THE ACTION OF HOMOTHALLISM GENES IN SACCHAROMYCES DIPLOIDS DURING VEGETATIVE GROWTH AND THE EQUIVALENCE OF *hm***a** and *HMα* LOCI FUNCTIONS

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ABSTRACT

The action of homothallism genes in vegetatively growing diploid cells was examined. The results demonstrate that homothallism genes function during regular vegetative growth cycles as well as during the first few divisions after spore germination. A procedure based on ultraviolet-induced reciprocal mitotic recombination monitored by homozygosity for cryptopleurine resistance (a recessive marker closely linked to the mating-type locus) allowed us to identify and recover *Saccharomyces cerevisiae* colonies sectored for the mating-type locus *i.e.*, **a**/**a** and *α*/*α*. Homothallism genes can switch **a**/**a** or *α*/*α* vegetative diploid cells, generated from a strain with genotype **a**/*α HO*/*ho HMα*/*HMα HM***a**/*HM***a**, to **a**/*α* diploids or **a**/**a**/*α*/*α* tetraploids during a given mitotic division cycle. We found that both **a**/**a** and *α*/*α* sectors generated from a strain with genotype **a**/*α HO*/*HO hmα*/*hmα hm***a**/*HM***a** switch to **a**/*α* diploids or **a**/**a**/*α*/*α* tetraploids. This finding supports NAUMOV and TOLSTORUKOV's suggestion (1973) that the *hm***a** allele provides for the same function as the *HMα* allele, namely, a switch at the mating-type locus from *α* to **a**. The *HO* allele is dominant to *ho* but *hm***a** and *HM***a** alleles are codominant. A loose linkage between the mating-type and the *HMα* loci (~55cM), confirming HARASHIMA, NOGI and OSHIMA (1974) data, was observed.

YEASTS are classified as heterothallic or homothallic with respect to mating capacity. Heterothallic baker's yeast, *Saccharomyces cerevisiae*, displays either **a** or *α* mating-type, and diploidization occurs principally through fusions between cells of opposite mating-type. In homothallic strains, however, diploidization can occur by mating between daughter cells originating from a single haploid ascospore. This process is regulated by a set of homothallism genes (*HO*, *HM***a**, *HMα*) that change the mating-type allele **a** to *α* and *vice versa* by specific directed changes or mutations at the mating-type locus during or soon after spore germination (WINGE and ROBERTS 1949; HAWTHORNE 1963a; TAKANO and OSHIMA 1967, 1970; HICKS and HERSKOWITZ 1976). A consequence of this process is that colonies grown from a single haploid spore contain mainly **a**/*α* diploid cells. A diploid strain of genotype **a**/*α HO*/*HO hmα*/*hmα HM***a**/*HM***a** produces tetrads containing 2 homothallic:2 *α* segregants and a diploid of genotype **a**/*α HO*/*HO HMα*/*HMα hm***a**/*hm***a** produces 2 homothallic:2 **a** segregants. These observations suggest that the *HO* and *HMα* genes are required for switching *α*

to **a** mating-type, and the *HO* and *HM***a** genes are required for the **a** to α switch (TAKANO and OSHIMA 1970; HARASHIMA, NOGI and OSHIMA 1974). Strains with genotype **a**/α *HO/HO HM*α*/HM*α *HM***a***/HM***a** are completely homothallic, *i.e.*, all four meiotic segregants in each tetrad are homothallic. Haploid strains containing the *ho* allele are heterothallic (HARASHIMA, NOGI and OSHIMA 1974; HICKS and HERSKOWITZ 1976). Recently it was reported that *HO hm*α *hm***a** as well as *HO HM*α *HM***a** strains are completely homothallic (NAUMOV and TOLSTORUKOV 1973; HARASHIMA, NOGI and OSHIMA 1974). The finding was explained by NAUMOV and TOLSTORUKOV (1973) who suggested that the *hm*α allele might provide the same function as the *HM***a** gene (*hm*$\alpha \equiv$ *HM***a**) and that the *hm***a** gene is functionally equivalent to the *HM*α locus (*hm***a** $\equiv$ *HM*α). The symbol $\equiv$ denotes functional equivalence.

Homothallism genes act during or soon after spore germination. Whether they can function during ordinary vegetative growth cycles as well, however, has not been previously reported. A technical difficulty in approaching this question experimentally lies in our inability to construct and maintain strains containing homothallism genes that also display an **a** or α mating-type. Typically, when ascospores carrying such a genetic combination are produced, their subsequent germination soon leads to diploidization, making it impossible to determine whether the homothallism genes act during vegetative growth. We circumvented this difficulty by inducing reciprocal mitotic crossovers between the centromere and the mating-type locus in an appropriately marked diploid strain. These events generate homozygosity for the mating-type locus in vegetatively growing **a**/α diploid cells. We could also assess the functional equivalence of the *hm***a** and *HM*α loci.

Our results suggest that homothallism genes act during vegetative growth as well as during spore germination, switching **a**/**a** or α/α diploid cells to **a**/α diploids or **a**/**a**/α/α tetraploids. Also, it seems clear that the *hm***a** allele provides the same or equivalent function as the *HM*α gene, a finding supportive of NAUMOV and TOLSTORUKOV's (1973) view.

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; GRANT, SANCHEZ and JIMENEZ 1974). Mitotic recombination was induced by exposing single cells plated on YEPD plates to ultraviolet light (UV, 380 ergs/mm^2, 95% survival) according to ROMAN and JACOB (1958). The UV light source consisted of a 25-watt Hanovia germicidal lamp (Hanovia, Newark, New Jersey). After 3 days of growth the mating-type of the UV induced sectors was determined by spraying standard strains of **a** and α mating-type with complementary markers on separate replica plates (FOGEL, CAMPBELL and LUSNAK. manuscript in preparation). After a 24 hr growth period, these plates were replica plated to appropriate selective medium. Only colonies which show mating capacity with tester strains grow confluently.

Matings: Diploids were generated by cell-to-cell, cell-to-spore or spore-to-spore matings. These matings were initiated by placing cells or spores in direct contact with each other on dissection agar. Subsequently, zygotes were isolated by micromanipulation. The hybrid nature of

zygotic clones was verified by appropriate complementation tests for the markers carried in the parent strains.

RESULTS

1) *Action of homothallism genes during vegetative growth*:

The diploid strain, J1, was constructed by mating spores obtained from homothallic strain S41 with fresh vegetative cells from heterothallic strain A68. Since *cry1* is a recessive antibiotic resistance marker (GRANT, SANCHEZ and JIMENEZ 1974), strain J1 is cryptopleurine sensitive. One hundred cells of this strain were plated on each of 10 YEPD plates and exposed to UV to induce mitotic crossovers. The plates were incubated in the dark at 30°. After three days of growth, the colonies were replicated to YEPD plates containing 5 μg/ml of cryptopleurine. Of the mitotic crossovers between the centromere and the *cry1* locus, only half result in a sectored colony (Figure 1). The sectors possess genotypes *cry1/cry1* or +/+; the former grow confluently on medium containing cryptopleurine. Since *cry1* is only 2.2 centimorgans proximal to the sex locus situated in chromosome III, an appropriate mitotic crossover event between the *cry1* locus and the centromere could yield a diploid *cry1*,α/*cry1*,α sector along with a reciprocal wild-type diploid +,*a*/+,*a* sector. If homothallism genes are functional during vegetative growth, we might expect these respective α/α and **a**/**a** sectors to be switched either to an **a**/α diploid state if only one sex locus is altered, or to an **a**/**a**/α/α tetraploid if both mating-type loci are switched and the corresponding **a**/**a** and α/α cells mate. Whether cells are diploid or tetraploid is readily ascertained from the meiotic segregation pattern for the three heterozygous auxotrophic markers carried in the J1 hybrid. Diploids yield 2+:2− segregations for each heterozygous marker while tetraploids generate 4+:0−, 3+:1− and

TABLE 1

List of strains used

Strain	Genotype*	Source
S41	**a**/α, *HO/HO*, *HM*α/*HM*α, *HM***a**/*HM***a**, *arg4-1/arg4-1*	M. and R. ESPOSITO
A68†	α, *ho*, *HM*α, *HM***a**, *cry1*, *his4*, *trp5*	J. HABER
T-1023-2C-1A	**a**, *HO*, *HM*α, *hm***a**, *gal1*, *his4*, *leu2*, *lys2 thr4*, *trp1*	I. TAKANO
S-14-9C-1A	α, *HO*, *hm*α, *HM***a**, *mal*, *suc*, *his4*, *leu2*, *leu2*, *lys2*	I. TAKANO
S2073B	**a**, *ho*, *HM*α, *HM***a**, *thr4*, *leu1*, *ade1*, *gal2*	Berkeley Stocks
LA178	α, *ho*, *HM*α, *HM***a**, *leu1*, *ade1*, *arg6*	Berkeley Stocks
J1	S41 × A68	This Study
J2	**a**/α, *HO/HO*, *hm*α/*hm*α, *hm***a**/*hm***a**, *his4/his4*, *leu2/leu2*, *gal1/gal1*	This Study
J3	α, *HO*, *hm*α, *HM***a**, *arg4*, *leu2*, *gal1*, *suc*	This Study

* The terminology for genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (PLISCHKE *et al.* 1976) except that the old terminology for the mating-types and homothallism genes is retained (HARASHIMA, NOGI and OSHIMA 1974).

† *cry1* is cryptopleurine resistance marker, recessive to wild type as described by GRANT, SANCHEZ and JIMENEZ (1974).

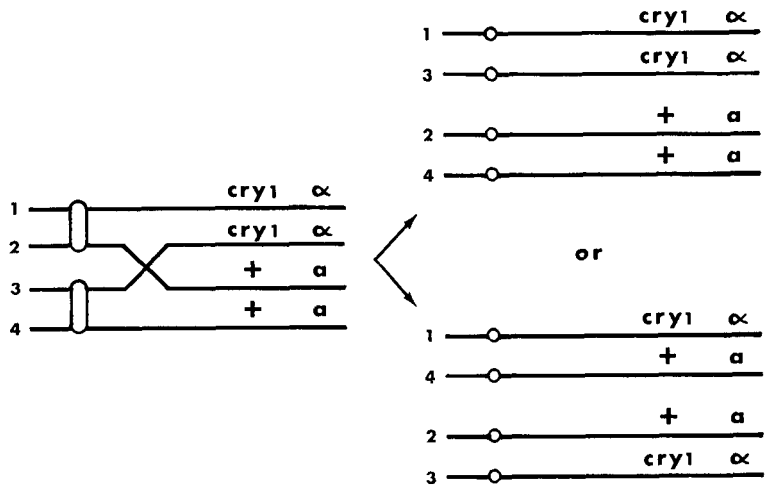

FIGURE 1.—Reciprocal mitotic crossing over in Chromosome III in J1. Mitotic crossing over results in two types of sectored colonies. These occur with equal frequencies and depend on the assortment of the centromeres. Upper (1,3:2,4): one sector is homozygous for *cry1* and α while the other is homozygous for + and **a**. Lower (1,4:2,3): both sectors are heterozygous for all markers. Only the 1,3:2,4 or upper pattern would yield a sectored colony on medium containing cryptopleurine. Cells derived from matings between **a**/**a** and α/α cells at the first division after the mitotic crossover event cannot grow on medium containing cryptopleurine. Hence they are not analyzed (modified from MORTIMER and HAWTHORNE 1969).

2+:2− segregation patterns for the same markers. Among approximately 1000 clones, we observed four sectored colonies. The *cry1* and wild-type sectors were separately grown, sporulated and dissected to ascertain their ploidy.

Table 2 shows that, except for colony 4, both sectors within each colony contain a mixture of diploid and tetraploid cells. In contrast, a control from the J1

TABLE 2

Ploidy levels and meiotic segregation patterns among UV induced cry1 *and* + *sectors from hybrid J1* (**a**/α HO/ho HMα/HMα HM**a**/HM**a**)

Colony no.	Sector phenotype	Tetraploids: diploids*	Cryptopleurine sensitivity segregation +:*cry1*
1	*cry1*	18:2	0:4
	+	4:13	4:0
2	*cry1*	7:11	0:4
	+	2:15	4:0
3	*cry1*	15:2	0:4
	+	5:10	4:0
4	*cry1*	0:20	0:4
	+	0:20	2:2
Control	J2 (untreated with UV)	0:40	2:2

* Tetraploid to diploid ratio ascertained from segregation patterns for heterozygous markers.

hybrid without induced mitotic recombination, gave only $2^+:2^-$ segregation in 40 tetrads analyzed. This finding suggests that the parental J1 strain is a stable diploid. Our procedure for assessing ploidy is selective since analysis is restricted to cells capable of sporulating. Homozygous **a**/**a** or α/α diploids cannot sporulate (MORTIMER and HAWTHORNE 1969). Accordingly, they are excluded from the totals. However, the sectors reported in Table 2 sporulate abundantly (>70%) and they do not display a mating-type reaction. We therefore suggest that the mating-type alteration process governed by homothallism genes is highly efficient and occurs in virtually all cells of the population. It seems that the homothallism genes act by altering or switching one or the other or both (*i.e.*, **a**/**a** → **a**/α or α/α) of the mating-type loci in vegetative diploid cells.

The different sectors and clones tetraploidized to various extents. This might reflect minor variations in the timing or efficiency of switching one mating-type to the other (*i.e.*, $\alpha \rightarrow \mathbf{a} > \mathbf{a} \rightarrow \alpha$). Moreover, the different clones probably switched both mating-types at different times after the initial mitotic crossover event. However, the observed variation in the tetraploid:diploid ratio may also represent sampling errors during sporulation and dissection as well as growth rate differences between diploid and tetraploid cells.

Sectored colony no. 4 did not contain tetraploid cells among 40 cells analyzed (Table 2). Most probably it arose as a consequence of gene conversion (FOGEL and MORTIMER 1971) of $+ \rightarrow$ *cry1* allele, rather than from a mitotic crossover. This origin is likely since the cryptopleurine sensitive sector of the colony yielded a 2 sensitive: 2 resistant segregation for the *cry1* marker (Table 2, column 4). Thus, we suppose that tetraploidization did not occur in colony 4 because **a**/**a** and α/α were not produced.

Since the J1 hybrid switches its mating-types in both **a**/**a** and α/α sectors where the *HO*/*ho* locus is in heterozygous condition, the *HO* allele is taken to be dominant over *ho*. HOPPER and HALL (1975) and HICKS (1975) have arrived at the same conclusion.

2) *Does the* hm**a** *allele contribute the same function as* HMα?

A. *Rationale and construction of strains.* The results obtained from genetic analyses of the homothallic strains by NAUMOV and TOLSTORUKOV (1973) and by HARASHIMA, NOGI and OSHIMA (1974) are simply explained by assuming, as suggested by the former authors, that strains with genotype *HO hm*α *hm***a** are also completely homothallic. But, as pointed out by HARASHIMA, NOGI and OSHIMA (1974), it is difficult to explain the homothallic switch observed in cells of genotype *HO hm*α *hm***a** by merely assuming the presence of inactive alleles of the *HM*α *and HM***a** *genes*. However, NAUMOV and TOLSTORUKOV (1973) explained the datum by suggesting that the *hm***a** allele might provide for the same function as the *HM*α gene and that the *hm*α gene might be functionally equivalent to the *HM***a** gene. A direct test of NAUMOV and TOLSTORUKOV's (1973) suggestion is lacking. Our results demonstrate that homothallism genes function in **a**/**a** or α/α diploids during vegetative growth. This approach allows us to test the proposed equivalence of *hm***a** and *HM*α gene functions. To test this suggestion,

a strain of genotype **a**/α *HO/HO hm*α*/hm*α *hm***a***/HM***a** is required. Mitotic recombination in chromosome III between the centromere and the mating-type locus could yield **a**/**a** and α/α sectored colonies (Figure 1). Since this strain carries two copies of *HO* and one copy of the *HM***a** gene, the **a**/**a** sector should produce **a**/α diploids or **a**/**a**/α/α tetraploids (see previous RESULTS). However, because *HM*α is absent in this hybrid (*i.e.*, *hm*α is homozygous) we should expect stable α/α sectors if *hm***a** and *HM*α are not functionally equivalent. Alternatively, if *hm***a** $\equiv$ *HM*α, then these α/α sectors should lose their α mating-type and yield **a**/α diploids or **a**/**a**/α/α tetraploids.

To construct the above diploid, strains of genotypes α *HO hm*α *HM***a** and **a**/α *HO/HO hm*α*/hm*α *hm***a***/hm***a** are required. Strain T-1023–2C–1A was crossed with S–14–9C–1A, sporulated and dissected to obtain segregants of the required genotype. Several segregants capable of sporulation (hence homothallic) were tested for their homothallism genotype. Segregant 10A was capable of sporulation and all the segregants obtained from 10 dissected asci were homothallic (Table 3). Segregant 10A-4B (designated J2) was sporulated again and all the segregants obtained from 10 dissected asci were capable of sporulation (Table 3). It is clear that segregant 10A and its progeny are completely homothallic. During tetrad analysis of the segregants obtained from 10A and J2, it was observed that 2 segregants from each tetrad exhibited a weak **a** mating-type response while the other 2 segregants were nonmaters. The mating-type response is attenuated though unambiguous. These "**a**" segregants are capable of sporulation (>30%) and upon dissection again produce 2 weak **a** and 2 nonmater segregants. One of the "**a**" segregants was streaked for single cells and the resultant colonies were tested for mating-type and sporulation. We found that only 5% of these secondary colonies show weak **a** mating-type response but all these clones are still capable of sporulation. In this homothallic genotype, segregants with an **a** mating-type allele do not switch to diploids with 100% efficiency. Some **a** mating-type cells are present and in turn these confer an **a** mating-type response on the clone as a whole. This response proved to be useful since we could observe the **a** mating-type sectors generated by mitotic recombination without depending on outside marker signals.

TABLE 3

Tetrad segregations from strains containing various combinations of homothallism genes

Strains	Tetrad segregation (Homothallic: **a**:α)								
	4:0:0	3:1:0	3:0:1	2:1:1	2:2:0	2:0:2	1:2:1	1:1:2	0:2:2
Segregant 10A	10	0	0	0	0	0	0	0	0
J2	10	0	0	0	0	0	0	0	0
J2 × S41*	3	0	0	4	0	0	0	0	1
S41 × J3†	5	0	47	0	0	18	0	0	0
J2 × J3†	3	0	3	0	0	3	0	0	0

* Cross was made by the spore-to-spore mating method.
† Cross was made by the spore-to-cell mating method.

Since strain J2 is a complete homothallic, it could have the genotype *HO HMα HM***a** or *HO hmα hm***a** (NAUMOV and TOLSTORUKOV 1973; HARASHIMA, NOGI and OSHIMA 1974). To distinguish between these two alternatives J2 was crossed with S41 (*HO HMα HM***a**). If J2 carries a genotype *HO HMα HM***a**, then all the segregants from this cross should be homothallic. However, if J2 has genotype *HO hmα hm***a** then some of the segregants should be stable heterothallic **a** or α mating-type, notably **a** *HO HMα hm***a** and α *HO hmα HM***a**. In all these crosses mating capacity, sporulation ability and segregation of the markers was tested. Hybrid J2 × S41 when sporulated and dissected, yielded some **a** and α mating-type segregants (Table 3). Consequently, the J2 strain was judged to possess genotype **a**/α *HO/HO hmα/hmα hm***a***/hm***a**. One of the segregants (designated J3) from the J2 × S41 cross had a stable α mating-type and it failed to sporulate: it should carry genotype α *HO hmα HM***a**. To confirm the J3 genotype it was crossed with S41. Hybrid J3 × S41 on tetrad analysis produced homothallic and α mating-type segregants only (Table 3) as expected if J3 had genotype *HO hmα HM***a**. The complete genotypes of J2 and J3 with respect to auxomarkers is given in Table 1.

B. hm**a** *can switch α to* **a** *in the presence of* HO: A hybrid between J2 × J3 (**a**/α *HO/HO hmα/hmα hm***a***/HM***a**) was constructed by spore-to-cell mating. About 1600 stationary phase cells from this hybrid were induced for mitotic recombination by UV (as described above) to produce **a**/**a** and α/α sectored colonies. After 3 days of growth these colonies were tested for their mating-type response. There was no α/α mating-type response observed. In all, 9 clones showed sectors of "**a**/**a**" mating-type. We suppose that these "**a**/**a**" mating type sectors are produced because the **a** to α switch is not 100% effective in these strains (see above, section 2A). From master plates the "**a**/**a**" sector and the opposite half of the colony were taken from the clone's periphery, grown, sporulated and dissected to ascertain their ploidy based on the meiotic segregation for markers heterozygous in the hybrid. As is clear from the results shown in Table 4, in all three of the sectored colonies tested there are diploids and tetraploids

TABLE 4

*Ploidy level achieved by the homothallism process in "***a**/**a***" and nonmater sectors induced by UV in hybrid J2 × J3* (**a** HO hmα hm**a** × α HO hmα HM**a**)

Colony no.	Sector*	Tetraploids:Diploids†
1	"**a**/**a**"	10:4
	nonmater	11:1
2	"**a**/**a**"	2:8
	nonmater	10:2
3	"**a**/**a**"	3:8
	nonmater	8:2
Control J2 × J3 (untreated with UV)		0:27

* See description of the sectors in the text.

† Tetraploid to diploid ratio was ascertained from the segregation patterns for heterozygous markers.

in both the "**a**/**a**" sector and the sector which should have been α/α if the *hm***a** gene was not functionally competent to switch the α to the **a** mating-type. The J2 × J3 hybrid, when dissected without prior mitotic cross-over events, gave 2+:2− segregation for all the heterozygous markers in 27 dissected asci (Table 4), suggesting that J2 × J3 is a stable diploid. As a control, in a heterothallic strain constructed by crossing S2073B with LA173 and treated as above with UV to induce mitotic recombination, we observed 10 colonies (in 1800 analyzed) containing **a** and α mating-type sectors. Among these sectors cosectoring for the *thr4* marker (located on chromosome III distal to the mating-type locus) occurred, suggesting that at the UV dose given we detect about 0.5% mitotic crossover events between the centromere and the mating-type locus. Our experiment with the homothallic J2 × J3 hybrid also produced approximately the same frequency (9 out of 1600) of colonies sectored for the "**a**/**a**" mating-type. This suggests that our inability to detect α/α sectors in the J2 × J3 hybrid may not reflect some trivial cause, *e.g.*, the insensitivity of this particular strain to UV induced mitotic recombination. Since we observed a relatively high frequency of tetraploids (Table 4), mitotic recombination must have occurred at customary rates in these clones. Tetrad analysis of J2 × J3 hybrid produced only homothallic and α mating-type segregants (Table 3). This pattern is expected if the J2 × J3 hybrid carries the genotype **a**/α *HO/HO hm*α*/hm*α *hm***a**/*HM***a**.

Since the J2 × J3 hybrid switches its mating-types in both **a**/**a** and α/α sectors of genetic constitution *HO/HO hm*α*/hm*α *hm***a**/*HM***a**, we suggest that the *hm***a** and *HM***a** alleles display codominance.

3) *Linkage between the* Hmα *and the mating-type loci*:

Tetrad segregation pattern obtained from hybrid J3 × S41 (α *HO hm*α *HM***a** × **a** *HO HM*α *HM***a**) suggested a loose linkage between the mating-type and the *HM*α locus. Presented in Table 3 are 18PD:5NPD:47TT asci (PD, parental ditype; NPD, nonparental ditype and TT, tetratype.) Based on these data we calculate a genetic distance of 55 cM (map distance) between the mating-type and the *HM*α loci. This observation confirms a similar linkage evidence reported by HARASHIMA, NOGI and OSHIMA (1974).

DISCUSSION

The mating-type locus in Saccharomyces yeasts seems to be a complex locus containing both **a** and α loci, though at any given time only a single mating type is expressed (CRANDALL, EGEL and MACKAY 1976). This notion is best supported by the homothallic process which can switch **a** to α mating-type and back. Also heterothallic strains can effect a switch at the mating-type at a very low frequency (HAWTHORNE 1963b). Two models have been proposed to explain the mating-type alterations. These involve DNA modification (suggested by D. HAWTHORNE; see HOLLIDAY and PUGH 1975) or insertion or removal of a "controlling element" into the mating-type locus (OSHIMA and TAKANO 1971). Recently, BROWN (1976) suggested another model based on an interrupted reverse repeat; the putative repeats flank a single promotor region common to

both the **a** and α loci. In Brown's model the promotor is shunted back and forth by crossover events within the reverse sequences. These recombination events are presumed to be mediated by the homothallism genes. The model predicts that homothallic mating-type switching might be blocked in a recombination negative (*rec*$^-$) genetic background.

Until now, it was not established whether the mating-type switch accomplished by homothallism occurs only during or soon after spore germination. Our experiments were designed to study whether this switch could be achieved during vegetative growth. Our results demonstrate that switching can occur during vegetative growth. It was observed that the switch occurs in haploids or in diploids (**a**/**a** or α/α) when only one mating-type is expressed. Homozygous **a**/**a** or α/α diploids can be switched to **a**/α diploids or to **a**/**a**/α/α tetraploids. It seems clear, then, that it is the mating-type expression rather than ploidy that controls the manifestations of the homothallic switch. The homothallic system must have a mechanism that senses the cell's mating-type expression. This notion led BROWN (1976) to propose a feedback loop in the homothallic process where the homothallism genes sense the mating phenotype displayed by the cell. His contention is further supported by the fact that **a**/α diploids do not display any mating-type (nonmaters) and hence are stable even in the presence of homothallism genes (TAKANO and OSHIMA 1970, and our unpublished observations).

Further, our data support the suggestion made by NAUMOV and TOLSTORUKOV (1973) that the *hm***a** allele is functionally equivalent to *HM*α locus. It remains to be seen whether the *hm***a** allele is actually another copy of the *HM*α locus. In any event, in view of these findings we suggest that the α mating-type can be switched to **a** by *HO hm***a** as well as by *HO HM*α gene combinations. The technique of UV-induced sectoring for the mating-type locus could not be used for investigating whether the *hm*α allele is functionally equivalent to the *HM***a** locus. The technical difficulty lies in the fact that *HM*α locus is located in the right arm of chromosome III distal to the mating-type locus (HARASHIMA, NOGI and OSHIMA 1974, and this study). Hence, mitotic recombination between the centromere and the mating-type locus would lead to cosectoring of the mating-type and the *HM*α locus. However, a test of the functional equivalence between *hm*α and *HM***a** could be approached by generating the required mating-type sectors by gene conversion or by obtaining the appropriate segregants from tetraploid strains.

Since the mating-type locus switch occurred during vegetative growth, we conclude that homothallism genes are not restricted in their action to the period immediately following spore germination. The homothallism genes can effectively convert vegetative **a**/**a** or α/α diploid cells to **a**/α diploids or **a**/**a**/α/α tetraploids. Also, the *hm***a** allele in the presence of *HO* gene can switch an α to an **a** mating type.

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