ALLELE SPECIFIC DETERMINANTS OF HOMOTHALLISM IN *SACCHAROMYCES LACTIS*[1]

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IN general, fertile yeasts are classified as being either heterothallic or homothallic. Heterothallic strains exhibit one of two alternate mating types (usually designated as *a* and α or + and −) with diploidization occurring between cells of opposite mating type only (e.g. *a* with α and + with −). In homothallic yeasts, on the other hand, restrictive mating systems are not observed and sister cells quite commonly fuse to form zygotes.

The known genetic controls regulating homothallism in yeast are numerous and apparently complex in their action. For example, LEUPOLD (1958) working with the fission yeast *Schizosaccharomyces pombe* strain *liquefaciens* found that mating behaviour was controlled by a complex locus. Two heterothallic strains, h^+ and h^-, were genetically determined by closely linked mutant sites within this complex locus. Homothallic strains, designated H^{40} and H^{90}, could be derived from these heterothallic cultures either through mutation or through recombination within the complex region. In the yeast *Saccharomyces chevalieri* the presence of a gene *D* in a single-spore culture committed the culture to homothallism (WINGE and ROBERTS 1949). The *D* gene was independent of the mating locus and was equally effective in the presence of either the *a* or α alleles. Heterothallic cells of *S. chevalieri*, on the other hand, possessed the recessive *d* allele and exhibited either one of the alternate mating types *a* or α. OESER (1962) and HAWTHORNE (1963b) have reported that the *D* gene produces homothallism by directing mutation of a mating type allele to the alternate form in some of the cells within a culture. Following this mutation, the affected culture becomes homothallic since it consists of a mixture of *a* and α sister cells capable of zygote formation.

In *Saccharomyces cerevisiae* homothallism results either from a high mutation rate of one mating type allele to the other (AHMAD 1952) or from the action of the complementary genes HM_1HM_2 and HM_2HM_3 (TAKAHASHI, SAITO, and IKEDA 1958; TAKAHASHI 1958). To compare the nature of the *D* and *HM* genes, TAKAHASI and IKEDA (1959) prepared hybrid Saccharomyces diploids containing both the HM_1HM_2 complement and the *D* gene of WINGE and ROBERTS (1949). The *D* gene and the *HM* genes segregated independently.

This paper reports the identification of factors affecting homothallism in

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selected strains of the yeast *Saccharomyces lactis*. Two unlinked independent homothallic genes were identified. These factors exert their effect by changing the mating type to the alternative form in a certain percentage of the cells. Moreover, these two genes are allele specific. One locus is effective in changing the mating type from *a* to *α*, the other in changing *α* to *a*.

MATERIALS AND METHODS

Parental stocks Y14 and Y123 were obtained from L. J. WICKERHAM (strains Y1140 and Y1118 respectively). All isolates used in this study were derived from matings in which these two strains were the original parents. The maintenance of stocks and the procedures used in tetrad dissections and carbohydrase assays were described previously (HERMAN and HALVORSON 1963). Cells grown for 24 hours on YM (WICKERHAM 1951) were used as mating inocula. Malt extract agar (ME) (WICKERMAN 1951) was used for mating and sporulation. Vegetative growth in *Saccharomyces lactis* consists essentially of haploid cells. The diploid phase is limited, in the majority of cases, to the zygote formed by the mating of two haploid cells. Usually the zygote forms four haploid ascospores per ascus. Since mating is immediately followed by sporulation, ME serves both as a mating and sporulating medium. In both homothallic cultures and heterothallic mating mixtures, zygote formation was confined exclusively to cells grown on ME. Cells inoculated onto ME do not immediately mate but undergo a period of multiplication (8 to 10 hr) before the first competent cells appear in the population. Whether the cells from the original inoculum ever become competent or whether the mating response is confined exclusively to cells formed during growth on ME is not known. Following the 8 to 10 hr period of vegetative growth the proportion of cells capable of producing zygotes gradually increases until a maximum degree of competency is attained after 3 to 5 days incubation.

Homothallic and heterothallic cultures are differentiated on the basis of differences in their mating response during growth on ME at 28°C. Homothallic cells form zygotes when grown alone on ME. Heterothallic strains exhibit no mating response under these same conditions but produce zygotes after inoculation in mixed culture with one of the two tester strains, either *a* or *α*. As will be seen later, most of the homothallic strains were weakly homothallic, i.e., less than 25% of the cells in a population formed zygotes.

During this study it was necessary to determine the homothallic potentialities of several segregants. This was done by counting the number of zygotes formed per 100 vegetative cells after 3 to 5 days incubation at 28°C and is referred to as the percent homothallism of a culture.

In addition to determining the percent homothallism, several cultures were also examined for the presence of cells possessing *a* or *α* mating potentialities. For this determination, standard inocula of the strain to be tested were mixed and plated with equal volumes of the *a* or *α* testers on ME. After 4 days incubation at 28°C, the ratio of zygotes to vegetative cells was determined in a sample of each cell mixture and compared with the ratio in the homothallic strain grown by itself. The rationale of this procedure was as follows: In the presence of an excess of *a* tester cells, each cell in the homothallic population possessing *α* characteristics should mate. Similarly, a challenge with an excess of *α* tester cells should initiate zygote formation by each cell with *a* qualities. Thus any marked increase in the zygote : vegetative-cell ratio in mixed matings compared to homothallic control implied the presence of cells possessing *a* or *α* mating potentialities in the homothallic culture. Usually 400 to 500 cells were counted per sample. Larger numbers were counted when the ratio of zygotes to vegetative cells was less than 1% in the homothallic strain. Generally the homothallic segregants contained an excess of competent *a* or *α* cells. Those possessing more *a* cells were designated as *a*-associated homothallic strains; those with more *α* cells as *α*-associated homothallic cultures.

Tetrads exhibiting normal segregation patterns for adenine, histidine, leucine and/or tryptophan nutritional markers as well as for the carbohydrases described previously (HERMAN and HALVORSON 1963) were used in this study. With the exception of one adenine requiring mutation, none of the markers used was closely linked to the mating locus. The adenine mutant controlled

the production of a red pigment and was linked to the mating locus with a recombination frequency of about 4%.

Each stock is identified by a tetrad number and a spore-culture letter A through D. The crosses and tetrads derived from each hybridization which were used in this study are listed in Table 1. In all the hybridizations considered in this paper, the complete genotype relating to mating type is listed. This is followed, in parentheses, by the stock number of the respective parents used in each cross.

RESULTS

The parental mating types: Parental cultures Y14 and Y123 mated and sporulated when they were mixed and grown on ME plates. Neither strain showed a mating response when grown alone on ME. They were, therefore, identified as heterothallic cultures. Strain Y14 was designated as the *a* mating type, strain Y123 as the α mating type.

Evidence for homothallism: When the mating type of progeny derived from the parental cross $a \times \alpha$ (Y14 × Y123) was determined, both homothallic and heterothallic segregants were identified. Segregation ratios for homothallism: heterothallism included 3:1 (3 tetrads), 2:2 (50 tetrads), 1:3 (77 tetrads), and 0:4 (26 tetrads). The 3 homothallic:1 heterothallic ratio indicated that the hybrid was heterozygous for more than one locus for homothallism while the 0 homothallic:4 heterothallic ratio implied that the homothallic condition required specific combinations of the homothallic factors and the mating type alleles. In the 26 tetrads in which all of the progeny were heterothallic, segregation of *a* and α mating types could be followed readily. The ratio of a:α was always 2:2 and indicated that *a* and α were segregating as alleles of the mating locus. It was inferred that *a* and α segregated in this manner in all of the tetrads even though

TABLE 1

Hybridizations and tetrads used in this investigation

Hybridization	Tetrad number
$ah_aH_\alpha \times \alpha H_a h_\alpha$ (Y14 × Y123)	155
	157
	311
	338
	599
	729
	1009
$\alpha h_a H_\alpha$ (729B*)	1216
$aH_a h_\alpha$ (155C*)	251
$aH_a h_\alpha$ (338C*)	953
$\alpha H_a h_\alpha$ (251A*)	1254
	1256
$\alpha H_a h_\alpha$ (1256C*)	1290
$aH_a h_\alpha \times ah_a H_\alpha$ (251A* × Y14)	1255
	1257
	1258

* Homothallic.

this segregation was obscured in many tetrads by the presence of the homothallic condition.

To account for the above observations, the following hypothesis is proposed: In addition to the mating type alleles a and α, there are two nonallelic genes for homothallism, H_a and H_α. The combinations αH_α (either $\alpha h_a H_\alpha$ or $\alpha H_a H_\alpha$) and aH_a (either $aH_a h_\alpha$ or $aH_a H_\alpha$) result in homothallism. Based on this hypothesis, the genotype of parent Y14 is $ah_a H_\alpha$ and Y123 is $\alpha H_a h_\alpha$ and both are therefore heterothallic.

On this interpretation, the cross $ah_a H_\alpha \times \alpha H_a h_\alpha$ (Y14 × Y123) would be expected to yield haploid progeny of the following genotypes: (1) $ah_a H_\alpha$, (2) $ah_a h_\alpha$, (3) $\alpha H_a h_\alpha$, (4) $\alpha h_a h_\alpha$, (5) $aH_a H_\alpha$, (6) $aH_a h_\alpha$, (7) $\alpha H_a H_\alpha$, and (8) $\alpha h_a H_\alpha$. The first four would be heterothallic and the last four homothallic segregants. All possible tetrad combinations and the frequency with which these tetrads were obtained are given in Table 2.

The linkage relations between the three genes can be determined from the number of parental ditype (PD), nonparental ditype (NPD) and tetratype (T) segregations shown in Table 2. The PD:NPD:T ratio for a in relation to h_a is 28:31:97; it is 129:0:27 for α in relation to h_α; and 48:28:80 for H_a in relation to H_α. Linkage between two genes is demonstrated when the parental ditypes are significantly more frequent than the nonparental ditypes (PERKINS 1953). It can be seen that a and h_a exhibit no linkage, whereas α and h_α are strongly linked ($27/156 \times 1/2 = 9\%$). There is also some indication of linkage between H_a and H_α but at best these are loosely linked.

TABLE 2

Type of segregations observed in the mating $ah_aH_\alpha \times \alpha H_a h_\alpha$ *(Y14 × Y123)*

	Type of tetrad								
	$aH_a h_\alpha$ $aH_a h_\alpha$ $\alpha h_a H_\alpha$ $\alpha h_a H_\alpha$	$aH_a h_\alpha$ $ah_a h_\alpha$ $\alpha H_a H_\alpha$ $\alpha h_a H_\alpha$	$aH_a H_\alpha$ $aH_a h_\alpha$ $\alpha h_a H_\alpha$ $\alpha h_a h_\alpha$	$ah_a h_\alpha$ $ah_a h_\alpha$ $\alpha H_a H_\alpha$ $\alpha H_a H_\alpha$	$aH_a H_\alpha$ $aH_a H_\alpha$ $\alpha h_a h_\alpha$ $\alpha h_a h_\alpha$	$aH_a h_\alpha$ $ah_a H_\alpha$ $\alpha H_a h_\alpha$ $\alpha h_a H_\alpha$	$ah_a H_\alpha$ $ah_a h_\alpha$ $\alpha H_a H_\alpha$ $\alpha H_a h_\alpha$	$aH_a H_\alpha$ $ah_a H_\alpha$ $\alpha H_a h_\alpha$ $\alpha h_a h_\alpha$	$ah_a H_\alpha$ $ah_a H_\alpha$ $\alpha H_a h_\alpha$ $\alpha H_a h_\alpha$
Numbers observed	0	0	3	0	28	22	2	75	26
Ratio of homothallism: heterothallism	4:0	3:1	3:1	2:2	2:2	2:2	1:3	1:3	0:4
Ratio of homothallism:a:α	4:0:0	3:1:0	3:0:1	2:2:0	2:0:2	2:1:1	1:2:1	1:1:2	0:2:2
PD, NPD or T* relationship for genes									
a:h_a	NPD	NPD	NPD	PD	NPD	T	PD	T	PD
a:H_α	NPD	NPD	T	NPD	PD	T	T	PD	PD
h_a:H_α	PD	T	T	NPD	NPD	PD	T	T	PD

* PD = parental ditype; NPD = nonparental ditype; T = tetratype.

If all three genes are on the same chromosome, the probable order is a-H_α-h_a. With these linkage relations in mind, it is easy to understand why some of the classes in Table 2 were not obtained. These would have required a four-strand double crossover between the mating-type locus and the H_α locus; such events would be expected to be rare. The other minority classes require a single crossover in this region.

Independent segregation of H_a *and* H_α*:* To test the foregoing hypothesis that there are two nonallelic genes governing homothallism, tetrads 157 and 1009 derived from the cross Y14 × Y123 were examined for the genotypes of some of their segregants.

In the first tetrad, 157, the mating segregation was 2 homothallic: 2α (Table 3). We can infer from this segregation that 157A and 157D, which are homothallic, each contained an a allele and H_a. Spores 157B and 157C, the heterothallic segregants, should therefore each possess an h_a allele and, since they are of α mating type, an h_α allele as well. The proposed genotypes of the four segregants are summarized in Table 3. Neither 157B nor 157C should produce a-associated homothallic progeny when mated with heterothallic ah_a cells.

Eighteen tetrads from the cross $\alpha h_a h_\alpha \times a h_a H_\alpha$ (157B × Y14) and 15 asci from the cross $\alpha h_a h_\alpha \times a h_a H_\alpha$ (157C × Y14) were dissected and the mating types of the segregants determined. The complete segregations for mating type observed in these crosses were as follows: The mating $\alpha h_a h_\alpha \times a h_a H_\alpha$ (157B × Y14) produced 2a:2α (16 tetrads) and 2a:1α:1H (2 tetrads) ratios, while from the cross $\alpha h_a h_\alpha \times a h_a H_\alpha$ (157C × Y14) 2a:2α (13 tetrads) and 2a:1α:1H (2 tetrads) ratios were obtained. As predicted, none of the a-bearing segregants were homothallic.

In the second tetrad, 1009, the mating types of the segregants were 2α:1a:1H. The proposed genotypes of the four spore-cultures are given in Table 3. Both 1009A and 1009D carried the h_α allele since they were heterothallic and of α mating type. One of these segregants should also contain an H_a gene. To determine which segregant contained H_a, each strain was mated with $ah_a H_\alpha$ (Y14) cells and their progeny examined for a-associated homothallism.

The segregations from the zygote $\alpha H_a h_\alpha \times a h_a H_\alpha$ (1009A × Y14) yielded 2a:2α (6 tetrads), 1a:2α:1H (18 tetrads) and 2α:2H (8 tetrads) ratios while the cross

TABLE 3

Mating type of spore isolates from tetrads 157 and 1009

Tetrad	Mating type	Proposed genotype
157A	H*	aH_aH_α
157B	α	$\alpha h_a h_\alpha$
157C	α	$\alpha h_a h_\alpha$
157D	H	aH_aH_α
1009A	α	$\alpha H_a h_\alpha$
1009B	H	aH_aH_α
1009C	a	ah_aH_α
1009D	α	$\alpha h_a h_\alpha$

* H = homothallic.

$\alpha h_a h_\alpha \times a h_a H_\alpha$ (1009D × Y14) gave $2a$:2α (31 tetrads) and $2a$:1α:$1H$ (4 tetrads) ratios. Twenty-six of the 32 tetrads derived from the mating of 1009A × Y14 thus contained a-associated homothallic segregants while none of the 35 tetrads obtained from the cross 1009D × Y14 possessed a-associated homothallic isolates. As predicted, only one of the two strains, 1009A, carried the H_a allele. Therefore the complete genotype of 1009A was $\alpha H_a h_\alpha$ while that of 1009D was $\alpha h_a h_\alpha$.

A further test of the genotypes of the 1009 tetrad was made by hybridizing 1009A × 1009C. The inferred genotypes are $\alpha H_a h_\alpha$ and $a h_a H_\alpha$ respectively. A mating of these two heterothallic cultures should be equivalent to that of Y123 × Y14 and should produce both a-associated and α-associated homothallic recombinants. This proved to be the case. Forty-five tetrads from the mating 1009A × 1009C were dissected and the mating types of the spore isolates determined. The segregations obtained from this cross were $2a$:2α (8 tetrads), $1a$:2α:$1H$ (24 tetrads), 2α:$2H$ (9 tetrads), $2a$:1α:$1H$ (1 tetrad), and $1a$:1α:$2H$ (3 tetrads). Thirty-six of the 45 tetrads contained a-associated homothallic segregants while 4 of the 45 contained α-associated homothallic spore cultures.

In summary, tests of the tetrads 157 and 1009 plus the original observations on homothallic distribution patterns (Table 2) served to identify the presence of two independent allele-specific homothallic factors (H_a and H_α) in the heterothallic parents Y123 and Y14 respectively.

Characteristics of homothallic cultures: As mentioned earlier, homothallism in *S. cerevisiae* and related species arises from a change in the expression of the mating locus from one allele to the other. If H_a and H_α produce homothallism in *S. lactis* through an analogous effect, one would expect the homothallic strains to consist of a mixture of competent a and α cells. To test this possibility, cells from each of the four homothallic genotypes, $\alpha h_a H_\alpha$, $\alpha H_a H_\alpha$, $a H_a h_\alpha$ and $a H_a H_\alpha$, were challenged with a and α testers as described under MATERIALS AND METHODS. The number of zygotes formed in the presence of excess a or α cells was an indication of the number of competent α or a cells present in the homothallic culture. The results are shown in Table 4. Each representative strain contained both a and α cells. A comparison of the ratio of competent α:a cells shows that in the two a-bearing classes, $a H_a h_\alpha$ and $a H_a H_\alpha$, cells of a mating type predominated. In the two α-bearing series, $\alpha h_a H_\alpha$ and $\alpha H_a H_\alpha$, on the other hand, there was an excess of cells of α mating type. Thus homothallic lines containing the a allele could be distinguished from those with the α allele by differences in the ratio of a:α cells present in the population.

As can be seen in Table 4, a marked difference in the α:a ratio exists between the two a-bearing homothallic lines $a H_a H_\alpha$ and $a H_a h_\alpha$. This difference can be used as a basis for distinguishing between these two genotypes. On the other hand, no significant differences were observed in the ratio of α:a cells between the two α-bearing homothallic genotypes $\alpha h_a H_\alpha$ and $\alpha H_a H_\alpha$ and, therefore, these two genotypes can not be distinguished from one another by this procedure.

It can be seen from Table 4 that the α homothallic members showed a lower percent homothallism than the a type. This was observed consistently, but at the present time no explanation for this behavior is evident. Perhaps αH_α is a more

TABLE 4

Mating response of representative clones from the four homothallic classes aH_ah_α, aH_aH_α, αh_aH_α, and αH_aH_α

	Genotype	Percent homothallism*	Percent competent a cells	Percent competent α cells	Ratio competent α:a cells
aH_ah_α					
	155C	4.4	7.1	2.0	0.3
	338C	0.6	7.2	0.2	0.03
aH_aH_α					
	1009B	12	4.7	4.2	0.9
	155B	11	7.4	6.3	0.9
	157A	6	3.5	2.5	0.7
	157D	20	15	12	0.8
	338D	7	6.1	3.9	0.6
αh_aH_α					
	729B	1.5	2.7	8	3
	155A	0.6	0.6	8	13
	338D	0.6	0.2	7.2	36
	599B	0.5	0.4	5.9	14
αH_aH_α					
	311C	1.9	1.1	3.6	3

* Procedures used for determining percent homothallism and percent competent a and α cells are described in MATERIALS and METHODS.

stable gene combination than aH_a. In all of these measurements, the percent homothallism expressed by the clones was stable and reproducible with a maximum error of 4 to 5%.

Expression of αh_aH_α *homothallism:* The preceding study indicated that each of the four classes of homothallic cultures (aH_aH_α, aH_ah_α, αH_aH_α, and αh_aH_α) contained mixtures of a and α cells. This suggested that H_a and H_α caused a shift in expression of the mating type allele from a to α or vice versa. The initial segregation patterns for homothallism had led to the assumption, furthermore, that the effects of H_a and H_α were directed in a specific manner against the a and α alleles respectively. If this were the case, one should be able to predict the mating response of segregants arising from the mating of sister cells in a homothallic culture. If H_α, for example, converted the α allele in cells of an αh_aH_α clone to the a allele, these newly derived a cells should be (1) unaffected by the H_α gene, (2) heterothallic, and (3) of a mating type. Consequently segregants derived from a mating of an αh_aH_α cell with such a newly derived a sister cell should display a 2:2 segregation for homothallism: a mating types.

To test these predictions, the mating response of αh_aH_α homothallic clone, 729B, as well as that of spore isolates obtained from one of its tetrads, 1216, was studied. The results are shown in Table 5. Most of the competent cells in the homothallic parent 729B were of α mating type. Two of the segregants (1216A and 1216C) derived from a self-sporulated ascus of 729B were homothallic and responded to tester cells in a manner analogous to the homothallic parent 729B. On the other hand, the other two segregants, 1216B and 1216D, formed zygotes with the α

TABLE 5

Percent homothallism and mating response of homothallic parent ah_aH_α (729B) and progeny derived from its self-sporulated tetrad 1216

Culture number	Genotype	Percent homothallism	Percent competent		Ratio competent α:a cells
			α cells	a cells	
729B	ah_aH_α	1.5	8	2.7	3
1216A	ah_aH_α	3.9	11	3	3
1216B	ah_aH_α	0	0	25	0
1216C	ah_aH_α	6.3	4	2.5	1.6
1216D	ah_aH_α	0	0	16	0

tester strain only, i.e., they behaved as stable heterothallic cells of a mating type. Since these a segregants were derived from a self-sporulated h_aH_α zygote, their complete genotype can be expressed as ah_aH_α. Further, the newly derived a cells showed a normal response to the effects of the H_a gene. For example, when one of the a segregants, 1216B, was mated with the heterothallic parent $\alpha H_a h_\alpha$ (Y123), typical aH_a recombinant homothallic progeny were obtained. The complete segregations for this cross $ah_aH_\alpha \times \alpha H_a h_\alpha$ (1216B × Y123) included 2α:$2a$ (4 tetrads), 2α:$1a$:$1H$ (2 tetrads), 2α:$2H$ (1 tetrad), and 1α:$1a$:2H (1 tetrad). From these results it is concluded that H_α renders α strains homothallic by changing the expressed mating type of some cells within the culture from α to a.

Expression of aH_ah_α *homothallism:* Since clones of aH_ah_α and aH_aH_α genotypes consisted of a mixture of a and α cells (Table 4), it was assumed that H_a, in a manner analogous to the action of the H_α gene, effects a shift of the mating allele from a to α. If this were true, one would expect a 2:2 segregation of homothallism: α mating type in tetrads derived from self-sporulated aH_ah_α cultures.

When self-sporulated tetrads obtained from two independent aH_ah_α clones (155C and 338C) were examined, the results shown in Table 6 were obtained.

TABLE 6

Comparison of percent homothallism and mating response of two homothallic aH_ah_α *clones (155C and 338C) and their progeny*

Culture number	Genotype	Percent homothallism	Percent competent		Ratio competent α:a cells
			α cells	a cells	
155C	aH_ah_α	4.4	2.0	7.1	3.5
251A	αH_ah_α	3.2	5.0	2.4	0.5
251B	aH_ah_α	5.9	2.8	10.0	3.6
251C	aH_ah_α	5.5	2.7	7.8	2.9
251D	αH_ah_α	3.0	5.3	2.6	0.5
338C	aH_ah_α	2.4	0.4	7.0	17.5
953A	αH_ah_α	1.5	4.3	0.4	0.1
953B	aH_ah_α	3.0	3.9	4.1	1.1
953C	aH_ah_α	4.2	2.6	2.8	1.1
953D	αH_ah_α	0.7	5.8	1.2	0.2

Tetrad 251 was derived from homothallic culture 155C and 953 from 338C. Contrary to expectation, it can be seen that instead of the predicted 2:2 ratio of homothallism to α mating type, all four segregants from each tetrad were homothallic. This discrepancy will be considered in more detail later. First let us compare the relative proportions of competent a:α cells present in each segregant. It should be noted that two of the segregants in 251 and 953, although homothallic, each exhibited a clear-cut excess of α-competent cells, whereas the parental cultures 155C and 338C both had an excess of a-competent cells. This suggests that H_a has induced a change from a to α but that this change is unstable in these tetrads.

Characteristics of newly derived $\alpha H_a h_\alpha$ cells: If, as the evidence indicates, H_a converts the mating potentialities of a fraction of the cell population from the a to the α form, then why do these H_a-induced α cells remain homothallic? It was possible that in the H_a mediated conversion of a to α something analogous to phenotypic lag occurred, i.e., after the shift from a to α, dilution of some remaining "a" type component was necessary before the H_a-induced α cells became phenotypically heterothallic and of α mating type.

To test this suggestion, the homothallic potentialities and mating response of the H_a-induced α strain 251A were examined at monthly intervals for a period of 3 months. Between examinations, the culture was maintained on stock media and transferred at weekly intervals. Consequently, numerous generations of vegetative growth occurred between consecutive comparisons. The percent homothallism varied from one test to another by 0.8% while the ratio of competent α:a cells remained at approximately two. Thus, no change in the homothallic potentialities or mating response occurred over this time interval. Therefore, dilution of some "a" mating type component via vegetative growth seemed unlikely.

The two following alternative possibilities were considered: (1) The instability might reflect some property unique to the $aH_a h_\alpha$ parents from which the α strains were derived rather than that of the new α allele itself; or (2) the instability resulted from characteristics specific to the newly derived α cell itself.

The first alternative was considered by examining the mating response of the α progeny derived from a cross of a homothallic $aH_a h_\alpha$ (155C) strain with a known heterothallic $\alpha H_a h_\alpha$ (Y123) line. Since the parents were homozygous for the h_α allele, all of the α-bearing progeny should be heterothallic. However, if the $aH_a h_\alpha$ (155C) parent possessed some additional unrelated property which rendered the α allele unstable, then some, if not all, of the α-bearing progeny should become homothallic. This was not observed experimentally. In each of six tetrads from the cross $aH_a h_\alpha \times \alpha H_a h_\alpha$ (155C $\times$ Y123) all of the α-bearing progeny were heterothallic. The segregation ratios in each case were 2α:$2H$. Thus, one could eliminate the first alternative.

To test the second alternative, that the observed instability in mating type was due to some quality unique to the newly derived α cell, the homothallic behavior of segregants derived from the self-sporulated unstable $\alpha H_a h_\alpha$ culture 251A (from 155C, Table 6) was examined for evidence that the instability was transmissible. In culture 251A the ratio of zygotes:100 vegetative cells averaged three, and there were approximately twice as many competent α cells as a cells within

TABLE 7

Comparison of percent homothallism and mating response of homothallic parent $\alpha H_a h_\alpha$ (251A) and progeny derived from two self-sporulated tetrads 1254 and 1256

Culture number	Genotype	Percent homothallism	Percent competent		Ratio competent α:a cells
			α cells	a cells	
251A	$\alpha H_a h_\alpha$	3.2	5.0	2.4	2.1
1254A	$\alpha H_a h_\alpha$	0.6	4.9	0.3	16.3
1254B	$\alpha H_a h_\alpha$	0.5	13.0	0.6	21.6
1254C	$\alpha H_a h_\alpha$	6.0	3.0	7.0	0.5
1254D	$\alpha H_a h_\alpha$	3.0	1.5	7.0	0.2
1256A	$\alpha H_a h_\alpha$	6.4	3.0	11.0	0.3
1256B	$\alpha H_a h_\alpha$	6.0	3.0	13.0	0.2
1256C	$\alpha H_a h_\alpha$	0.6	11.9	0.3	39.6
1256D	$\alpha H_a h_\alpha$	1.4	6.7	1.0	6.7

the population. When asci from 251A were dissected and the mating potentials of the segregants were compared to those of 251A, one observed marked differences in the mating response of some of the segregants. The results from two such self-sporulated tetrads, 1254 and 1256, are shown in Table 7. In 1254, the homothallic potentialities of two of the segregants, 1254A and 1254B, displayed a marked decrease fivefold to sixfold from that of parent 251A. Further, when these isolates were challenged with a and α testers, they exhibited a 16-fold to 22-fold excess of competent α cells compared to a cells. Thus, following one meiotic passage the unstable α segregants of presumed $\alpha H_a h_\alpha$ genotype had undergone a fivefold reduction in their homothallic potentialities while the ratio of competent α:a cells had increased by a factor of 8 to 11 over the $\alpha H_a h_\alpha$ parent 251A.

In the second tetrad, 1256, the α segregants, 1256C and 1256D, displayed a similar pattern of behavior (Table 7). Each isolate showed a reduced percentage of homothallism and an increased ratio of competent α:a cells. However, the degree of stabilization exhibited by these two α sister segregants was noticeably different. In 1256C the percent homothallism had been reduced by a factor of five (from 3% in parent 251A to 0.6% in the segregant) while the ratio of competent α:a had risen 20-fold (from 2% in parent 251A to 39.6 in 1256C). In isolate 1256D, on the other hand, the difference between parent and segregants was not as marked. The percent homothallism had been reduced by a factor of two and the ratio of competent α:a had shown a threefold increase only. These results indicated that, whatever the nature of this meiotically induced stabilization, it was not directed with equal intensity against the two α sister segregants.

The percent homothallism continued to decrease following additional meioses. For example, in tetrad 1290, derived from a self-sporulated zygote of 1256C, the α segregants 1290A and 1290B showed a further reduction in homothallic behavior (0.2% compared to 0.6% in parent 1256C) while the ratio of competent α:a cells remained high (20- to 22-fold excess) (Table 8). Thus, following three generations of self-sporulation the percent homothallism in H_a-induced α segre-

TABLE 8

Comparison of the percent homothallism and mating response of homothallic parent $\alpha H_a h_\alpha$ *(1256C) and its progeny 1290*

Culture number	Genotype	Percent homothallism	Percent competent		Ratio competent α:a cells
			α cells	a cells	
1256C	$\alpha H_a h_\alpha$	0.6	11.9	0.3	39.6
1290A	$\alpha H_a h_\alpha$	0.2	4.0	0.2	20
1290B	$\alpha H_a h_\alpha$	0.2	4.0	0.16	22
1290C	$aH_a h_\alpha$	11.0	1.5	7.0	0.2
1290D	$aH_a h_\alpha$	6.0	1.2	4.0	0.3

gants decreased from 3% in culture 251A through 0.6% in culture 1256C to 0.2% in strains 1290A and 1290B.

This tendency of homothallic $\alpha H_a h_\alpha$ segregants to progress toward heterothallism occurred in tetrads arising from homothallic × heterothallic crosses also. As shown in Table 9, each of the α segregants arising from the mating of homothallic $\alpha H_a h_\alpha$ × heterothallic $ah_a H_\alpha$ cells (251A × Y14) was more strongly heterothallic than the parent 251A. Further, and as was observed above in the self-sporulated α segregants, the stabilizing effects exerted on sister α segregants were not identical and did not appear to follow any pattern. However, disregarding these inequalities in effect, it is evident that the reduction in the homothallic tendencies of $\alpha H_a h_\alpha$ cultures depends, apparently, upon some basic mechanism associated with or following meiosis.

This continued decrease in homothallic behavior associated with sequential mating and sporulation suggests that, if these processes were continued long enough, one might eventually obtain completely stabilized $\alpha H_a h_\alpha$ heterothallic strains of α mating type such as the α parent Y123 and implies that this may have been the mechanism through which this parent was formed. Possible explanations for the homothallism associated with newly derived H_a-induced α cells will be considered in the DISCUSSION.

TABLE 9

Comparison of the percent homothallism and mating response of homothallic parent $\alpha H_a h_\alpha$ *(251A) and homothallic α segregants from the mating* $\alpha H_a h_\alpha \times a h_a H_\alpha$ *(251A × Y14)*

Culture number	Genotype	Percent homothallism	Percent competent		Ratio competent α:a cells
			α cells	a cells	
251A	$\alpha H_a h_\alpha$	3.2	5.0	2.4	2.1
1255C	$\alpha H_a h_\alpha$	0.4	8.0	0.1	80
1255D	$\alpha H_a h_\alpha$	1.8	4.0	0.4	10
1257A	$\alpha H_a h_\alpha$	0.1	7.0	<0.1	>70
1257D	$\alpha H_a h_\alpha$	1.1	4.0	0.7	5.7
1258C	$\alpha H_a h_\alpha$	0.9	9.5	0.5	19
1258D	$\alpha H_a h_\alpha$	3.1	2.9	0.3	10

DISCUSSION

Evidence from this study indicates that H_a and H_α produce homothallism by shifting the expressed mating type to the alternate form in a certain portion of the cell population. Except for the allele specificity of H_a and H_α, their mode of action parallels that of the *D* gene (OESER 1962; HAWTHORNE 1963b) quite closely. *Saccharomyces lactis* does not hybridize with *S. cerevisiae* and related strains; therefore, one cannot test the homology of the two systems.

The nature of the mechanism through which H_a and H_α produce their effects remains a point of speculation. A possible explanation is that H_a and H_α produce the shift in mating type by increasing the rate of mutation at the mating locus from one allele to the other. This would, however, require specific mutagenic effects by products from H_a and H_α and such effects are as yet unknown.

Are H_a and H_α acting as regulatory genes (JACOB and MONOD 1961)? If, as in other yeasts, the mating locus is complex (LEUPOLD 1958 and HAWTHORNE 1963a) and consists of *a*- and α-determining regions one might postulate that H_a and H_α produce specific repressors that determine which region of the complex locus will be expressed. Such a model proves inadequate, however, since it does not explain the heterothallic nature of the $\alpha h_a h_\alpha$ and $a h_a h_\alpha$ recombinants which were obtained (Table 2) nor does it account for the stability of the "mutation" once it is induced. Thus no satisfactory explanation for the action of H_a and H_α is evident at this time.

Also still obscure is the significance of the paradoxical observation relating to the homothallic behavior of H_a-induced α cells. Contrary to expectation, these H_a-induced α cells were homothallic, and the degree of homothallism remained constant during an extended period of vegetative propagation; however, the homothallic potentialities of these H_a-induced α cells were not transmitted intact to their α-bearing progeny as evidenced by the marked decline in the percent homothallism expressed by the α segregants. This instability in mating type did not reflect a generalized effect of the $aH_a h_\alpha$ genome on all α alleles as shown by the fact that normal stable heterothallic segregants of α mating type were consistently derived from the mating of $aH_a h_\alpha$ cells with known heterothallic cells of $\alpha H_a h_\alpha$ genotype. Thus, the observed instability of the newly derived H_a-induced α cultures apparently reflected an interaction between the $\alpha H_a h_\alpha$ parental cells and the newly derived α clones.

These observations suggest (1) that the H_a-induced α cells retained the $aH_a h_\alpha$ parental capacity to form *a* mating component even though they lacked the necessary genetic information and (2) that this capacity was propagated in some autonomous manner during vegetative growth but decreased during sporulation. This capacity could reflect either a delay in the accumulation or distribution of a product determining α mating type or a reduction in the ability of the newly derived α cells to destroy information carried over from the $aH_a h_\alpha$ parent for determining synthesis of *a* mating type product.

One region of the cell which may serve as the vehicle for transmission and expression of the homothallic potentialities observed in H_a-induced α strains is

the cell wall. It has been demonstrated that reactions between specific cell wall components implements mating between some heterothallic yeasts (BROCK 1959). EDDY and WILLIAMSON (1959) have suggested that during vegetative propagation yeast cell wall structure is copied from existing cell wall. A continuous flow of nuclear information directing cell wall synthesis may not be necessary.

If specificity for *a* mating type resides in some cell wall component, it is easy to see how, once the *a*-forming capacity of $\alpha H_a h_\alpha$ cells was expressed in cell wall structures, its continued synthesis could remain constant during extended periods of vegetative growth.

Since it is known that yeast spores possessing normal cell walls are formed from diploid protoplasts devoid of cell walls (EDDY and WILLIAMSON 1959), it is apparent that spore wall synthesis must be nuclear controlled rather than initiated from vegetative cell wall primer. The switch from vegetative to nuclear control at this time would, of course, permit full expression of cell wall determinants quite different from those expressed in the original diploid. In the case of the H_a-induced α homothallic cells, any preceding events leading to the inactivation of the nonnuclear *a*-determining information could be expressed at this time as a reduction in the homothallic potentialities of clones derived from the α spores.

Finally, one further implication which may be derived from these experiments should be considered briefly. H_a and H_α cause the mating locus to shift from one allele to the other. However, this shift in mating type reflects only one aspect of the overall process. As was indicated earlier, the percent homothallism expressed by representatives of the four homothallic classes, aH_aH_α, aH_ah_α, αH_aH_α and αh_aH_α was reproducible to within a few percent. This constancy in percent homothallism would not be expected if the H_a- and H_α-directed shift in mating type had occurred at random and over a period of time in a population of vegetatively growing cells. Under such circumstances, one would have observed an alteration in the percent homothallism due to changes in the number of derived *a* or α cells which would accumulate in the population. On the contrary, the observed constancy in percent homothallism implies that the H_a- and H_α-directed shift did not occur during vegetative growth, but appeared after the cells had been transferred to ME only. Thus, it is evident that another independent system of controls must determine when H_a and H_α will exert their effect. Since nothing is known about the nature of the physiological changes which occur in *S. lactis* during growth on ME, speculation concerning the nature of the controls which allow H_a and H_α to become active during growth on this medium would be fruitless at this time.

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SUMMARY

Two unlinked independent genes designated H_a and H_α produce homothallism in strains of *S. lactis*. These factors exert their effects by changing the mating type to the alternate form in a small proportion of a cell population. Both H_a and H_α are allele specific. H_α changes α mating type to *a* while H_a changes *a* to α.

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