MAR1—A REGULATOR OF THE *HM***a** AND *HM*α LOCI IN *SACCHAROMYCES CEREVISIAE*

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Manuscript received September 29, 1978
Revised copy received May 21, 1979

ABSTRACT

A mutation in the *MAR1* (mating-type regulator) locus causing sterility in *Saccharomyces cerevisiae* is reported. The mutation maps on the left arm of linkage group IV between *trp1* and *cdc2* at a distance of about 27 cM from *trp*1 and about 31 cM from *cdc*2. Haploid strains with genotype *MAT*α *HM*α *HM***a** *mar1–1* and *MAT***a** *HM*α *HM***a** *mar1–1* are sterile. However, *MAT*α *hm*α *HM***a** *mar1–1* and *MAT***a** *HM*α *hm***a** *mar1–1* strains exhibit α and **a** mating type, respectively. The sterile strains can be "rare mated" with standard strains as a consequence of mutational changes at *HM***a** and *HM*α. It is proposed that the *MAR1* locus blocks the expression of *MAT*α and *MAT***a** information thought to exist at *HM***a** and *HM*α loci, respectively (HICKS, STRATHERN and HERSKOWITZ, 1977). In a *mar1–1* mutant, the expression of both *HM*α and *HM***a** information leads to a nonmating phenotype similar to that of *MAT***a**/*MAT*α diploids. The genetic evidence reported here is consistent with a central feature of the "cassette model", namely that *HM*α and *hm***a** carry *MAT***a** information and *HM***a** and *hm*α carry *MAT*α information.

HETEROTHALLIC (*ho*) strains of bakers yeast, *Saccharomyces cerevisiae*, display **a** or α mating type. The mating type is controlled by two stable allelic forms of the mating-type locus, *MAT***a** and *MAT*α, although rare switches between the two allelic states occur at low frequencies of about 10^{-6} in standard laboratory strains (HAWTHORNE 1963a; RABIN 1970). In contrast, homothallic (*HO*) strains can change their mating types as often as every generation (WINGE and ROBERTS 1949; HAWTHORNE 1963b; OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1976; STRATHERN 1977). These switches comprise stable heritable changes at the mating-type locus (*MAT*). The continued presence of the *HO* gene is not required for the maintenance of the altered allele. Mitotic products of a single haploid *HO* cell may express opposite mating types due to switching and therefore fuse to produce *MAT***a**/*MAT*α diploids that are not subject to further switching.

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Two other genes, *HM***a** (alternate allele *hm***a**) and *HM*α (alternate allele *hm*α) control the direction of switching. *HO* and *HM*α or *hm***a** are required for *MAT*α to *MAT***a**, and *HO* and *HM***a** or *hm*α are required for *MAT***a** to *MAT*α interconversions (TAKANO and OSHIMA 1970; NAUMOV and TOLSTORUKOV 1973; HARASHIMA, NOGI and OSHIMA 1974; KLAR and FOGEL 1977; HICKS, STRATHERN and HERSKOWITZ 1977). The *HM*α and *HM***a** loci are located on opposite arms of chromosome 3; and to date *HO* has not been mapped. The *HM*α locus displays loose linkage to *MAT* (HARASHIMA and OSHIMA 1976; KLAR and FOGEL 1977), which is situated about 20 map units from the centromere on the right arm of chromosome 3 (MORTIMER and HAWTHORNE 1969).

Several molecular models have been proposed to explain interconversions at *MAT*. Variously they posit DNA modification of the regulatory site at *MAT* (HAWTHORNE, quoted in HOLLIDAY and PUGH 1975), inversion of such a regulatory site by recombination events (BROWN 1976; HICKS and HERSKOWITZ 1977), insertion of a "controlling element" into *MAT* (OSHIMA and TAKANO 1971), or gene replacement (HICKS, STRATHERN and HERSKOWITZ 1977). The models are summarized in HICKS and HERSKOWITZ (1977) and KLAR, FOGEL and RADIN (1979). OSHIMA and TAKANO (1971) proposed that *HO* controls insertion or removal of a regulatory element into *MAT*. The regulatory elements are proposed to function in a manner analogous to that of the controlling elements described for maize (MCCLINTOCK 1956). The *HM***a** and *HM*α loci are hypothesized to produce the mating-type-specific controlling elements. The attachment of an *HM*α element differentiates the *MAT* locus to an **a** allele and the attachment of an *HM***a** element differentiates the *MAT* locus into an α allele. HICKS, STRATHERN and HERSKOWITZ (1977) proposed a similar but more specific scheme, the "cassette model." Here, the *HM***a** and *hm*α loci are presumed to be blocks of unexpressed *MAT*α information and the *HM*α and *hm***a** loci are blocks of unexpressed *MAT***a** information. It is suggested that the switch is brought about by insertion of this silent information or its copy (i.e., "cassette") into *MAT* by the action of the *HO* gene. It is proposed that the *MAT* information located at the "silent genes," *HM***a** and *HM*α (and alternate *hm***a** and *hm*α), are silent perhaps due to the lack of an essential regulatory site.

We isolated a spontaneous mutation at a locus designated *MAR1* (mating-type regulator). Overall, the genetic evidence provided by an analysis of the *mar1–1* mutant and the alterations of *HM***a** and *HM*α is consistent with the suggestion that the loci *HM*α and *hm***a** carry *MAT***a** information and that *HM***a** and *hm*α carry *MAT*α information.

MATERIALS AND METHODS

Strains: All strains used are described in Table 1.

Techniques: All media for growth and sporulation and techniques for micromanipulation and tetrad analysis have been described (MORTIMER and HAWTHORNE 1969). For rare-mating experiments, freshly grown cells of the parental strains were mixed at 10^8 cells per ml (1:1) in sterile water, and 0.2 ml of the suspension was spread on each of five plates containing a complex, rich, nonselective medium. After 48 hr of growth, the confluent lawn was trans-

TABLE 1

List of strains used

Strain	*Genotype**	*Source*
2180–1A	*MAT***a**, *HMα*, *HM***a**, *MAR1*, *gal2*, *mal*, *SUC*, *CUP1*	Berkeley stocks
D–2180–1A diploidized variant	*MAT***a**/*MATα*, *HMα*/*HMα*, *HM***a**/*HM***a**, *mar1–1*/*mar1–1*, *gal2*/*gal2*, *mal*/*mal*, *SUC*/*SUC*, *CUP1*/*CUP1*	This study
Y382	*MAT***a**, *HMα*, *HM***a**, *MAR1*, *gal10*, *ade6*	Berkeley stocks
Y386	*MATα*, *HMα*, *HM***a**, *MAR1*, *gal10*, *trp1*, *ade6*, *ura1*, *his1*	Berkeley stocks
KM2B–36B	*MAT***a**, *HMα*, *hm***a**, *mar1–1*, *his1*, *gal10*, *SUC*+	This study
KM2B–36C	*MAT***a**, *HMα*, *hm***a**, *mar1–1*, *ade6*, *gal10*, *gal2*	This study
T–1074–38C	*MATα*, *HMα*, *hm***a**, *MAR1*, *gal1*, *his4*, *leu2*, *thr4*, *trp1*	I. Takano
KM2C–43B	*MATα*, *HMα*, *HM***a**, *mar1–1*, *trp1*, *his1*, *ade6*	This study
K14	*MAT***a**, *HMα*, *HM***a**, *MAR1*, *cry1*, *thr4*, *leu2*, *his4*, *his2*, *ura1*	This study
K15	*MATα*, *HMα*, *HM***a**, *MAR1*, *cry1*, *thr4*, *leu2*, *his4*, *ura1*, *ade6*	This study
KM30–32A	*MATα*, *HMα* mutant, *HM***a**, *mar1–1*, *ura1*, *his1*, *leu2*	This study
KM30–32D	*MATα*, *HMα* mutant, *HM***a**, *mar1–1*, *trp1*, *his2*, *gal2*	This study
J38	*MAT***a**, *hmα*, *HM***a**, *MAR1*, *cry1*, *thr4*, *ura3*, *lys2*, *metX*, *his4*, *leu2*	This study
#370	*MAT***a**,*HMα*, *HM***a**, *MAR1*, *cdc2*, *ade1*, *ade2*, *ura1*, *his7*, *lys2*, *tyr1*, *gal1*	Berkeley stocks
K40	*MAT***a**, *HMα*, *HM***a**, *MAR1*, *aro1D*, *his2*	This study
KM2C–43C	*MATα*, *HMα*, *HM***a**, *mar1–1*, *ade6*, *gal10*	This study
KM31	KM2C–43C × K14	This study

*The genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (PLISCHKE, VON BORSTEL, MORTIMER and COHN 1976) except the old terminology for the mating types and homothallism genes is retained (HARASHIMA, NOGI and OSHIMA 1974). All the strains used are *ho*.

ferred by replica-plating onto selective media. These strains carried multiple complementary auxtrophic markers, and only the rare fusions grow on the selective media. Control plates containing each of the unmixed parents were also prepared.

RESULTS

Isolation of the mar1–1 *mutant*: Ordinarily, heterothallic haploid strains switch their mating type at a low frequency of about 10^{-6} (HAWTHORNE 1963a; RABIN 1970). The switched cells mate with their sister cells to produce isogenic *MAT***a**/*MATα* diploids, which in turn yield 2**a**:2*α* meiotic products when their tetrads are analyzed. However, during routine transfers a spontaneous diploid arose from a heterothallic strain 2180-1A (**a** mating type). This diploid sporulated abundantly with nearly 100% spore survival and yielded only sterile (nonmating) meiotic products in every tetrad analyzed. All spores or spore

progenies tested were insensitive to α factor and displayed a polar budding pattern, characteristic of *MAT***a**/*MAT*α diploids. The sterile segregants exhibit incipient sporulation, a characteristic of haploids disomic for chromosome 3 with *MAT***a**/*MAT*α constitution at *MAT* (ROTH and FOGEL 1971). Also they yield X-ray survival kinetics characteristic of haploids (FOGEL and MACLEOD, unpublished observation).

To explain these results, we hypothesize that the parental 2180-1A strain accumulated a spontaneous mutation at a locus designated *MAR1*, whose function normally is to inhibit the expression of proposed mating-type information situated at the *HM***a** and *HM*α *loci* (Figure 1). The *mar1-1* mutation might then allow the expression of *MAT* information at these loci. Such a cell would express at least one *MAT***a** (located at *HM*α) and one *MAT*α (located at *HM***a**) locus and would exhibit sterility, a phenotype similar to that of *MAT***a**/*MAT*α diploids. We further postulate that the mutant diploidized by endomitosis to establish a diploid D-2180-1A variant. Endomitosis is attributed to replication of the genetic material without ensuing cell division and results in the production of diploid cells possessing the same genotype as the haploids (ROMAN and SANDS 1953). We presume that the D-2180-1A diploid variant with *MAT***a**/*MAT***a** constitution sporulates, since the α function for sporulation is provided by the expression of *MAT*α information at the *HM***a** loci. The following genetic studies contributed to the development of this hypothesis.

Mapping the mar1–1 *mutation*: Rare matings between segregants from a single tetrad of D-2180-1A (*gal2*) were attempted with tester strains Y382 (*MAT***a**, *gal10*) and Y386 (*MAT*α, *gal10*). One such hybrid between sterile segregant C and Y386 yielded the following segregation pattern: 13 (2*ste*:2**a**) : 13 (2*ste*:2α) : 23 (2*ste*:1**a**:1α). Apparently, a cell free of secondary mutations rare-mated with Y386 since the hybrid produced only tetrads containing 2*ste*:2 maters. This result demonstrates that *mar1* segregates as a single Mendelian marker. Linkage between *trp1*, a marker located on chromosome 4, and *mar1* was indicated by the tetrad segregation ratio of 25 parental ditype (PD) : 0 nonparental ditype (NPD) : 30 tetratype (TT). Based on PERKINS (1949) for-

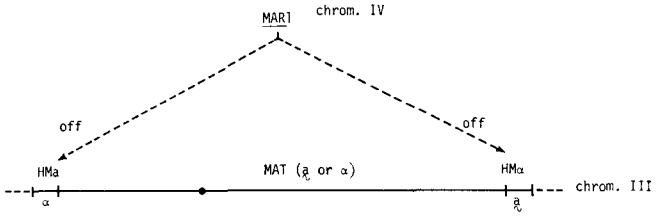

Fig. 1 Model for MAR1 as the regulator of HMa and HMα MAT loci

FIGURE 1.—The *MAR1* locus gene product is postulated to keep the mating type information situated at the loci *HM***a** unexpressed by a negative control. The information at *MAT* is expressed constitutively and is not regulated by the *MAR1* gene product. In a *mar1* mutant, the expression of α (at *HM***a**) and **a** (at *HM*α) information leads to sterile phenotype similar to that exhibited by the *MAT***a**/*MAT*α diploids.

mula, we calculate a map distance of 27.3 centimorgans (cM) between these markers.

Strain KM31 constructed by rare-mating the cells from KM2C-43C (*MATα mar1*) with K14 (*MAT***a** *MAR1*) yielded the segregation pattern: 8 (2*ste*:2**a**): 6 (2*ste*:2α) : 21 (2*ste*:1**a**:1α). Clearly, the *ste* phenotype segregates as a single marker. In these studies, mating phenotype was determined by testing the clonally derived progeny of each spore. We wondered, however, whether the *mar1* spores derived from a *mar1/MAR1* (*e.g.*, KM31) hybrid are able to express their respective mating types at the spore stage due to *MAR1* function in the hybrid prior to spore formation. Consequently, at the spore stage, even the *mar1* spore segregants may act as though they carry the *MAR1* allele. Subsequent growth of such spores may dilute and/or inactivate the *MAR1* function, resulting in sterility. To investigate this possibility, 20 tetrads from strain KM31 were dissected and the spores allowed to grow in the presence of α factor. (α factor is an oligopeptide secreted by the α cells that specifically arrests the growth of **a** cells; DUNTZE, MACKAY and MANNEY 1970.) The sterile segregants of D-2180-1A, apparently of genotype *MAT***a** *mar1*, are insensitive to the α factor. However, two spores from each KM31 tetrad were arrested in their growth. Eleven of the arrested spore cells were allowed to grow in the absence of α factor. Seven, presumably carrying the *mar1* allele, grew to establish sterile clones, and the balance exhibited an **a** mating type. This observation suggested that the spores inheriting the *mar1* allele may express their respective mating type at the spore stage and thus be mated by spore-to-cell mating.

Individual spores derived from strain KM31 were placed adjacent to cells from strain K40 (*MAT***a** *MAR1 aro1*). Five zygotes were isolated, grown and subjected to tetrad analysis. Three hybrids yielded 2**a**:2α, and the rest segregated 2*ste*:2 mater. Clearly, the hybrids producing *ste* segregants, resulted from matings between KM31 spores with genotype *MATα mar1* and K40 cells. Strain K40 carries *aro1* marker, which maps on the right arm of chromosome 4 (MORTIMER and HAWTHORNE 1969). The results indicate that *mar1* and *aro1* are unlinked since they yield 7PD:7NPD:30TT tetrads (Table 2). Similarly, spores from KM31 were mated with strain #370 (*MAT***a** *cdc2*) cells. Two of six hybrids tested segregated 2*ste*:2 mater products. Data resulting from analysis of

TABLE 2

Mapping of the mar1–1 *mutation*

Cross	Markers pair	Ascus typs* PD	NPD	TT	(cM) Map distance
D–2180–1A C× Y386	*mar1* and *trp1*	25	0	30	27.3
KM31 spore × K40	*mar1* and *aro1*	7	7	30	unlinked
KM31 spore × #370	*mar1* and *cdc2*	22	1	21	30.7

*The entires in the table correspond to the numbers of asci that displayed the various segregation patterns.

these hybrids are presented in Table 2. The *mar1* and *cdc2* markers are linked by 30.7 cM. Since *cdc2* has been assigned to the left arm of chromosome 4 (MORTIMER and HAWTHORNE 1969), we conclude that the *MAR1* locus maps to the left arm of this linkage group between *cdc2* and *trp1*.

Isolation of alterations at HM**a**: Rare-matings between segregants from a single tetrad of D-2180-1A (*gal2*) were attempted with tester strains Y382 (*MAT***a**, *gal10*) and Y386 (*MAT*α, *gal10*). The individual prototrophic hybrids were selected on media containing galactose as the sole carbon source. The *GAL*$^+$ clones were picked, purified and tested for mating and sporulation. Such clones could arise by reversion of either the *gal2* mutation in D-2180-1A segregants or the *gal10* deficiency in Y386 or Y382 strains, or by fusions between the sterile segregants and the tester strains. Most of the *GAL*$^+$ clones (26/32) isolated from rare-matings between segregant A and the α strain sporulate and appear to result from *bona fide* fusions. However, all such clones (80 tested) isolated from rare-matings with the **a** strain fail to sporulate and represent revertants of the *gal10* ($\sim$1/3) or *gal2* ($\sim$2/3) alleles. In control experiments, the *gal2* and *gal10* mutations exhibit a reversion frequency of about 10^{-7}. Segregants B, C and D displayed a similar bias with respect to mating with the α strain. In subsequent studies using multiple marked auxotrophic strains, such nonmater strains were observed to rare-mate only with α strains. These observations support the view that the diploid D-2180-1A arose by endomitosis from the 2180-1A (*MAT***a**) haploid, since each segregant appears to carry an **a** allele at *MAT*.

A single rare-mated hybrid between Y386 and each sterile segregant from a single tetrad was sporulated and the asci dissected. Analysis of the tetrads yielded a complex segregation pattern for mating type and the sterile pheno-

TABLE 3

Numbers of different tetrad classes from hybrids between Y386 (α) and the four sterile meiotic segregants obtained from D-2180-1A

Segregant	Tetrad classes and numbers						Total spores
	2**a**:1α:1*ste*	2**a**:2*ste*	1**a**:2α:1*ste*	1**a**:1α:2*ste*	2α:2*ste*	2**a**:2α	**a**:*ste*:α
A	8	7	3	12	0	1	47:49:28
B	6	14	6	8	1	2	58:58:32
C	17	14	11	15	1	2	92:88:60
D	9	10	5	7	1	0	50:50:28
Total	40	45	25	42	3	5	
Observed frequencies	0.25	0.28	0.16	0.26	0.02	0.03	
Predicted frequencies	0.27	0.22	0.17	0.28	0.03	0.03	

type. As presented in Table 3, six classes of tetrads, each containing two or more maters, were observed. Heterozygous markers on six other chromosomes *i.e.*, *trp1*, *ade6*, *ura1*, *his1*, *gal2* and *gal10* segregated 2+:2−, thereby establishing the haploid nature of the original sterile segregants from D-2180-1A and the Y386 strain. The *ste* segregants can rare-mate with α cells (1) if fusion occurs without any secondary mutation; (2) if *mar1–1* reverts to *MAR1*; or (3) if a *mar1* suppressor appears in the **a** strain. Rare-matings attributed to possibility (1) should result in hybrids yielding 2*ste*:2 mater tetrads; while in possibility (2), only 2**a**:2α meiotic products are expected. Suppressors of *mar1–1* could be of two types: (1) those that represent nonspecific suppressors of *mar1–1* allele, *e.g.*, transfer RNA translational suppressors; assuming that the suppressors of this class segregate independently of *mar1* and *MAT*, the hybrids *HM***a**/*HM***a** *MAT***a**/*MATα* *HMα*/*HMα* *mar1–1*/*MAR1* *SUP*/+ should yield a 3**a**:2*ste*:3α segregant ratio on a total spore basis, and (2) those that represent alterations of *HM***a** if *mar1–1* permits expression of the *HM***a** and *HMα* loci as we hypothesized. In the latter case, two types of alterations at *HM***a** would suppress the *mar1–1* phenotype: mutation at the locus inactivating the [α] information normally present at *HM***a**, or switches from *HM***a** to *hm***a** so that [**a**] information is present at the locus (NAUMOV and TOLSTORUKOV 1973; KLAR and FOGEL 1977; HICKS, STRATHERN and HERSKOWITZ 1977). In both instances, the *mar1–1* strain would express an **a** mating type since normally silent copy [α] information is absent. The results presented in Table 3 are consistent only with possibility (2).

The explanation for sterility we proposed above, based upon the cassette model, was suggested by the 3**a**:3*ste*:2α segregant ratio (last column, Table 3) as follows. Suppose we write the *MAT* constitution of the nonmating segregants in cassette terminology as [α] **a** [**a**] *mar1* (*i.e.*, *HM***a** *MAT***a** *HMα* *mar1*). The genotypes are presented on a line to indicate the order and linkage of these markers. Bracket [α] signifies silent *MATα* information located at *HM***a** and [**a**] represents silent *MAT***a** information at the *HMα* locus. Since the mutant allele *mar1–1* is also present, we reasoned that the otherwise silent *MAT* information at the *HM***a** and *HMα* loci is expressed and confers a *ste* phenotype on these segregants. The [α^m] signifies a defective *MATα* allele or its switch to [**a**] at *HM***a**, *i.e.*, *HM***a** to *hm***a**. Assuming that the loci *HM***a**, *MAT*, *HMα* and *mar1* essentially segregate independently, the observed 3**a**:3*ste*:2α ratio could be predicted.

Given the hybrid:

$$\frac{[\alpha]\ \mathbf{a}\ [\mathbf{a}]\ \textit{mar1–1}}{[\alpha]\ \alpha\ [\mathbf{a}]\ \textit{MAR1}} \quad \xrightarrow[\textit{HM}\mathbf{a}\text{ in the sterile strain}]{\text{rare-mating by changes at}} \quad \frac{[\alpha^m]\ \mathbf{a}\ [\mathbf{a}]\ \textit{mar1–1}}{[\alpha]\ \alpha\ [\mathbf{a}]\ \textit{MAR1}}$$

the parental and recombinant *MAT* configurations of the segregants possess the following phenotypes, depending upon whether they carry *mar1–1* or *MAR1*.

MAT configurations of the segregants	Phenotype *mar1–1*	Phenotype *MAR1*
$[\alpha^m]$ **a** [**a**]	**a**	**a**
$[\alpha^m]$ α [**a**]	*ste*	α
[α] **a** [**a**]	*ste*	**a**
[α] α [**a**]	*ste*	α

In effect, changes at *HM***a** could act as suppressors of the *mar1–1* mutation and allow an otherwise sterile cell to mate as an **a** cell. Based on these assumptions, the frequencies of different tetrad classes can be calculated. As displayed in Table 3, the predicted frequencies are in excellent agreement with the observed frequencies.

Mapping of a sterile suppressor isolated in HM**a** MAT**a** HMα mar1-1 *strain*: In order to test whether suppressor of *mar1–1* is a mutational change of *HM***a** resulting in a nonfunctional [α] copy as proposed above, its map position with respect to *HM***a** was determinetd. Both mating type **a** segregants from a 2**a**:2α tetrad obtained from the above-described hybrids between D-2180-1A segregants and Y386 should carry the proposed *mar1–1* suppressor, as well as the mutant allele *mar1–1*. Hybrids between two such segregants, strains KM2B-36B and KM2B-36C (both *HM***a**? *MAT***a** *HM*α *mar1–1*) with T-1074-38C (*hm***a** *MAT*α *HM*α *MAR1*, courtesy of I. TAKANO) were constructed and subjected to tetrad analysis. The results (Table 4) demonstrate that, in both hybrids, each tetrad yields 2**a** segregants and half of the segregants with an α allele at *MAT* grow to establish *ste* clones and the other half express the α mating type. These results are easily accommodated within the cassette and the *MAR1* hypotheses. If we assume that the suppressor is a mutational change of *HM***a** resulting in a nonfunctional [α] copy, we may write the genotype of the KM2B-36B and KM2B-36C segregants in cassette terminology as $[\alpha^m]$ **a** [**a**] *mar1–1*. The cassette genotype of the test strain T-1074-38C may be written as [**a**] α [**a**] *MAR1* (*i.e.*, *hm***a** *MAT*α *HM*α *MAR1*; *hm***a** carries silent [**a**] information according to the cassette hypothesis). The genotype of the hybrids may be denoted as $[\alpha^m]$ **a** [**a**] *mar1*. If the sterile suppressor (*i.e.*, $[\alpha^m]$) mutation maps at *HM***a**,

[**a**] α [**a**] *mar1*

TABLE 4

Numbers of different tetrad classes from hybrids between KM2B–36B and KM2B–36C with T–1074–38C

Hybrids	Tetrad Classes PD	NPD	TT
	2**a**:2α	2**a**:2*ste*	2**a**1*ste*:1α
KM2B–36B × T–1074–38C	3	4	12
KM2B–36C × T–1074–38C	4	5	9

both segregants with an **a** allele at *MAT* in each tetrad must exhibit an **a** phenotype regardless of which *mar1* allele is present. According to our hypothesis, segregants carrying an α allele at *MAT*, when assorted with the *mar1–1* allele, are expected to be sterile but of α cell type when the wild-type allele *MAR1* is inherited. Since *mar1–1* is located on a different chromosome from *MAT*, an equal number of parental ditype (2**a**:2α) and nonparental ditype (2**a**:2*ste*) tetrads are predicted. The results (Table 4) are in excellent agreement with the prediction. Should the suppressor not map at *HM***a**, then some of the segregants with an **a** allele at *MAT* with genotype *HM***a** *MAT***a** *HMα mar1* must exhibit *ste* phenotype. Since each tetrad displayed in Table 4 carry two **a** spores, we conclude that a mutation or a change that allows the *HM***a** *MAT***a** *HMα mar1–1* sterile cell to mate as an **a** maps at or close to the *HM***a** locus. These results are consistent with the suggestion that *HM***a** carries *MATα* and that *hm***a** is equivalent to *MAT***a** information (HICKS, STRATHERN and HERSKOWITZ 1977). Furthermore, 25% of the **a** mating-type segregants obtained from this hybrid must have the genotype *hm***a** *MAT***a** *HMα mar1–1*. Hence, the *hm***a** *MAT***a** *HMα mar1–1* mutants are not sterile, and they exhibit an **a** mating type. This is not surprising since such strains, according to cassette hypothesis, carry only **a** information at three loci *i.e.*, [**a**] **a** [**a**] and are expected to express an **a** mating type.

Isolation of alterations at HMα: One rare-mated hybrid between sterile segregant C from D-2180-1A and Y386 (*MATα*) produced the following tetrad segregation pattern: 13 (2*ste*:2**a**) : 13 (2*ste*:2α) : 23 (2*ste*:1**a**:1α). Apparently, a sterile cell, free of any secondary mutations, rare-mated with Y386 since the hybrid produced ascus types containing only 2*ste*:2 maters. Both sterile segregants from a tetrad with 2*ste*:2**a** products should have an α allele at *MAT* and should carry the *mar1–1* allele to confer sterility. Rare matings between one of these segregants, strain KM2C-43B, with K14 (*HM***a** *MAT***a** *HMα MAR1*) and K15 (*HM***a** *MATα HMα MAR1*) were attempted. KM2C-43B was observed to rare-mate with K14 at a frequency of about 10^{-7}, but not with K15. The resulting hybrid (Table 5) yielded a ratio of 2**a**:3*ste*:3α segregants based on pooled data from six ascus classes observed. All other heterozygous markers (*ura1*, *his1*, *his4*, *leu2*, *ade6*, *thr4*, *cry1* and *trp1*) segregated 2+:2− in 28 tetrads ana-

TABLE 5

Numbers of different tetrad classes from a hybrid between KM2C–43B and K14

	Tetrad classes						Total spores
	2**a**:1α:1*ste*	2**a**:2*ste*	1**a**:2α:1*ste*	1**a**:1α:2*ste*	2α:2*ste*	2**a**:2α	**a**:*ste*:α
No.	4	1	8	8	6	1	28: 42: 42
Observed frequencies	0.14	0.04	0.28	0.29	0.21	0.04	
Predicted frequencies	0.17	0.03	0.27	0.28	0.22	0.03	

lyzed. This result established the haploid nature of the KM2C-43B and K14 strains.

The 2**a**:3*ste*:3α ratio can be explained readily by postulating that spontaneous changes occur at *HM*α in a sterile strain allowing cells to exhibit an α mating type. The [α] α [**a**] (cassette terminology for *HM***a** *MAT*α *HM*α) *mar1–1* sterile strain can rare-mate with the standard **a** strain by spontaneous alterations at *HM*α, so that it does not carry a functional copy of *MAT***a**. The resulting hybrid $\frac{[\alpha]\ \alpha\ [\mathbf{a}^m]\ \textit{mar1–1}}{[\alpha]\ \mathbf{a}\ [\mathbf{a}]\ \textit{mar1}}$ should yield a ratio of 2**a**:3*ste*:3α segregants on a total spore basis by a rationale similar to that used to explain the 3**a**:3*ste*:2α ratio of the segregants observed from the hybrids between sterile segregants from D-2180-1A and a standard α strain, as detailed in the previous sections. In effect, changes at *HM*α can act as suppressors of the *mar1–1 allele* and allow an otherwise sterile strain to exhibit an α mating type. Assuming that *HM*α, *MAT* and *mar1–1* segregate independently of each other and that the hypothesized suppressor inactivates the *MAT***a** information at *HM*α, expected frequencies of different tetrad classes can be calculated. The predicted frequencies (Table 5) are in accord with the observed frequencies.

Mapping of a sterile suppressor isolated in HM**a** MATα HMα mar1–1 *sterile strain*: In order to test whether the *mar1–1* suppressor is a mutational change of *HM*α, resulting in a nonfunctional [**a**] copy as proposed above, its map position with respect to the *HM*α locus was determined. Both α segregants in the single 2α:2**a** tetrad among 28 analyzed from the KM2C-43B (*HM***a** *MAT*α *HM*α *mar1–1*) and K14 (*HM***a** *MAT***a** *HM*α *MAR1*) rare-mated hybrid must carry a sterile suppressor and the *mar1–1* mutant allele. Hybrids between two such segregants, strains KM30-32A and KM30-32D (both *HM***a** *MAT*α *HM*α? *mar1–1*), with J38 (*HM***a** *MAT***a** *hm*α *MAR1*) were constructed and subjected to tetrad analysis. Results displayed in Table 6 demonstrate that, in both hybrids, each tetrad yields 2α segregants and half of the segregants with an **a** allele at *MAT* grow to establish *ste* clones and the other half express an **a** mating type. These results are easily accommodated within the cassette and the *MAR1* hypotheses. If we assume that the suppressor is a mutational change of *HM*α resulting in a nonfunctional [**a**] copy, we may write the genotype of the KM30-32A and KM30-32D segregants in cassette terminology as [α] α [$\mathbf{a}^m$]

TABLE 6

Numbers of different tetrad classes from hybrids between KM30–32A and KM30–32D with J38

Hybrids	Tetrad Classes PD	NPD	TT
	2**a**:2α	2*ste*:2α	1**a**:1*ste*:2α
KM30–32A × J38	4 :	3 :	9
KM30–32D × J38	3 :	2 :	15

mar1–1. Cassette genotype of the test strain J38 may be denoted as [α] **a** [α] *MAR1* (*i.e.*, *HM***a** *MAT***a** *hmα MAR1*; *hmα*, carries silent [α] information according to the cassette hypothesis). If the sterile suppressor (*i.e.*, [$\mathbf{a}^m$]) mutation maps at *HMα*, both segregants with an α allele at *MAT* in each tetrad must exhibit an α phenotype regardless of which *mar1* allele is present. According to our hypothesis, segregants carrying an **a** allele at *MAT*, when assorted with the *mar1–1* allele, are expected to be sterile but of **a** type when *MAR1* is inherited. Since *mar1–1* is located on a different chromosome from *MAT*, an equal number of parental ditype (2**a**:2α) and nonparental ditype (2*ste*:2α) tetrads are predicted. The results (Table 6) agree completely with the prediction. Should the suppressor not map at *HMα*, then some of the segregants with an α allele at *MAT* with genotype *HM***a** *MATα HMα mar1–1* must exhibit *ste* phenotype. Since each tetrad displayed in Table 6 carries two α spores, we conclude that a mutation or a change that allows the *HM***a** *MATα HMα mar1–1* sterile cell to mate as α maps at or close to the *HMα* locus. These results are consistent with the proposal that *HMα* and *hmα* carry structural information respectively equivalent to *MAT***a** and *MATα* (HICKS, STRATHERN and HERSKOWITZ 1977). Moreover, one-fourth of the α mating type segregants obtained from this hybrid must have the genotype *HM***a** *MATα hmα mar1–1*. Hence, the *HM***a** *MATα hmα mar1–1* strains are not sterile, and they exhibit an α mating type. This is understandable since such strains, according to cassette hypothesis, carry only α information at three loci *i.e.*, [α] α [α] and are expected to express an α mating type.

DISCUSSION

Regulation of mating type in *Saccharomyces cerevisiae* seems to be complex, since a given haploid cell can express one or the other mating type. These phenotypes can alternate at low but detectable frequencies in *ho* cells, but at very high frequencies in *HO* cells. Hence, yeast must possess the information or the capacity to generate both varieties of mating-type information. HICKS, STRATHERN and HERKOWITZ (1977) proposed that *HMα* and *HM***a** loci carry blocks of silent *MAT***a** and *MATα* information, respectively. They suggested that these loci are silent presumably due to the lack of some "essential regulatory site, *e.g.*, promoter." We propose a variation on this scheme by supposing that yeast employs a mechanism to turn off the *MAT* information at these silent or storage loci. Specifically, we propose a model where a product(s) of the *MAR1* locus is assumed to repress the *MAT* information located at *HMα* and *HM***a** by a negative control mechanism. A newly discovered spontaneous mutation at *MAR1* locus allows their expression, resulting in a sterile phenotype. The *MATα HMα HM***a** *mar1–1* sterile strains rare-mate preferentially or almost exclusively with standard α strains as a consequence of additional mutations or changes at the *HMα* locus. Similarly, *MAT***a** *HMα HM***a** *mar1–1* sterile strains rare-mate preferentially or exclusively with standard α strains by virtue of mutations or changes at the *HM***a** locus. This rationale allowed us to isolate spontaneous variants of the *HMα* and *HM***a** genes. The *MATα hmα HM***a** *mar1–1* and

*MAT***a** *HMα hma mar1-1* strains exhibit α and **a** mating types, respectively. These results are entirely consistent with the notion that *HMα* and *hma* carry *MAT***a** structural information and that *HM***a** and *hmα* carry *MATα* structural information. However, it must be realized that these results do not establish the above contention, since the possibility exists that the *HMα and HM***a** loci may be regulatory genes that control the expression of *MAT* information located elsewhere in the yeast genome.

As indicated above, *MATα HMα HM***a** *mar1-1* sterile strains rare-mate preferentially or exclusively with standard **a** strains. This distinctive bias can be readily explained on the assumption that sterile cells rare-mate with **a** cells as a consequence of mutations or changes at the *HMα* locus (*i.e.*, *MAT***a** information). Such alterations would allow the cell to mate as an α since at least two α loci are expressed, one at *MAT* and the other at *HM***a**. In order to mate with α strains, two coincident events are required to inactivate *MATα* information at *MAT* and at *HM***a** (proposed site for *MATα* information). Preferential rare-matings of *MAT***a** *HMα HM***a** *mar1-1* sterile strains with α strains is explained in a similar fashion.

In the RESULTS, we suggested that the suppressors of the *mar1-1* sterile phenotype are attributable to changes at the *HM***a** and *HMα* loci. The changes could be mutations of the MAT information stored at *HM***a** and *HMα* loci or their switching to *hm***a** or *hmα*, respectively. Both kinds of events are observed. Furthermore, when a *HMα* mutant is used for switching *MATα* to *MAT***a**, only defective *MAT***a** alleles are obtained (KLAR and FOGEL, unpublished observations). These results may strongly support the cassette model proposed for mating-type interconversion.

We cannot, at present, suggest a plausible mechanism whereby the *MAR1* locus regulates the *HM***a** and *HMα* loci. The *mar1-1* mutation is recessive to the wild-type *MAR1* allele (KLAR and FOGEL, unpublished observations); hence, we presume that *MAR1* provides for a function that is impaired or absent in the *mar1-1* mutant. In any case, it is important to determine whether other available sterile mutations are allelic to the *MAR1* locus and also if they allow for the expression of the silent loci. Unlike the *MAR1* locus, none of the well-characterized sterile mutations described by MACKAY and MANNEY (1974) exhibit centromere linkage. Also *ste2*, *ste3*, *ste4* and *ste5* mutants do not act in a fashion analogous to the *mar1-1* mutant (KLAR, unpublished observations). The *nul3* mutation confers a sterile phenotype; however, it maps on the right arm of chromosome 4 (HAWTHORNE, personal communication).

It is interesting to note that the *MAT***a**/*MATα mar1-1*/*MAR1* hybrids yield spores all of which express their mating types at the spore stage. Presumably, the *MAR1* function is distributed to all of the spores and consequently, at that stage, even the *mar1-1* spores are able to mate. This observation is consistent with the idea that *MAR1* is dominant over *mar1-1*. Most rare-matings of standard strains with *mar1-1 ste* strains are due to secondary mutations. We can avoid this problem by mating the *mar1* spores derived from *mar1*/*MAR1* hybrids. With this technique, we crossed the *mar1-1* mutation into *hm***a** and *hmα*

strains. The results obtained are consistent with the conclusions derived in this paper (KLAR, unpublished observations). We tested whether other *ste2*, *ste4* and *ste5* mutations (MACKAY and MANNEY 1974) are "conditional," similar to *mar1* at the spore stage. *ste2* mutants gave positive results. Their observations suggest that the conditional nature of *ste* mutations may be exploited for hybridizing *ste* strains with each other.

In an independent study, RINE, STRATHERN, HICKS and HERSKOWITZ (personal communication) suggested that another locus, *SIR1*, acts in a manner analogous to *MAR1*. Subsequently, HABER and GEORGE (personal communication) observed that the *cmt* mutant (HOPPER and HALL 1975), also allows the expression of *HMα* and *HM***a** loci. Apparently, several genes may function to repress the *HM***a** and *HMα* loci, either independently or cooperatively. Thus, there might be a sequence of events to produce a repressor as a final product. Any mutational block in this pathway could lead to the constitutive expression of the silent loci.

This investigation was supported by Public Health Service Grant No. GM-17317 awarded to S. FOGEL. We thank J. STRATHERN, and J. HICKS for criticizing the manuscript and J. RINE, J. STRATHERN, J. HICKS and I. HERSKOWITZ for communication of their results before publication. We also thank L. DALESSANDRO for preparation of the manuscript.

Note added in proof: In recent experiments we have demonstrated that strains possessing amber and ochre mutations with the *HMα* locus yield defective *MAT***a** alleles by switching. The defective *MAT***a** alleles carry the corresponding amber and ochre mutations originally present in *HMα* (KLAR, submitted for publication). Thus, the coding sequence for the mating-type **a** allele exists at *HMα*, and a copy of that information is transposed to *MAT* during *MAT* interconversion. Therefore, this result confirms the *MAR1* hypothesis since that was based on the assumption that the unexpected mating-type information exists at *HM***a** and *HMα*.

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