INTERCONVERSION OF YEAST MATING TYPES III. ACTION OF THE HOMOTHALLISM (*HO*) GENE IN CELLS HOMOZYGOUS FOR THE MATING TYPE LOCUS

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ABSTRACT

Mating type interconversion in homothallic *Saccharomyces cerevisiae* has been studied in diploids homozygous for the mating type locus produced by sporulation of **a**/**a**/**a**/α and **a**/**a**/α/α tetraploid strains. Mating type switches have been analyzed by techniques including direct observation of cells for changes in α-factor sensitivity. Another method of following mating type switching exploits the observation that **a**/α cells exhibit polar budding and **a**/**a** and α/α cells exhibit medial budding.——These studies indicate the following: (1) The allele conferring the homothallic life cycle (HO) is dominant to the allele conferring the heterothallic life cycle (*ho*). (2) The action of the *HO* gene is controlled by the mating type locus—active in **a**/**a** and α/α cells but not in **a**/α cells. (3) The *HO* (or *HO*-controlled) gene product can act independently on two mating type alleles located on separate chromosomes in the same nucleus. (4) A switch in mating type is observed in pairs of cells, each of which has the same change.

SACCHAROMYCES CEREVISIAE strains exhibit two kinds of life cycles, heterothallic and homothallic, which differ in the frequency at which mating types (**a** or α) are interconverted. In heterothallic strains the mating type is stable, although rare changes can be observed (HAWTHORNE 1963a; HICKS and HERSKOWITZ 1976, 1977). In homothallic strains the mating type of a cell is unstable (HAWTHORNE 1963b; TAKANO and OSHIMA 1970), switching from **a** to α or from α to **a** as often as every cell division (HICKS and HERSKOWITZ 1976; STRATHERN and HERSKOWITZ, in preparation). These two types of strains differ by a single Mendelian gene, *HO* ("homothallism"), which was introduced into *S. cerevisiae* from *S. chevalieri* by genetic crosses (WINGE and ROBERTS 1949; see also TAKANO and OSHIMA 1967). Other genes involved in mating type switching are described in HARASHIMA, NOGI and OSHIMA (1974).

The relationship between the homothallic (*HO*) and heterothallic (*ho*) alleles is not clear; for example, is *ho* a defective *HO* or is it perhaps the complete

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absence of *HO*? In characterizing a new gene involved in homothallism, HOPPER and HALL (1975) have presented some evidence indicating that *HO* is dominant to *ho*. We have used essentially the same technique as HOPPER and HALL in order to confirm the dominance of *HO* and to ask an additional question concerning mating type interconversion. It should be noted that the dominance relationship between *HO* and *ho* cannot be assessed by behavior of **a**/α *HO/ho* strains, as the *HO* gene does not act in **a**/α cells (that is, **a**/α *HO/HO* cells are stable **a**/α diploids) (TAKANO and OSHIMA 1970; HICKS and HERSKOWITZ 1977). We therefore have constructed *HO/ho* strains homozygous for the mating type locus as segregants from tetraploid strains and determined by direct observation (HICKS and HERSKOWITZ 1976) that the mating type loci in such cells do switch. In addition we have determined that homothallic **a/a** strains give rise to both **a**/α and α/α cells, and that homothallic α/α strains give rise to both **a**/α and **a/a** cells. KLAR and FOGEL (1977) have made similar observations by analysis of **a/a** and α/α cells derived from homothallic **a**/α cells by UV-induced mitotic recombination.

MATERIALS AND METHODS

Yeast Strains: Strains are described in Table 1. The homothallic strain X10-1B was confirmed as a type II homothallic strain (*i.e.*, *HO HM***a** *HM*α) by crosses with Hp and Hq type strains (*HO HM***a** *hm*α and *HO hm***a** *HM*α, respectively) provided by DR. Y. OSHIMA (data not shown).

XJ21-1 (**a/a/a**) was constructed as follows: An **a/a** diploid (XJ7-1) was isolated from **a**/α strain XJ7 by screening single colonies of XJ7 for those which had acquired an **a** phenotype, presumably a result of mitotic recombination. XJ7-1 was mated within α strain X57-3D to form **a/a**/α strain XJ21. The **a/a/a** triploid strain XJ21-1 was isolated from XJ21 by screening single colonies of XJ21 for those which had acquired an **a** phenotype. The **a/a/a** genotype of XJ21-1 was confirmed by crossing to haploid α strain XT1172-S245c and analyzing the meiotic products (see RESULTS). The strains used in the construction of XJ7 and X57-3D were confirmed as *ho HM***a** *HM*α by crosses to X10-1B (data not shown).

TABLE 1

Strain list

Strain	Genotype	Source or reference
X10-1B	**a**/α *HO/HO his5-2/his5-2 ade5/ade5 ura4/ura4 met4/met4 met13/met13*	HICKS and HERSKOWITZ 1976
70	α *thr3-10*	
73	**a** *ilv3-x*	
XT1172-S245c	α *ade6 his6 leu1 trp5 met1 gal2 can1*	T. R. MANNEY
XJ21	**a**/**a**/α *cry1-3/cry1-3/cry1-3 his4/his4/his4 leu2-1/leu2-1/leu2-1 thr4/thr4/THR+ ade6/ade6/ade6 lys2/lys2/lys2 ho/ho/ho*	
XJ21-1	**a/a/a** *thr4/thr4/thr4* (all other markers identical to XJ21)	Mitotic recombinant from XJ21 (see MATERIALS AND METHODS)
X104	XJ21a $\times$ X10-1B-4 (an α *HO* spore derived by sporulation of X10-1B)	
XJ7	**a**/α *cry1-3/cry1-3 his4/his4 leu2/leu2 thr4/THR+ ade6/ade6 lys2/lys2*	
XJ7-1	**a/a** *thr4/thr4* (all other markers identical to XJ7)	
X57-3D	α *cry1-3 his4 leu2 thr4 ade6 lys2*	

Media: YEPD, SPOR and YM-1 Dissection Agar are described in HICKS and HERSKOWITZ (1976).

Mating procedures: All crosses were accomplished by spore-cell or cell-cell mating on YM-1 Dissection Agar (HICKS and HERSKOWITZ 1976). Zygotes were isolated by micromanipulation.

Mating type tests: Mating type phenotype was assayed according to the prototroph complementation test (HICKS and HERSKOWITZ 1976). Mating type tester strains used were 70 (α) and 73 (**a**). Sporulation was assayed after 4 days on SPOR agar at 30°.

Sensitivity to α-factor was determined by microscopic observation of individual spores or cells placed near a heavy streak of growing α cells (strain 70) on an agar slab (see MATERIALS AND METHODS in HICKS and HERSKOWITZ 1976).

Assay for HO: The homothallic phenotype (HO) was assayed as follows: (1) by microscopic observation of a change in sensitivity to α-factor in progeny derived from a single spore, (2) by microscopic observation of formation of zygotes among siblings, (3) by sporulation proficiency of cells derived from a single spore, (4) by poor mating proficiency of patches derived by growth of a single spore (assayed by prototroph complementation test), and in some cases (5) by a change in budding pattern from medial to polar (described in text).

RESULTS

1. *Dominance of the HO gene*

The heterothallic triploid XJ21–1 (**a**/**a**/**a** *ho/ho/ho*) was mated with an α spore from homothallic X10–1B to form the tetraploid strain X104 (**a**/**a**/**a**/α *ho/ho/ho/HO*). According to the data presented by ROMAN, PHILLIPS and SANDS (1955), an **a**/**a**/**a**/α tetraploid such as X104 should segregate 2(**a**/**a**):2(**a**/α) spores in nearly 100% of its asci. Other spore genotypes, such as **a**/0, α/0, **a**/**a**/α, or α/α, may also occur as a result of chromosome nondisjunction or tetravalent pairing and subsequent segregation of sister chromatids into the same spore. However, these types should make up less than 10% of the total asci (ROMAN, PHILLIPS and SANDS 1955). Consistent with these expectations, we have observed 2(**a**/**a**):2(**a**/α) segregation in 14 of 14 tetrads derived by sporulation of an **a**/**a**/**a**/α *ho/ho/ho/ho* strain (unpublished observations). In tetraploid X104 (**a**/**a**/**a**/α *ho/ho/ho/HO*), the homothallic allele is present in one copy and thus should be heterozygous in two of the four spores in nearly every ascus, the other two spores in each ascus being homozygous for the heterothallic allele.

The above arguments predict that approximately one-half of the spores from X104 should have the **a**/**a** genotype and, since the homothallic locus is unlinked to the mating type locus, approximately one-half of these **a**/**a** spores should be heterozygous for the homothallic allele. We expect that homothallic **a**/**a** spores will produce clones containing cells capable of sporulation (either **a**/α, **a**/**a**/α/α, or both). The various tetrad types expected and the phenotypes predicted for each tetrad under both possible dominance relationships for homothallism and heterothallism are shown in Table 2 (see HOPPER and HALL 1975 for similar analysis). If the heterothallic allele were dominant, none of the heterozygotes would exhibit the homothallic phenotype, and nearly all tetrads would exhibit 2(**a**/**a**):2(**a**/α) segregation. Alternatively, dominance of the homothallic allele would produce an excess of phenotypically **a**/α segregants. The observed frequencies of tetrad types (Table 2) conform to the latter hypothesis, and indicate that homothallism (*HO*) is dominant.

TABLE 2

Segregation of homothallic phenotype in diploid spores derived from **a**/**a**/**a**/α ho/ho/ho/HO

Genotype of tetrads	Frequency expected*	HYPOTHESIS I Heterothallic allele (*ho*) dominant: phenotype expected	HYPOTHESIS II Homothallic allele (*HO*) dominant: phenotype expected
a/**a** *HO/ho*	.67	Mta†	Mt(**a**/α)†
a/**a** *ho/ho*		Mta	Mta
a/α *HO/ho*		Mt(**a**/α)	Mt(**a**/α)
a/α *ho/ho*		Mt(**a**/α)	Mt(**a**/α)
a/**a** *HO/ho*	.17	Mta	Mt(**a**/α)
a/**a** *HO/ho*		Mta	Mt(**a**/α)
a/α *ho/ho*		Mt(**a**/α)	Mt(**a**/α)
a/α *ho/ho*		Mt(**a**/α)	Mt(**a**/α)
a/**a** *ho/ho*	.17	Mta	Mta
a/**a** *ho/ho*		Mta	Mta
a/α *HO/ho*		Mt(**a**/α)	Mt(**a**/α)
a/α *HO/ho*		Mt(**a**/α)	Mt(**a**/α)
		[2Mta:2Mt(**a**/α)]	[1Mta:3Mt(**a**/α)]	[0Mta:4Mt(**a**-α]
HYPOTHESIS I		1.		
HYPOTHESIS II		.17	.67	.17
OBSERVED (X104)‡		.17 (3/18)	.61 (11/18)	.17 (3/18)

* Frequency expected is calculated assuming bivalent pairing of chromosomes and independent assortment of *HO* and the mating type locus (HARASHIMA, NOGI and OSHIMA 1974). This simplifying assumption allows one to ignore α/α and *HO/HO* spores, whose occurrence is rare (ROMAN, PHILLIPS and SANDS 1955; unpublished observations).

† Mta indicates mating behavior of **a** cells. Mt(**a**/α) indicates mating and sporulation behavior of **a**/α (or **a**/**a**/α/α) cells present in heterothallic clones or occurring in homothallic clones.

‡ Of 18 tetrads analyzed, the single unexpected tetrad [1Mta:2Mt(**a**/α):1Mtα] may have resulted from recombination in a tetravalently paired first meiotic division followed by segregation of both α alleles into the same spore.

2. *Switching of* **a**/**a** *to* α/α *and* **a**/α

The action of the *HO* gene in homozygous **a**/**a** diploid segregants of X104 was examined by microscopic observation by techniques described in HICKS and HERSKOWITZ (1976). Switching of **a**/**a** to **a**/α or α/α cells was observed in two ways: (i) by formation of zygotes between siblings descended from an **a**/**a** spore, and (ii) by production of cells insensitive to α-factor (hence **a**/α or α/α) descended from an **a**/**a** cell (which is sensitive to α-factor). Tetrads from X104 were dissected on an agar slab in the presence of α-factor. As expected from the segregation data for X104 shown in Table 2, two spores from each tetrad were sensitive to α-factor (**a**/**a** diploids) and two were not (**a**/α diploids). Seven pairs of α-factor sensitive cells were then moved to a second agar slab not containing α-factor and allowed to divide. The dividing cells (half of which are expected to be of genotype *HO/ho*) were inspected at the four-cell stage or later for zygote formation, that is, for the homothallic phenotype. Microcolonies scored for homothallic or heterothallic behavior were then grown into macrocolonies and tested for mating and sporulation.

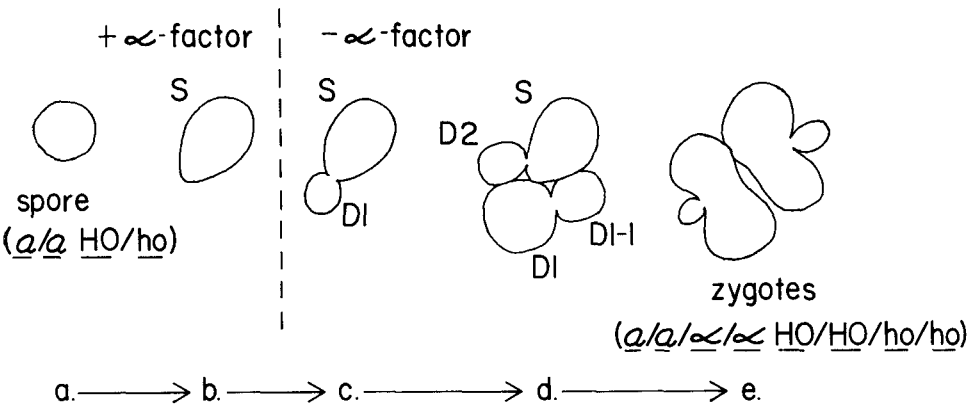

FIGURE 1.—Zygote formation among progeny of a homothallic **a**/**a** spore. Asci from X104 were dissected and allowed to germinate in the presence of α-factor. The figure shows the development of a single spore (17A) from dissection (a) through zygote formation (e) at approximately two hour intervals. The spore exhibited sensitivity to α-factor within two hours (b) and was then removed from α-factor in order to allow cell division to take place (c, d). Zygote formation took place at the four-cell stage.

a. *Zygote formation at the four-cell stage*: In 2 of 14 clones derived from α-factor sensitive (**a**/**a**) spores (clones X104–17A and X104–11A), mating occurred at the four-cell stage to form two zygotes (see Figure 1). This double zygote formation is the same as that frequently exhibited by **a** *HO* and α *HO* cells (HICKS and HERSKOWITZ 1976). These zygotes presumably are formed by matings between **a**/**a** cells and newly-arisen α/α cells, and are thus expected to produce tetraploid cells of genotype **a**/**a**/α/α. To determine whether tetraploids were formed, cells from clones 17A and 11A were sporulated and resultant tetrads analyzed. Efficiency of germination of the spores was greater than 95%, indicating that the zygotes were not triploid (PARRY and COX 1970). The spores were then tested for sensitivity to α-factor. In tetrads derived from these clones, sensitivity to α-factor segregated as expected for an **a**/**a**/α/α tetraploid (Table 3). These data support the conclusion that zygote formation occurred between **a**/**a** and newly formed α/α cells in clones 17A and 11A. The **a**/**a**/α/α tetraploid derived from X104–11A was used in later experiments to produce homothallic α/α cells.

b. *Zygote formation at later stages*: Of the clones which did not mate at the four-cell stage, two had formed at least one zygote by the sixteen-cell stage. Additional growth produced colonies which did not mate strongly with either **a** or α testers and contained cells which sporulated, indicating that they were homothallic. The sporulating cells were presumably **a**/**a**/α/α tetraploid and/or **a**/α diploid cells (see below).

c. *Clones lacking zygotes*: Ten of fourteen clones showed no zygote formation by the sixteen-cell stage. These clones may, therefore, lack the *HO* gene or be cases in which **a**/**a** has switched to **a**/α. In order to determine whether these cells

TABLE 3

Segregation of α-factor sensitivity from homothallic tetraploids

Tetrad types expected from **a**/**a**/α/α	Expected frequency‡ A	B	C	Sensitivity to α-factor (Sensitive: Insensitive)	Observed segregation of α-factor sensitivity 17A	11A	Budding pattern* Pattern expected	Observed frequency
I **a**/α, **a**/α, **a**/α, **a**/α	.48	.62	.55	0:4	.47 (7/15)	.57 (54/95)	polar, polar, polar, polar	.57 (34/61)
II **a**/**a**, **a**/**a**, α/α, α/α	.10	.15	.09	2:2	.20 (3/15)	.15 (13/95)	shmoo†, shmoo, medial, medial	.07 (4/61)
III **a**/**a**, α/α, **a**/α, **a**/α	.42	.20	.36	1:3	.33 (5/15)	.29 (28/95)	shmoo, medial, polar, polar	.34 (21/61)

Spores were tested for sensitivity to α factor by the confrontation assay. Details are described in MATERIALS AND METHODS.

* An individual tetraploid cell was isolated from clone 11A and denoted as TX104–11A. 61 tetrads from TX104–11A were analyzed for polar or medial budding pattern in the presence of α-factor as described in the text and in Figure 2. Two exceptional tetrads gave patterns 2 medial: 2 polar and 2 shmoo:2 polar. These may correspond to tetrads of genotypes (α, α, **a**/α, **a**/α) and (**a**,**a**,α/α, α/α) respectively, due to nondisjunction of chromosome III, or they may be false tetrads. Other details are described in the Legend to Figure 2.

† Characteristic morphological change exhibited by **a** cells arrested by α-factor. See Figure 2.

‡ Listed are the observed frequencies of tetrad types produced by sporulation of an **a**/**a**/α/α tetraploid: A. ROMAN, PHILLIPS and SANDS (1955); B. LEUPOLD (1956); C. RABIN (1970).

were **a**/**a** or **a**/α, six to ten individual cells from each were placed in the presence of α-factor by micromanipulation, and some tested for ability to sporulate.

In eight of the ten clones tested in this manner, all individual cells retained the sensitivity to α-factor originally exhibited by the parent spore. These clones were presumed to be of genotype **a**/**a** *ho/ho*. This presumed genotype was later confirmed as each of the resultant colonies was found to possess the **a** phenotype for mating and sporulation (data not shown).

Cells from the other two clones retested for α-factor sensitivity (3A, 5B) showed a very different response. Although in each clone one or two cells were sensitive, the others were insensitive, thereby suggesting that these cells contain the *HO* allele and have switched mating types to become **a**/α, α/α, or possibly **a**/**a**/α/α. Since the α-factor insensitive cells dividing in the presence of α-factor did not segregate sensitive progeny (**a**/**a**) as expected if they were α/α diploids, it was hypothesized that these insensitive cells were actually **a**/α diploids. This hypothesis was confirmed by the tests described below.

Three presumed **a**/α *HO/ho* cells from clone 5B were isolated by micromanipulation, grown into colonies and sporulated. Tetrads were dissected from each and tested for sensitivity to α-factor. In each case all tetrads contained two

TABLE 4

Identification of **a**/α *cells in clone X104-5B-1 produced by homothallic conversion of an* **a**/**a** *cell*

Tetrad type	Mate with **a**	Mate with α	Sporulation	Inferred genotype	Expected† frequency (**a**/α *HO/ho*)	Observed frequency X104-5B-1
I	+/—	+/—	+	* *HO*	.67	.56 (6/11)
	+/—	+/—	+	* *HO*		
	+	—	—	α *ho*		
	—	+	—	**a** *ho*		
II	+/—	+/—	+	**a** *HO*	.17	.36 (4/11)
	+/—	+/—	+	**a** *HO*		
	+	—	—	α *ho*		
	+	—	—	α *ho*		
III	+/—	+/—	+	α *HO*	.17	.09 (1/11)
	+/—	+/—	+	α *HO*		
	—	+	—	**a** *ho*		
	—	+	—	**a** *ho*		

* Original mating type of spore cannot be determined in these tests.
† Based on independent segregation of *HO* and the mating type locus (HARASHIMA, NOGI and OSHIMA 1974).

sensitive and two insensitive spores. Representative tetrad analysis of segregants from isolate 5B-1 is shown in Table 4. Homothallism segregates 2:2 in these tetrads, and the heterothallic segregants are equally divided between **a** *ho* and α *ho*, thus confirming the presumed genotype for 5B.

d. *Summary of* **a**/**a** *to* α/α *and* **a**/α: In the fourteen **a**/**a** clones derived from sporulation of X104 (**a**/**a**/**a**/α *ho/ho/ho/HO*), six were observed to switch mating types. 11A and 17A gave rise to α/α cells, 3A and 5B gave rise to **a**/α cells, and two others gave rise to sporulating cells (**a**/α or **a**/**a**/α/α) which were not further characterized. The other eight **a**/**a** clones appeared to be heterothallic.

3. *Switching of* α/α *to* **a**/**a** *and* **a**/α

Homothallic α/α cells were produced as segregants from the tetraploid TX104-11A (α/α/**a**/**a** *HO/ HO /ho/ho*) which was derived by mating between **a**/**a** *HO/ho* and α/α *HO/ho* cells as described earlier. The expected tetrad types with **a**/**a**, α/α and **a**/α diploid spores, and the observed frequency with respect to sensitivity to α-factor have been given in Table 3. α/α spores identified on the basis of insensitivity to α-factor and by their budding pattern (described below) were then scored for the activity of the *HO* allele.

a. *Identification of* α/α *spores from a tetraploid*: As noted by MACKAY (CRANDALL, EGEL and MACKAY 1976) **a**, **a**/**a**, α and α/α cells exhibit "medial budding." That is, the first bud (D1–1) of daughter cell (D1) is located near the junction of the original cell and the first daughter (D1) (Figure 2). Buds D2 and D1–1 develop asynchronously. In contrast, **a**/α cells exhibit "polar budding", with the

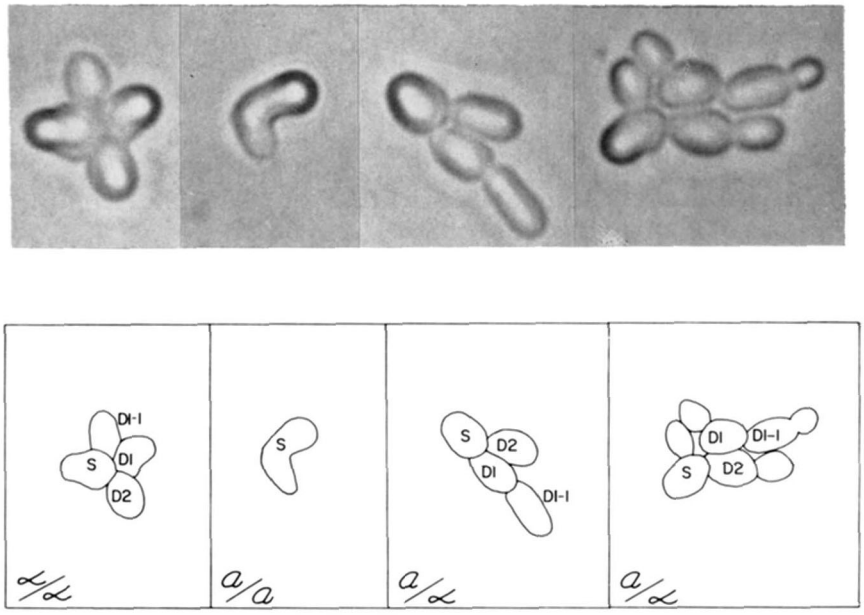

FIGURE 2.—Budding pattern of **a**/**a**, α/α, and **a**/α cells in the presence of α-factor. Asci from tetraploid TX104-11A were dissected and allowed to germinate in the presence of α-factor supplied by a streak of α cells (strain 70). Under these conditions, **a**/**a** spores are arrested as unbudded cells and exhibit a characteristic morphological change ("shmoo"). α/α and **a**/**a** cells are not sensitive to α-factor and bud. α/α cells can be distinguished from **a**/α cells by the position of the first bud (D1-1) from the spore's first daughter (D1). In **a**/α cells D1-1 emerges on the side of D1 opposite to the spore cell (polar budding). In α/α cells D1-1 emerges near the junction of D1 with the spore (medial budding). **a**/**a** cells exhibit the same budding pattern as α/α cells when germinated in the absence of α-factor (data not shown). The examples shown here have been chosen from two separate tetrads for visual clarity.

D1–1 bud at the end of the D1 cell (Figure 2). Buds D2 and D1–1 develop synchronously. Asci from the tetraploid TX104–11A were dissected and allowed to germinate in the presence of α-factor. **a**/**a** spores were arrested at the first cell division. The others (**a**/α and α/α) were scored for α-factor sensitivity and budding pattern at the four-cell stage. The 61 tetrads fell into three major expected types: Type I (34/61) had four α-factor insensitive cells, all of which exhibited the polar budding pattern and are presumed to be 0(**a**/**a**):0(α/α):4(**a**/α). Type II (4/61) had two α-factor sensitive and two α-factor insensitive cells with the medial budding pattern [2(**a**/**a**):2(α/α):0(**a**/α)]. Type III (21/61) had one α-factor sensitive and three insensitive spores, two with polar budding pattern and one with the medial pattern [1(**a**/**a**):1(α/α):2(**a**/α)]. (Two exceptional tetrads are described in the Legend to Table 3). The agreement between the expected and observed segregations of the mating type loci and α-factor sensitivity with budding pattern indicates that budding pattern is an accurate reflection of the mating type of these spores.

b. *Observation of switching in α/α cells*: 213 α-factor insensitive spores derived from TX104–11A were subjected to pedigree analysis in the presence of α-factor. None of the 174 clones with cells exhibiting polar budding (presumably **a**/α cells) gave rise to α-factor sensitive cells or formed zygotes at the four-cell stage. This observation provides additional support for the interpretation that these cells are **a**/α diploids. Of 39 clones with cells exhibiting medial budding (presumably α/α cells), fifteen produced α-factor sensitive cells by the four-cell stage. The budding pattern and α-factor insensitivity of the original cells and the fact that **a**/α cells do not switch mating type indicate that the original cells in these clones were α/α and gave rise to **a**/**a** cells. In each case the switch to **a** phenotype was observed in the original cell and its second daughter, the same pattern seen in homothallic haploids (HICKS and HERSKOWITZ 1976; STRATHERN and HERSKOWITZ, in preparation). In two of the clones derived from α/α spores, a switch from medial to polar budding was observed within three generations. These cells were confirmed as **a**/α by sporulation and segregation of α-factor sensitivity (data not shown). At least some of the other 22 clones, those which did not change response to α-factor or form zygotes, are expected to be *ho*/*ho*.

DISCUSSION

Four conclusions may be drawn from the results described above: (1) The allele conferring the homothallic life cycle (*HO*) is dominant to the allele conferring the heterothallic life cycle (*ho*). (2) The action of the *HO* gene is controlled by the mating type locus—active in **a**/**a** and α/α cells but not in **a**/α cells. (3) The *HO* (or *HO*-controlled) gene product can act independently on two mating type alleles located on separate chromosomes in the same nucleus. (4) A switch in mating type is observed in pairs of cells, each of which has the same change.

Most models for the action of *HO* propose that the *HO* gene product is or controls an enzyme which interacts specificially with the mating type locus DNA in order to interconvert the **a** and α alleles (OSHIMA and TAKANO 1971; D. HAWTHORNE, personal communication; HICKS and HERSKOWITZ 1977). Our observation that the *HO* allele is dominant to *ho* supports the work of HOPPER and HALL (1975) and is consistent with this view.

Earlier work (TAKANO and OSHIMA 1970; HICKS and HERSKOWITZ 1977) has shown that mating type interconversion does not occur in diploids which are **a**/α. Not only do the progeny of **a**/α *HO*/*ho* (or *HO*/*HO*) strains remain genetically **a**/α, but also the position of each individual mating type locus on chromosome III is maintained. That is, in a diploid formed by mating between **a** *cry*^{r} *ho* and α *CRY*^{s} *HO*, the mating type alleles retain their linkage to their respective *cry* marker through more than twenty generations of growth (HICKS and HERSKOWITZ 1977; KLAR and FOGEL 1977). Thus, *HO* does not act to interconvert mating type alleles in an **a**/α *HO*/*ho* strain. Experiments described here show that failure of *HO* to act in such strains is due to the presence of both **a** and α alleles rather than diploidy *per se* since **a**/**a** and α/α diploids do switch mating types. Support-

ing this view is the recent finding that mutants defective in sporulation due to a mutation in the α mating type locus also fail to "turn off" action of the *HO* gene (J. STRATHERN, unpublished observations). The *HO* inhibition phenomenon must be added to the list of functions, such as sporulation, specific to **a**/α cells (FRIIS and ROMAN 1968; HOPPER *et al.* 1974).

The action of *HO* results in a change in the mating type locus which is genetically stable after removal of *HO* to prevent further switches (OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1976, 1977). In this work we have seen that *HO* can independently alter the mating type allele expressed on one or both chromosomes in an **a**/**a** cell to form either an **a**/α or an α/α diploid. Similarly, α/α cells can give rise to **a**/α or **a**/**a** cells. Our experiments do not allow us to determine whether the pathway of switching—for example, from α/α to **a**/**a**—is direct, or whether α/α is switched first to **a**/α in one cell division cycle and subsequently to **a**/**a** in the next cell division cycle before inhibition of *HO* is established. Our observations are consistent with the notion that *HO* acts on the mating type DNA itself rather than on the cell as a whole. Previous work has shown that *HO* need not act to interconvert mating types at *every* generation in homothallic haploid cells (HICKS and HERSKOWITZ 1976; STRATHERN and HERSKOWITZ, in preparation.) Similarly, *HO* is not obligated to alter both mating type alleles in an **a**/**a** or α/α diploid simultaneously.

The pattern of switching in homothallic **a**/**a** and α/α cells is the same as observed in haploid homothallic cells. For example, at the four-cell stage after germination, only the spore cell and its most recent daughter exhibit the change. Interestingly, the two switched cells derived from an homothallic α/α cell appear to exhibit the same change, for example, both becoming **a**/**a** rather than one **a**/**a** and the other **a**/α. This observation supports our earlier conclusion that the change in mating type occurs before or during DNA replication of the second cell division cycle.

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