zygotic cells as they become incapable of sporulation. Similarly, zygotic cells constructed by mating strain *HML***a** *mat***a** *hmr***a** *mar1* (**a** phenotype) with *MATα MAR1* were successfully induced to sporulate.

A combination of these two procedures allowed us to manipulate and construct strains with mutations in the *MAR1*, *HML*, *HMR* and *MAT* loci.

## RESULTS

*The* HML *locus maps on the left arm of chromosome* III: Strain K95, possessing the genotype *HMLα mat***a** *hmr***a** *mar1*, expresses an α phenotype. Strain K96, with genotype *HML***a** *mat***a** *hmr***a** *mar1*, expresses an **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 2α:2**a** segregants, a result similar to that of the wild-type strains. But, unlike the wild-type strains, the mating type maps distal to *his4* by about 26.8 cM and is located farther away from *leu2* (Table 2). Since *his4* and *leu2* are located on the left arm of chromosome *III* (MORTIMER and HAWTHORNE 1969), *HML* locus maps on that arm. We presume that in this cross *HMLα* acts as *MATα* and *HML***a** acts as *MAT***a**. The mutant information at *MAT* and *HMR* does not contribute to the cell phenotype.

*The* HMR *locus maps on the right arm of chromosome* III: Similar to the experiment discussed above, the *HMR* locus was mapped by mapping mating type directly to that locus. Cells from strain K97 (*hml***a** *mat***a** *HMRα mar1*, α pheno-

TABLE 2

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

| Marker pairs | Ascus type and numbers | | | Map distance (cM)* |
|---|---|---|---|---|
| | PD | NPD | TT | |
| Mat and *his4* | 55 | 1 | 52 | 26.8 |
| Mat and *leu2* | 31 | 2 | 77 | 40.5 |
| Mat and *thr4* | 20 | 9 | 79 | *essentially unlinked* |
| *his4* and *leu2* | 80 | 0 | 28 | 13.0 |

* 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 (*hml***a** *mat***a** *HMR***a** *mar1*, ***a*** phenotype). The hybrids were sterile and capable of sporulation, both phenotypes being identical to those of *MATα/MAT***a** cells. When analyzed by tetrad analysis, the K97 × K98 hybrids produced 2α:2**a** 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, *HMRα* acted as *MATα* and *HMR***a** acted as *MAT***a**. 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 3

*Mapping mating type to the* HMR *locus; analysis of tetrads produced by a K97* (hmla mata HMRα mar1) × *K98* (hmla mata HMRa mar1) *hybrid*

| Marker pairs | Ascus type and numbers | | | Map distance (cM) |
|---|---|---|---|---|
| | PD | NPD | TT | |
| Mat and *MAL2* | 105 | 0 | 2 | 1.0 |
| Mat and *thr4* | 42 | 4 | 62 | 39.8 |
| Mat and *leu2* | 14 | 11 | 72 | unlinked |
| *thr4* and *leu2* | 36 | 6 | 67 | 47.7 |

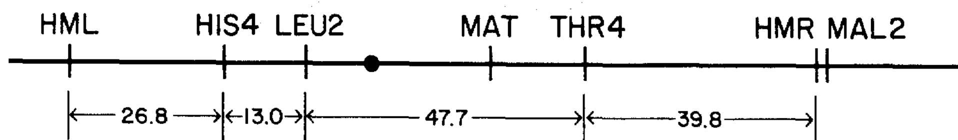


FIGURE 1.—Location of *HML* and *HMR* on chromosome *III*. The dot signifies the centromere, and the map distances are expressed in centimorgans. The interval between *HMR* and *MAL2* is about 1 cM.

lend additional support to the model that yeast has functional mating information at *HML* and *HMR*. Recently, we have demonstrated that the information at *HML* and *HMR* is kept unexpressed by a negative transcriptional control, since *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 *HML* and *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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