MUTATION OF A HETEROTHALLIC STRAIN TO HOMOTHALLISM

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ABSTRACT

Upon mutagenesis, a heterothallic $\alpha\alpha$ diploid strain mutated to homothallism. The gene confering homothallism is nuclear, recessive, and unlinked to mating type. This gene is not allelic to the *HO* gene, which is responsible for previously described instances of homothallism in yeast. We have designated this new gene for homothallism as *cmt* (change of mating type).

IN homothallic strains of *S. cerevisiae*, diploidization (WINGE 1949) occurs by virtue of a "mutation" from **a** to α mating type, or *vice versa*. Subsequently the variant cells mate with siblings of the original mating type, giving rise to $\boldsymbol{a}\alpha$ diploids. This change occurs during the first few mitotic divisions following ascospore germination (HAWTHORNE 1963a, b). As a result, colonies grown from single spores of homothallic strains are generally found to contain only $\boldsymbol{a}\alpha$ diploid cells.

Previously-studied instances of homothallism in yeast were shown to involve three unlinked genes, *HO*, *HM*α, and *HM***a***. *HM*α and *HM***a** control the specificity of mutation at the mating-type locus, while *HO* determines whether or not mutation may take place (TAKANO and OSHIMA 1970; OSHIMA and TAKANO 1971; 1972). The existence of these three genes has been inferred from the observations that crosses between various heterothallic Saccharomyces species produce hybrid progeny which are homothallic (TAKAHASHI 1958; OSHIMA and TAKANO 1972). Consequently, the genes for homothallism are defined not by mutant and wild-type alleles, but by their presence in some species or strains and absence from others.

By mutagenesis of an $\alpha\alpha$ diploid strain, we isolated a number of yeast mutants which gained the ability to sporulate (HOPPER and HALL 1975). In this paper we present an analysis of one of these mutant strains, D-135a, which is a nonmater. Upon sporulation of D-135a, all progeny clones behave as $\boldsymbol{a}\alpha$ diploids. Marker segregation data and dominance marker tests indicate that D-135a is homozygous for a recessive nuclear gene for homothallism. This gene is not allelic to *HO*.

MATERIALS AND METHODS

Strains: the mutant strain, D-135a, the triploid strain homozygous for mating type, AU-155 (**aaa**), and mating tester strains were described in the previous communication.

* An alternate nomenclature for these loci has been proposed (PLISCHKE *et al.* 1975) in which *HO* has been renamed *HTH1*; *HM*α—*HTH2-*α and *HM***a**—*HTH3*-a.

S41 (*HO/HO HMa/HMa HMα/HMα arg4/arg4, cyh1/cyh1*) is a homothallic strain previously described (ESPOSITO *et al.* 1970).

Techniques: All media and techniques are as previously described (HOPPER and HALL 1975).

RESULTS

Mating and sporulation behavior of D-135a: In preliminary studies (HOPPER and HALL 1975) mutant D-135a, isolated from AP-1-*αα*, was found to sporulate with high efficiency, but to be incapable of mating with all tester strains. These properties suggest that D-135a may have undergone mutation at the mating-type locus, changing from *αα* to ***aα***. If that were the only mutational alteration, sporulations of D-135a would produce two ***a*** and two *α* segregants from each ascus. An analysis of segregants from asci produced by D-135a makes it evident that this is not the case; apparently some more complex genetic alteration has occurred.

Dissection of nine asci yielded 29 viable spores. When cultures were grown from the spores, none of the 29 was able to mate with any one of 3***a*** and 3*α* tester strains (HOPPER and HALL 1975) as analyzed either by zygote formation or prototroph production. Of the 29 segregant cultures 20 were capable of sporulation, producing microscopically visible asci within 3 days (at 32°) after being replicated onto sporulation agar. Included in these 20 were 3 segregants from each of the asci that yielded 4 viable spores (Table 1).

The two most obvious explanations for our observation that meiotic segregants of D-135a are non-maters which are able to sporulate are: (1) that the original mutation changed AP-1-*αα* into an ***aa*** diploid which then proceeded to mate with an *αα* sibling, thereby giving rise to an ***aaαα*** tetraploid strain. Such a tetraploid would be a non-mater and would be capable of sporulation. *Some* of the asci produced would contain 4 ***aα*** diploid spores, which would themselves be capable of sporulation, producing haploid segregants capable of mating, but incapable of sporulating. (2) that the original mutation was not simply a change from one functional allele to the other at the mating-type locus. Instead, the strain has changed its conjugal behavior from heterothallism to homothallism. Such a change might result in either a diploid or tetraploid homothallic strain.

Segregation of mating-type and nutritional markers: The ratios of auxotrophic to prototrophic spores in individual asci allow a direct determination of the ploidy of D-135a. If D-135a were a diploid, when it sporulated the recessive nutritional markers for which it is heterozygous should have segregated 2:2. If, however, D-135a were tetraploid, 4:0 or 3:1 ratios of phenotypically wild-type to mutant segregants should have been found for many asci. For centromere-linked markers, tetraploid segregation should give 4:0 ratios in 2 out of 3 asci; for non-centromere-linked markers, 8/9 of the asci should be either 4:0 or 3:1 (ROMAN, PHILLIPS and SANDS 1955). D-135a is heterozygous for *gal1*, *lys2*, *tyr1*, *his7*, *leu1*, and *cyh2* mutations [*gal1* and *leu1* are centromere-linked; the other loci are neither linked to their respective centromeres nor to each other (MORTIMER and HAWTHORNE 1973)]. For each of these loci, segregation of 2

wild type : 2 mutant spores was observed in all asci (4) that gave 4 viable spores. Marker segregation is thus precisely that expected for a diploid (2:2) and shows no instances of the 4:0 or 3:1 ratios of wild-type : mutant segregants expected for tetraploid meiosis (Table 1).

If D-135a had been an ***aaαα*** tetraploid and had undergone meiotic pairing and disjunction in the same manner as other ***aaαα*** tetraploids, the distribution of ascus types would have been 48.2% Type I (4 ***aα*** spores): 9.5% Type II (2***aa*** and 2***αα*** spores): 42.3% Type III (2***aα***, 1***aa***, and 1***αα*** spores) (ROMAN, PHILLIPS and SANDS 1955). Averaging over the three ascus types, 31% of all spores should be either **aa** or ***αα***. Of the 29 F_1 segregant cultures grown from spores of D-135a,

TABLE 1.

Summary of D-135a spore characteristics

	Segregation of genetic markers						Mating		
Spores	*gal1*	*lys2*	*tyr1*	*his7*	*leu1*	*eyh2*	56a S400-D M25-3D	S2072α M25-18B(α) S288C-27	Sporulation
D-135a-1a	+	−	+	+	−	+	−	−	+
D-135a-1b	+	+	+	+	−	−	−	−	+
D-135a-1c	−	+	−	−	+	−	−	−	−
D-135a-1d	−	−	−	−	+	+	−	−	+
D-135a-2a	+	+	−	+	−	−	−	−	+
D-135a-2b	+	+	+	−	−	+	−	−	−
D-135a-2c	−	−	+	+	+	+	−	−	+
D-135a-2d	−	−	−	−	+	−	−	−	+
D-135a-3a	+	−	−	+	+	−	−	−	+
D-135a-3d	−	−	−	−	−	−	−	−	+
D-135a-4a	+	−	+	−	+	+	−	−	+
D-135a-4b	+	−	−	+	+	−	−	−	+
D-135a-5a	+	+	+	+	+	+	−	−	−
D-135a-5b	−	−	+	−	+	−	−	−	−
D-135a-5d	+	+	−	+	−	+	−	−	−
D-135a-6a	+	−	−	−	−	+	−	−	+
D-135a-6b	−	+	+	+	−	+	−	−	−
D-135a-6c	−	−	+	+	+	−	−	−	+
D-135a-7a	+	+	−	−	+	−	−	−	+
D-135a-7b	−	−	−	+	+	+	−	−	+
D-135a-7d	+	+	+	+	−	−	−	−	−
D-135a-8a	+	−	−	−	−	+	−	−	+
D-135a-8b	+	+	+	+	−	+	−	−	−
D-135a-8c	−	−	−	−	+	−	−	−	+
D-135a-8d	−	+	+	+	+	−	−	−	+
D-135a-9a	−	+	+	+	−	+	−	−	+
D-135a-9b	−	−	−	+	−	−	−	−	+
D-135a-9c	+	+	+	−	+	+	−	−	−
D-135a-9d	+	−	−	−	+	−	−	−	+

none were able to mate with any of the six tester strains. Twenty of the 29 can be positively identified as $a\alpha$ because they are able to sporulate. From these data, it is clear that the ratios of $a\alpha$:aa and $\alpha\alpha$ among F_1 segregants of D-135a are not those expected for an $aa\alpha\alpha$ tetraploid.

Transmission of the diploidization trait and homozygosity of F_2 segregants: The observations that D-135a is diploid, yields four-spored asci upon sporulation and that these in turn give rise to diploid segregant cultures can all be reconciled by postulating the action of a diploidization gene in the F_1 segregants of D-135a. If diploidization is the consequence of a heritable trait, then the F_2 and succeeding generations of spores produced by D-135a should also be capable of self-diploidization and sporulation. To test for this, F_1 segregants (D-135a-1a, D-135a-1b, and D-135a-1d; Table 1) from a single D-135a ascus were dissected, and segregant colonies were grown from the spores. Of 25 such F_2 colonies tested, none contained cells capable of mating and 23 contained cells capable of undergoing sporulation. Dissection of the asci produced by one of these gave a high yield of viable F_3 spores. The ability of clones descended from D-135a to repeatedly undergo diploidization followed by meiosis indicates the transmission of a diploidization gene. The presence of such a gene in D-135a would have another testable consequence: from the F_1 generation, all the descendants of D-135a should be homozygous for all genetic markers.

To test for homozygosity, the F_1 segregants from a single D-135a ascus; D-135a-1a, D-135a-1b, D-135a-1d (Figure 1) were sporulated, asci dissected, and the resulting colonies were tested for marker segregation at six loci. The phenotype of each F_2 segregant was the same as that of its F_1 parent (Figure 1), indicating that the F_1 segregants of D-135a are homozygous at all six loci. Therefore it appears likely that these F_1 strains became diploid by the fusion of two cells of identical genotype, as occurs in homothallic yeasts.

Figure 1.

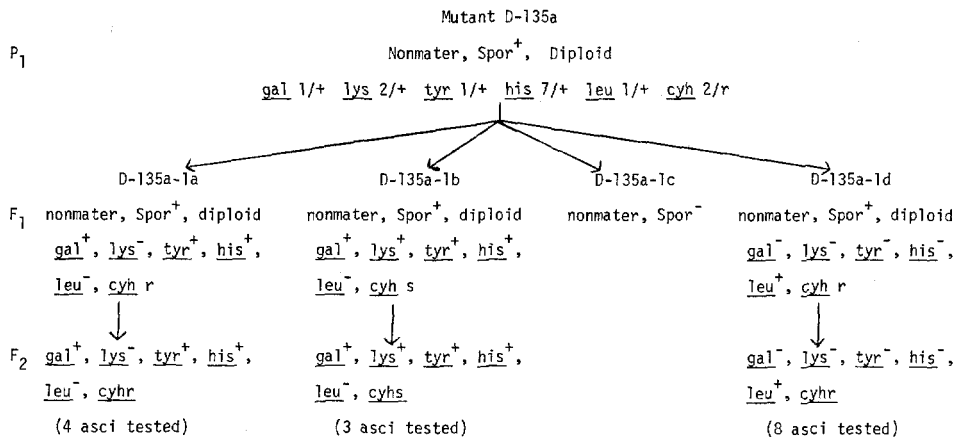


FIGURE 1.—Diagrammatic representation of the pedigrees of D-135a progeny strains.

Nuclear inheritance of the D-135a gene: The homothallic trait is inherited by all of the progeny of D-135a. Such 4:0 segregation implies either that the homothallism gene is cytoplasmic or that D-135a is homozygous for a nuclear homothallism gene. To determine which is the case, segregation of the homothallism gene was studied by mating D-135a spores* to an ***a*** heterothallic strain and then sporulating the resulting ***aα*** diploids. A 2:2 segregation of homothallic spores : heterothallic spores would be expected for a nuclear gene and either 4:0 or 0:4 for a cytoplasmic gene. For the mating, D-135a spores were allowed to germinate in YEP medium in the presence of heterothallic ***a*** cells (strain M-25-3D). Three hybrid colonies (selected by complementary markers) were sporulated and five asci from each dissected. For the asci with four viable spores, (13 of the 15 asci) homothallism segregated 2:2. Furthermore, the segregants included 14 heterothallic **a** and 12 heterothallic α strains. The results indicate that D-135a is homozygous for a nuclear gene for homothallism and that this gene is unlinked to the mating-type locus.

The mutation in D-135a is not allelic with the HO gene: It was conceivable that the mutation to homothallism in our strain occurred simply as a result of a mutation of *ho* to *HO*. Such a mutation has been previously reported (TAKAHASHI 1964). In order to study the possible allelism, a cross was made to test for segregation of the two homothallism genes. Spores from D-135a were mixed with spores from homothallic strain S41 and allowed to germinate in YEP medium. Hybrid diploids were selected by plating on minimal agar. Asci produced by sporulation of the hybrid diploids were dissected and segregant colonies from the resulting spores were tested for the ability to mate with tester strains. If the homothallism gene in D-135a and the *HO* gene are allelic or closely linked, then all spores produced by the hybrid diploids should have received a homothallism gene (i.e., four non-mating segregants should be obtained from all asci). If the two genes are non-allelic and unlinked then independent marker segregation should lead to asci containing two, three, or four homothallic spores in an ascus. Out of 13 asci produced by sporulation of the S41 × D-135a hybrid, 2 had 2 maters : 2 non-maters, 10 or 11 had 1 mater : 3 non-maters and 0 or 1 had 4 non-mating segregant colonies.** These data indicate that the mutation responsible for homothallism in the progeny of D-135a is non-allelic with the classical *HO* gene (HAWTHORNE 1963a, b) for homothallism in *S. cerevisiae*. We use the abbreviation *cmt*† (change of mating type) to designate this new homothallism gene.

Recessiveness of the cmt homothallism gene: For dominance tests, diploids homozygous for mating type and heterozygous for a homothallism gene were made by crosses of homothallic α haploids spores × heterothallic ***aaa*** triploids followed by sporulation of the ***aaaα*** tetraploids. Haploid spores from D-135a-1d (*cmt*) and from strain S41 (*HO*) were each mated with the triploid AU-155-(***aaa***) (+ for both homothallism genes). Upon sporulation of the resulting

* In homothallic strains it is possible to mate spores; diploidization does not occur until 1–2 divisions after germination.

** Uncertainty in numbers is due to one ascus in which only three spores gave rise to colonies; all three colonies were non-maters.

† According to the new nomenclature *cmt* would be referred to as *hth4*.

TABLE 2

Dominance and recessiveness of homothallic loci

A. Predictions:				
	mα × *MMM* **aaa** → **aaa***α* *MMMm* tetraploid → sporulation →			
	MM **aa**	*MM* **aa**	*mM* **aa**	
	MM **aa**	*mM* **aa**	*mM* **aa**	
	mM **a***α*	*mM* **a***α*	*MM* **a***α*	
	mM **a***α*	*MM* **a***α*	*MM* **a***α*	
	1 :	4 :	1	% non-mating spores
If recessive:*	2 non-maters:	2 non-maters:	2 non-maters:	50
	2 maters	2 maters	2 maters	
If dominant:	2 non-maters:	3 non-maters:	4 non-maters	83
	2 maters	1 mater		
B. Results:	2 maters:	3 non-maters	4 non-maters	
	2 non-maters	1 mater		
Spores S41 × **aaa**	1	3	1	78
Spores D-135a-1d × **aaa**	13	2	0	52

* If *m* is unlinked to its centromere and undergoes tetravalent pairing, approximately 3.5% of the asci would have 1 *mm* **aa** spore and therefore have 3 nonmaters : 1 mater.

aaaα tetraploid, the great majority of asci should contain 2 ***aα*** and 2***aa*** spores.** The homothallism gene (either *cmt* or *HO*), initially present in one of the four homologous chromosomes, will segregate, independently of mating type, into two of the four spores. Consequently, the three ascus types (Table 2) should occur in the ratio 1:4:1. Diploid ***aa*** spores heterozygous for a dominant homothalism gene will undergo a change of mating type, yielding non-mater (***aα*** or ***aaaα***) segregants. Therefore, for a dominant homothallism gene, ascus types I, II, and III will yield, respectively, 2, 3, and 4 non-mating segregants.

The observed frequencies of asci yielding 2, 3, and 4 non-mating segregant cultures are 1:3:1 (5 asci dissected) for the cross S41 (*α*, *HO*) × AU155 (***aaa***, +) and 13:2:0 (15 asci dissected) for the cross D-135a-1d (*α*, *cmt*) × AU155 (***aaa***, + (Table 2). The data for the cross involving the *HO* gene agree with the finding that *HO* is a dominant homothallism gene (HAWTHORNE, personal communication). The data for the *cmt* × wild-type cross agree with the expectation for a recessive homothallism gene.

When such a cross involves a recessive homothallism gene, the vast majority of asci will yield two ***aa*** segregants capable of mating and two non-mating ***aα*** segregants. Although the homothallism gene will be transmitted to 50% of the segregants of both classes, it will be present in heterozygous condition and therefore not expressed. Asci yielding three non-mating and one ***aa*** segregant will occur only in those rare instances where both of the homothallism genes, present in only two copies per octet, are meiotically segregated into the same spore nucleus which, in addition, happens to be ***aa***. This unlikely event requires

** Exceptions could occur because of tetravalent rather than bivalent pairing and crossing over between the mating-type locus and its centromere.

tetravalent pairing and a crossover between the homothallism gene and its centromere.

The ratios of mating to non-mating segregants in asci from the cross D-135a-1d × AU-155 agree qualitatively with those expected for a recessive homothallism gene. Thus it appears that *cmt*, unlike *HO*, is a recessive gene for homothallism.

The genesis of a homozygous recessive mutation ($+/+ \rightarrow cmt/cmt$) required the occurrence of both a mutation ($+/+ \rightarrow cmt/+$) and subsequent homozygosis to *cmt/cmt*. Such events would necessarily be rare. We have found one such mutant among 135,000 mutagenized colonies.

DISCUSSION

The experiments we have described above show that a heterothallic yeast strain can become homothallic as a consequence of a homozygous *cmt* mutation. In order to discuss the mode of action of this mutant gene, we will first review previous studies on homothallism in *S. cerevisiae*.

For diploid strains homozygous for the *D* gene complex (equivalent to *HO* + *HMa* + *HMα* of OSHIMA and TAKANO 1972), HAWTHORNE (1963 a, b) has shown that asci contain two spores of ***a*** and two spores of α mating type. When such asci are dissected and the spores allowed to grow, half the progeny of a given spore change in mating type. These mate with cells of the original mating type and thus give rise to a segregant clone which consists mainly of ***aα*** cells which are non-maters capable of undergoing sporulation.

Although the *cmt* mutation is not allelic with *HO*, diploidization occurs similarly. Upon sporulation of *cmt/cmt* homozygous diploids, two ***a*** and two α haploid segregants are obtained. When colonies are grown from these individual spores, not only have the cells in them diploidized; they appear also to have become ***aα*** at the mating-type locus, since they are unable to mate and can form asci which contain both spores of ***a*** and α mating-types. Because *D* gene and *cmt*-mediated homothallism follow the same pattern, we infer that *cmt* acts by changing ***a*** to α or α to ***a***, with subsequent diploidization by mating.

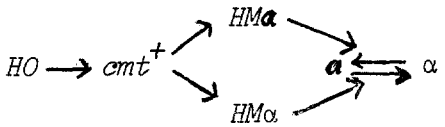
It is probable that AP-1-$\alpha\alpha$, the strain from which D-135a was isolated, contains the *HMa* and *HMα* genes, but lacks *HO*.* Both AP-1-$\alpha\alpha$, and the related ***aα*** and ***aa*** strains are heterothallic without exception; no diploid colonies have been found among segregants dissected from 50 AP-1-***aα*** asci. Moreover, AP-1-***aa*** and AP-1-$\alpha\alpha$ have been stably maintained for two years and have shown no tendency to tetraploidize. The very low frequency of sporulation exhibited by these strains apparently results from "leakiness" of mating-type control over sporulation, rather than from mutability at the mating-type locus. The few asci produced by AP-1-***aa*** contain only ***a*** spores and those from AP-1-$\alpha\alpha$ only α spores.

Mutation at a single genetic locus caused homothallism to appear in a strain (D-135a) whose ancestors were all heterothallic. It seems unlikely either that

* It has been observed by D. HAWTHORNE (personal communication) that virtually all of the heterothallic *S. cerevisiae* strains in common laboratory use have the genotype *ho HMa* $HM\alpha$. This genotype is consistent with all our data. Alternative *HO*, *HMa*, $HM\alpha$, and cmt^+ phenotypes for AP-1-$\alpha\alpha$ are either inconsistent with our data or would require four mutational events in order to undergo the change from heterothallic to homothallic phenotype.

this mutation would have created a new functional gene from non-functional "spacer" DNA or that mutation would have modified the specificity of a previously functional gene product in such a way that it could carry out a new function, related to homothallism. It seems rather more probable that the *cmt* mutation involves the mutational loss of a gene activity which was expressed in the heterothallic parental strains. This can be rationalized on two grounds: (1) mutations that inactivate a gene product are the type most frequently observed and (2) the *cmt* gene is recessive to *cmt*$^+$, suggesting that *cmt* corresponds to absence and *cmt*$^+$ to presence of a functional gene product.

In order to conceptualize the relationship between the various homothallism genes and to account for the postulated *cmt*$^+$ function in heterothallic strains, we propose the following tentative model for the genetic control of homothallism. For a wild-type strain which is *HO HMa HM*α, the control circuit is:



HO negatively controls *cmt*$^+$; *cmt*$^+$ negatively controls *HMa* and *HM*α; these in turn act to change mating type. The *cmt*$^+$ gene product is central to the model. Its presence in heterothallic strains prevents the expression of *HMa* and *HM*α, leading to stability of the mating type. Homothallism results from lack of functional *cmt*$^+$ gene product, either because the gene has mutated to a non-functional (*cmt*) configuration or because it is turned off by the presence of an *HO* gene.

This model of sequential negative control of homothallism genes accounts both for the dominance of *HO* and for the recessiveness of *cmt*. Furthermore, it predicts that haploid segregants which are *HO cmt* will be homothallic. The fact that the cross *HO cmt*$^+$ $\times$ *ho cmt* can yield 3 nonmating : 1 ***a*** or α haploid progeny confirms this prediction.

Alternative models for the action of homothallism genes might envision a parallel but separate control of *HO* and *cmt* over the *HMa* and *HM*α genes, or a mode of action of *cmt* upon mating type which involves neither *HMa* or *HM*α. These three possibilities may be tested by constructing diploids heterozygous at the *cmt*$^+$, *HO*, *HMa* and *HM*α loci and then testing the various segregants for homothallism.

Note added in proof: After this manuscript was submitted for publication NAUMOV, G. I. and TOLSTORUKOV (Genetika **9**: 82) and HARASHIMA, NOGI and OSHIMA (Genetics **77**: 639–650) reported that a spore having *HO hma hm*α genotype is homothallic. This new finding is compatible with the model proposed here.

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LITERATURE CITED

ESPOSITO, M. S., R. E. ESPOSITO, M. ARNAUD and H. O. HALVORSON, 1970 Conditional mutants of meiosis in yeast. J. Bacteriol **104**: 202–210.

HAWTHORNE, D. C., 1963a A deletion in yeast and its bearing on the structure of the mating type locus. Genetics **48**: 1727–1729. ——, 1963b Directed mutation of the mating type allele as an explanation of homothallism in yeast. Proc. XI Intl. Congr. Genet. **I**: 34–35.

HOPPER, A. K. and B. D. HALL, 1975 Mating type and sporulation in yeast. I. Mutations which alter mating-type control over sporulation. Genetics (this issue)

MORTIMER, R. K. and D. C. HAWTHORNE, 1973 Genetic mapping in Saccharomyces. IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. Genetics **74**: 33–54.

OSHIMA, Y. and I. TAKANO, 1971 Mating types in Saccharomyces: Their convertibility and homothallism. Genetics **67**: 327–355. ——, 1972 Genetic controlling system for homothallism and a novel method for breeding triploid cells in *Saccharomyces*. Proc. IV IFS: Ferment. Technol. Today, pp. 847–852.

PLISCHKE, M. E., R. C. VON BORSTEL, R. K. MORTIMER and W. E. COHN, 1975 Selective markers and associated gene products in *Saccharomyces cerevisiae*. In: The Handbook of Biochemistry. Third edition. Edited by G. D. EASMAN. The Chemical Rubber Co., Cleveland, Ohio. Manuscript in preparation.

ROMAN, H., M. M. PHILLIPS and S. M. SANDS, 1955 Studies of polyploid *Saccharomyces*. I. Tetraploid segregation. Genetics **40**: 546–561.

TAKAHASHI, T., 1958 Complementary genes controlling homothallism in Saccharomyces. Genetics **43**: 705–714. ——, 1964 Gene conversion of the mating-type locus in *Saccharomyces*. Bulletin of Brewing Science **10**: 11–22.

TAKANO, I. and Y. OSHIMA, 1970 Allelism test among various homothallism-controlling genes and gene systems in Saccharomyces. Genetics **64**: 229–238.

WINGE, O. and C. ROBERTS, 1949 A gene for diploidization in yeast. C. R. Trav. Lab. Carlsberg **24**: 341–346.

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