SWITCHING OF A MATING-TYPE **a** MUTANT ALLELE IN BUDDING YEAST *SACCHAROMYCES CEREVISIAE*[1]

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ABSTRACT

Aimed at investigating the recovery of a specific mutant allele of the mating type locus (*MAT*) by switching a defective *MAT* allele, these experiments provide information bearing on several models proposed for *MAT* interconversion in bakers yeast, *Saccharomyces cerevisiae.* Hybrids between heterothallic (*ho*) cells carrying a mutant *MAT***a** allele, designated *mat***a***-2*, and *MAT*α *ho* strains show a high capacity for mating with *MAT***a** strains. The *MAT*α/*mat***a***-2* diploids do not sporulate. However, zygotic clones obtained by mating *MAT*α homothallic (*HO*) cells with *mat***a***-2 ho* cells are unable to mate and can sporulate. Tetrad analysis of such clones revealed two diploid (*MAT*α/*MAT***a**):two haploid segregants. Therefore, *MAT* switches occur in *MAT*α/*mat***a***-2 HO/ho* cells to produce *MAT*α/*MAT***a** cells capable of sporulation. In heterothallic strains, the *mat***a***-2* allele can be switched to a functional *MAT*α and subsequently to a functional *MAT***a**. Among 32 *MAT*α to *MAT***a** switches tested, where the *MAT*α was previously derived from the *mat***a***-2* mutant, only one *mat***a***-2* like isolate was observed. However, the recovered allele, unlike the parental allele, complements the *mat*α *ste1–5* mutant, suggesting that these alleles are not identical and that the recovered allele presumably arose as a mutation of the *MAT*α locus. No *mat***a***-2* was recovered by *HO*-mediated switching of *MAT*α (previously obtained from *mat***a***-2* by *HO*) in 217 switches analyzed. We conclude that in homothallic and heterothallic strains, the *mat***a***-2* allele can be readily switched to a functional *MAT*α and subsequently to a functional *MAT***a** locus. Overall, the results are in accord with the cassette model (HICKS, STRATHERN and HERSKOWITZ 1977b) proposed to explain *MAT* interconversions.

HETEROTHALLIC (*ho*) strains of the budding yeast, *Saccharomyces cerevisiae*, display **a** or α mating types. Diploidization typically occurs by fusion between cells of opposite mating type. The mating-type locus (*MAT*)* exists in two stable allelic forms (*MAT***a** and *MAT*α); rare switches from one allelic state to the other occur at frequencies of about 10^{-6} in standard laboratory strains (HAWTHORNE 1963a; RABIN 1970). In contrast, homothallic (*HO*) strains can

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MAT*a** and *MAT*α signify the original **a** and α mating-type alleles and *Mat***a** and *Mat*α the respective phenotypes of the cells harboring those *MAT* alleles. *mat* signifies a defective allele, *e.g.*, *mat***a***-2*.

switch *MAT* as often as every cell division (WINGE and ROBERTS 1969; HAWTHORNE 1963b; OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1976a; STRATHERN 1977). Consequently, the mitotic products of a single *HO* cell can express opposite mating types and therefore fuse to produce *MAT***a**/*MAT*α diploids. *MAT***a**/*MAT*α cells are not subject to further *HO*-induced *MAT* switches. These switches comprise stable heritable changes at *MAT* and are promoted by three loci: *HO*, *HM*α (alternate allele *hm*α) and *HMa* (alternate allele *hma*). *HO* and *HM*α (or *hma*) are required for *MAT*α to *MAT***a** interconversions, and *HO* and *HMa* (or *hm*α) are required for *MAT***a** to *MAT*α interconversions (OSHIMA and TAKANO 1971; NAUMOV and TOLSTORUKOV 1973; HARASHIMA, NOGI and OSHIMA 1974; KLAR and FOGEL 1977). Standard heterothallic laboratory strains have the genotype *ho HM*α *HMa* (HAWTHORNE, quoted in HICKS and HERSKOWITZ 1976a; KLAR and FOGEL 1977).

The *HM*α and *HMa* loci are located on opposite arms of chromosome *III*, but to date *HO* has not been mapped. The *HM*α locus displays loose linkage with *MAT*, which is situated about 20 map units from the centromere (HARASHIMA and OSHIMA 1976; KLAR and FOGEL 1977).

Several molecular models have been proposed to explain the *MAT* interconversion. These models involve DNA modification, intrachromosomal recombination, insertion and removal of controlling elements or gene replacement. According to the DNA modification model, both *MAT***a** and *MAT*α loci are present at the *MAT* region, and the expression of these genes is dictated by *HO*-directed sequence modification or specific methylation of the promoter or some other regulatory site (HAWTHORNE, cited in HOLLIDAY and PUGH 1975; LODISH cited in HICKS and HERSKOWITZ 1977). Recently, a model based on reverse-repeat sequences has been proposed (BROWN 1976; HICKS and HERSKOWITZ 1977; D. BOTSTEIN, personal communication). In this model, *MAT* is a complex locus containing both *MAT***a** and *MAT*α loci, but at any given time only one locus is expressed. It is postulated that these loci share a common regulatory site (*e.g.*, promoter), which is flanked by reverse-repeat sequences. In this model, the promoter is shunted back and forth by crossover events within the reverse-repeat sequences, resulting in flipping the promoter; hence, a *MAT* switch. These recombination events are presumed to be mediated by homothallism genes. Recently such a "flip-flop" mechanism has been implicated for "phase variation" of bacterial flagella (ZIEG *et al.* 1977) and has been proposed for controlling mating types in fission yeast (EGEL 1977).

OSHIMA and TAKANO (1971; see also HARASHIMA, NOGI and OSHIMA 1974) proposed that *HO* controls insertion (or removal) of a regulatory element analogous to controlling elements in maize (MCCLINTOCK 1956) into (or from) *MAT*. The mating-type specific controlling elements are proposed to be produced by *HM*α and *HMa* loci. HICKS, STRATHERN and HERSKOWITZ (1977b) proposed a modification of the controlling element model, the "cassette" model, where the *HMa* and *hm*α loci are presumed to be silent blocks of a *MAT*α gene and *HM*α and *hma* of a silent *MAT***a** gene. They suggest that the switch is brought about by insertion of this silent information (or its copy, *i.e.*, "cassette") into *MAT* by

the action of *HO*. *MAT***a** is switched to *MAT*α by insertion of the *MAT*α cassette (obtained from *HMa* or *hm*α) and correspondingly, *MAT*α is switched to *MAT***a** by insertion of the *MAT***a** cassette (obtained from *HM*α or *hma*) into *MAT*. Essentially, the resident *MAT* information is replaced by the opposite *MAT* cassette to generate a switch. It is suggested that the *MAT* alleles at the "master" genes, *HM*α and *HMa* (and alternate alleles *hm*α and *hma*), are silent because, for example, these loci do not carry a promoter or another regulatory site. An equally likely possibility exists where the cells contain a mechanism to repress these loci (KLAR, FOGEL and MACLEOD, in preparation). The cassette model was designed to explain the observation that defective *MAT*α alleles (*mat*α-sterile) are readily interconverted to functional *MAT***a** and subsequently to functional *MAT*α (HICKS and HERSKOWITZ 1977; D. HAWTHORNE, personal communication). However, the recombination model can also account for the same observation if it is assumed that the tested *mat*α-sterile mutations map in the stem of a recombination region. Thus, two successive crossovers could generate a functional *MAT*α (BROWN 1976; HICKS and HERSKOWITZ 1977). The other models do not account for "healing" of the defective *MAT* alleles by switching.

It seems worthwhile to determine whether the defective *MAT* allele is ever recoverable by interconversion. The cassette model predicts that the defective alleles will be repaired, while the recombination-shunt model allows for frequent recovery of the mutant allele. HICKS and HERSKOWITZ's (1977) data "provide no information as to whether the *mat*α-sterile allele is even recovered." The problem seems to be in the difficulty of scoring the *mat*α-sterile phenotype. Recently, we isolated a mutant allele of *MAT***a**, designated *mat***a**-*2* (RADIN 1976), which has a readily testable phenotype. Thus, we have been able to determine (1) whether the *mat***a**-*2* mutant can be switched to function *MAT*α and subsequently to functional *MAT***a**, and (2) whether *mat***a**-2 is recoverable by switching.

Our results demonstrate that the *mat***a**-*2* mutant allele can be readily switched to functional *MAT*α and subsequently to functional *MAT***a**. STRATHERN (1977) and WYGAL and HABER (personal communication) also observed a similar healing for two different *MAT***a** alleles.

MATERIALS AND METHODS

Strains: The strains of *S. cerevisiae* used are listed in Table 1.

Media and techniques: All media for growth and sporulation and techniques for micromanipulation and tetrad analysis have been described (MORTIMER and HAWTHORNE 1969). Cryptopleurine resistance (*cry*) was scored on YEPD plates supplemented with 1 mg/L-crytopleurine (GRANT, SANCHEZ and JIMENEZ 1974). Cryptopleurine was obtained from Chemasea Pty., Ltd., Sydney, N.S.W., Australia. Mitotic recombination to produce *cry* papillae was induced by exposing freshly replicated cells on *cry* plates to UV (380 ergs mm^2, 25-watt Hanovia germicidal lamp, 95% survival) according to ROMAN and JACOB (1958). Mating type was routinely determined by spraying complementing tester strains of *Mat***a** or *Mat*α mating type on separate replica plates of strains to be tested on rich GNA-containing plates (FOGEL, CAMPBELL and LUSNAK, manuscript in preparation). After a 24-hr growth period the plates were replica-plated onto the appropriate selective media. Temperature growth and sporulation was 30°.

TABLE 1

Strain list

Strain	Genotype/origin*	Source or Reference
D24I	*MATα ade8 his4 thr1 ilv3 trp4 can1 cyh2*	RADIN (1976)
D24II	*MAT***a** *ade8 ilv3 trp4 ura3 met13 can1 cyh2*	RADIN (1976)
D24III	*MAT***a** *ade8 his4 thr1 ura3 met13*	RADIN (1976)
D4	*mat***a***-2 ade8 ade2 his4 met13 trp4*	RADIN (1976)
K14	*MAT***a** *cry1 thr4 his4 leu2 his2 ura1*	Lab stocks
K15	*MATα cry1 thr4 his4 leu2 ura1 ade6*	Lab stocks
K16	K15 × D4× D4 × K14	This study
J7	*MAT***a***/MATα HO/HO cry1/cry1 his4/his4 leu2/leu2 arg4/arg4 metx/metx suc/suc*	This study
J32	D4 × *MATα HO* spore from J7	This study
BZ5276-3-1295D	*MATα his4 met13 ade2 ade8 ura1 trp1 arg4*	Lab stocks
K17	Spontaneous *cry1* mutant of D4	This study
T-1023-23B-1A	*MAT***a** *HO HMα hma gal1 ade1 his4 lys2 leu2 arg4 trp1*	I. TAKANO
J33	BZ5276-3-1295D × K17 × K17 × T-1023-23B-1A	This study
K18	*MATα' cry1 thr1 leu2 met? his4 can1*	This study
J20	*MAT***a** *HO HMα hma cry1 his4 leu2 lys2 his2 met? gal1*	This study
J34	*MAT***a***'/MATα' HO/HO HMα/HMα hma/hma leu2/leu2 lys2/lys2 arg4/arg4 his4/his4 his2/his2 met2/met2*	This study
3628-6D	*MATα ade1 ura3 his2 leu1 arg4 gal2 mal*	Lab stocks

* The terminology for genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (PLISCHKE *et al.* 1976) except that the older terminology for homothallism genes is retained (HARASHIMA, NOGI and OSHIMA 1974). Unless otherwise noted, *ho* strains are *ho HMα HMa* and *HO* strains are *HO HMα HMa*.

Matings: Hybrids were generated by cell-to-cell, cell-to-spore or spore-to-spore matings. These matings were initiated by placing cells or spores in direct contact with each other on complex nutrient agar plates. Subsequently, zygotes were isolated by micromanipulation. The hybrid nature of the zygotic clones was verified by appropriate complementation tests for the markers carried in the parent strains. For "rare mating" tests, freshly grown cells of the strains to be tested were mixed at 10^8 cells per ml (1:1) in sterile water and a small portion (0.2 ml) of the suspension was spread on each of five plates containing complex, rich nonselective media. After 48 hr of growth, the plates were replica-plated onto selective media where only the rare mated clones were able to grow. A control plate containing each of the unmixed parents was also prepared.

α-factor and barrier tests: To test strains for α-factor response, individual cells were arranged about 3 mm away from a streak of *Matα* cells that had grown overnight on YEP or GNA agar. Production of α-factor by the cells in the streak causes growth arrest of *Mat***a** cells and changes them to a characteristic "shmoo" figure within about three hr of incubation (DUNTZE, MACKAY and MANNEY 1970). The Bar+ (barrier) phenotype is an inhibition of response to α-factor exhibited by the *Mat***a** cells. The Bar+ phenotype was determined by the barrier test designed by HICKS and HERSKOWITZ (1976b).

Mutagenesis: Haploid stationary phase cells at a density of 3×10^8 cells per ml were exposed to 3.1% ethyl methanesulfonate (EMS) in 0.2 M phosphate buffer (pH 8.0) containing 2% glucose (LINDGREN *et al.* 1965). After 70 min of treatment, the cells were washed twice with water and then suspended in 6% sodium thiosulfate for ten min to inactivate any remaining

EMS. About 15% of the cells survived this treatment. The EMS used for mutagenesis was inactivated with 6% sodium thiosulfate before discarding.

RESULTS

Isolation of a mat**a**-2 *mutant*

During a screening of mutants defective in various phases of the mating process, an unusual mutant class was observed (RADIN 1976). Mutations in this class were generated as follows: strain D24II (*Mat***a**, also see Table 1 for complete genotype) was mutagenized with EMS as described in MATERIALS AND METHODS. The mutagenized cells were spread on GNA plates at a density of 3×10^7 cells per plate. After growth for two days, the resulting thick cell lawn was replica-plated onto fresh GNA-containing plates and sprayed with a dense (3×10^8 cells per ml) suspension of freshly grown mixture (1:1) of cells from strains D24I (*Mat*α) and D24III (*Mat***a**). After two days of mating and growth, the cells were replicated onto plates containing selective media where only cells with genetic inputs from all three parents could grow. We anticipated that a *Mat***a** mutant would still mate with a *Mat*α strain, but that the *Mat*α/ mutant *Mat***a** diploid, unlike the normal *Mat*α/*Mat***a** hybrid, would express a *Mat*α phenotype and thus be able to mate with another *Mat***a** cell. From 9×10^7 viable cells originally plated, 200 presumptive triploid colonies were obtained. Of 24 unselected triploid isolates tested, eight produced segregants exhibiting the expected mutant phenotype. In all instances the altered mating behavior was confined to *Mat***a** segregants. In the present study only a single mutant (strain D4) is analyzed since we cannot assert that the eight mutations originated independently.

Mutant characterization

As described above, hybrids between the mutant and a *Mat*α cell display *Mat*α behavior. Such hybrids mate readily with another mutant cell and the resulting triploid exhibits a *Mat*α phenotype. When the above zygotes or zygotic clones are mated to normal *Mat***a** cells, the resulting hybrids are nonmaters. The mutant cell mates with normal *Mat***a** cells only rarely, *i.e.*, at frequencies similar to those of wild-type *Mat***a** strain. Mutant *Mat***a**/*Mat*α hybrids do not show detectable sporulation. Apparently, the mutant has simultaneously lost the capacity to turn off mating and to turn on sporulation in mutant/*MAT*α hybrids.

The mutant *MAT***a** allele is designated as *mat***a**-*2* to signify a defective *MAT***a** locus. A mutant, *mat***a***, displaying similar properties has been described previously (KASSIR and SIMCHEN 1976).

Since the *mat***a**-*2*/*Mat*α diploid does not sporulate, meiotic mapping of the mutation cannot be accomplished. However, the tetraploid *Mat*α/*mat***a**-*2*/*mat***a**-*2*/ *Mat***a** does sporulate; hence, mapping and tetrad analysis could be undertaken. Such a tetraploid, strain K16, was sporulated and the asci were dissected. The mating-type locus is situated about 20 map units from the centromere on chromosome *III* (MORTIMER and HAWTHORNE 1969). Assuming that the *mat***a**-*2*

mutation maps at or close to *MAT*, and employing the equations applicable to tetraploids that were developed by ROMAN, PHILIPS and SANDS (1955), we calculated the relative frequencies of the various ascus types—2 *Mat***a**:2 *Mat*α, 2 *Mat***a**:2 nonmater (*Mat***a**/*MAT*α), 2 *Mat***a**:1 nonmater:1 *Mat*α as 0.48, 0.15 and 0.37, respectively. Among 31 complete tetrads analyzed from strain K16, the corresponding classes contained 14, 4 and 13 tetrads, numbers which agree closely with the predicted numbers, 14.9, 4.7 and 11.5. These results suggest that the *mat***a**-*2* mutation maps close to or within the *MAT* locus. Moreover, this mutation is readily switched and "healed" by *HO* (next section), it is not surprising that it is located at *MAT*, since *HO* is presumed to induce changes only at *MAT* (HAWTHORNE 1963b; HICKS and HERSKOWITZ 1977).

The *mat***a**-*2* mutation is not suppressed by numerous unidentified amber or ochre suppressors. Strains carrying the *mat***a**-*2* allele "schmoo" in the presence of α-pheromone and exhibit a Bar^+ phenotype (HICKS and HERSKOWITZ 1976b). These reactions are characteristic of *Mat***a** strains.

Switching of the mat**a**-2 *allele by* HO *in* MATα/mat**a**-2 *hybrids*

*MAT*α spores from a homothallic strain J7 (*cry1 MAT***a**/*cry1 MAT*α *HO*/*HO*) were mated with strain D4 (*CRY mat***a**-*2 ho*) cells by spore-to-cell mating, as described in MATERIALS AND METHODS. The resulting zygotic clones (strain J32) were tested for mating and sporulation capacity. They were observed to be nonmaters and sporulated abundantly. Since these clones can sporulate, the cells must carry at least one functional copy of both the *MAT*α and the *MAT***a** alleles. If the *mat***a**-*2* allele cannot be switched by *HO*, these clones should be tetraploids, assuming that *MAT*α/*mat***a**-*2* switches to *MAT***a**/*mat***a**-*2* and then the switched and unswitched cells mate. But, if *mat***a**-*2* can be switched, then some J32 clones could be diploids. Whether cells are diploids or tetraploids is readily ascertained from the meiotic segregation patterns for a number of heterozygous markers carried in this hybrid. Diploids should yield 2+:2− segregations for each heterozygous marker, while tetraploids yield 4+:0−, 3+:1− and 2+:2− segregation patterns for the same markers. Marker segregation data for the J32 hybrid are presented in Table 2 for 47 tetrads. The data indicate that *HO*, *cry1* (data shown), *leu2*, *trp4* (data not shown) segregate 2+2−. This finding demonstrates the diploid nature of the hybrid. Hence, we may conclude that *HO* mediates the switch of *MAT*α/*mat***a**-*2 HO*/*ho* cells to *MAT*α/*MAT***a** *HO*/*ho*.

How this interconversion might occur was suggested by an analysis of the data presented in Table 2, particularly by the segregation patterns for *cry1* and *MAT*. *cry1* is a recessive cryptopleurine-resistance marker closely linked (2.2 map units) to *MAT* on chromosome *III* (GRANT, SANCHEZ and JIMENEZ 1974). Close linkage of the *cry1* locus to *MAT* allowed us to monitor which *MAT* allele was switched. The *cry1* allele was coupled with *MAT*α in eight tetrads (classes I and V) and to *MAT***a** in nine tetrads (classes II and IV). These data can be rationalized if the J32 zygotic clone contained a mixture of *cry1 MAT*α/*CRY MAT***a** and *cry1 MAT***a**/*CRY MAT*α cells, or if *cry1* is not linked to *MAT*. To test these possibilities, single-cell isolates from J32 hybrid were obtained and subjected to

TABLE 2

Segregation of cry1, HO *and* MAT *in hybrid J32* (cry1 MATα/CRY mata-2 HO/ho)

Ascus type		*CRY/cry1*	*Mat*	*HO/ho*	Number	Ascus type		*CRY/cry1*	*Mat*	*HO/ho*	Number
I	A	*CRY*	nm*(**a**)	*HO*	1	VI	A	*CRY*	**a**	*ho*	1
	B	*CRY*	nm(**a**)	*HO*			B	*CRY*	α	*ho*	
	C	*cry1*	α	*ho*			C	*cry1*	nm(**a** or α)	*HO*	
	D	*cry1*	α	*ho*			C	*cry1*	nm(**a** or α)	*HO*	
II	A	*CRY*	nm(α)	*HO*	6	VII	A	*CRY*	nm(α)	*HO*	0
	B	*CRY*	nm(α)	*HO*			B	*CRY*	**a**	*ho*	
	C	*cry1*	**a**	*ho*			C	*cry1*	nm(α)	*HO*	
	D	*cry1*	**a**	*ho*			D	*cry1*	**a**	*ho*	
III	A	*CRY*	nm(**a** or α)	*HO*	0	VIII	A	*CRY*	nm(**a**)	*HO*	1
	B	*CRY*	nm(**a** or α)	*HO*			B	*CRY*	α	*ho*	
	C	*cry1*	**a**	*ho*			C	*cry1*	nm(**a**)	*HO*	
	D	*cry1*	α	*ho*			D	*cry1*	α	*ho*	
IV	A	*CRY*	α	*ho*	3	IX	A	*CRY*	nm(**a** or α)	*HO*	15
	B	*CRY*	α	*ho*			B	*CRY*	**a**	*ho*	
	C	*cry1*	nm(**a**)	*HO*			C	*cry1*	nm(**a** or α)	*HO*	
	D	*cry1*	nm(**a**)	*HO*			D	*cry1*	α	*ho*	
V	A	*CRY*	**a**	*ho*	7	X	A	*CRY*	nm(**a** or α)	*HO*	13
	B	*CRY*	**a**	*ho*			B	*CRY*	α	*ho*	
	C	*cry1*	nm(α)	*HO*			C	*cry1*	nm(**a** or α)	*HO*	
	D	*cry1*	nm(α)	*HO*			D	*cry1*	**a**	*ho*	

* nm stands for nonmaters *MAT***a**/*MAT*α diploids. The bracketed genotype of the nm(*HO*) segregants is inferred from the *Mat* genotype of the sister *ho* segregants in each tetrad.

tetrad analysis to assess the linkage of *cry1* to *MAT*. The individual secondary clones from J32 show close linkage of *cry1* and *MAT*, as depicted in Table 3, since mainly parental ditype tetrads were observed. In four of five clones tested, *cry1* is coupled with *MAT*α and in the fifth clone with *MAT***a**. Hence, we suggest that the *cry1 MAT*α/*CRY mat***a**-*2 HO/ho* zygote (J32) produced a mixture of

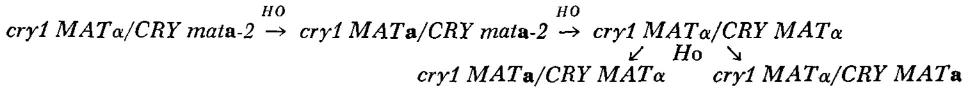
TABLE 3

Coupling of cry1 *and* MAT *in single clones of hybrid J32* (cry1 MATα/CRY mata-2 HO/ho)

Clone no.	Genotype* of the clone	*HO*:*ho*	Ascus types		
			PD	NPD	TT
1	*cry1 MAT*α/*CRY MAT***a**	2:2	17	0	2
2	*cry1 MAT*α/*CRY MAT***a**	2:2	15	0	1
3	*cry1 MAT*α/*CRY MAT***a**	2:2	12	0	1
4	*cry1 MAT*α/*CRY MAT***a**	2:2	18	0	0
5	*cry1 MAT***a**/*CRY MAT*α	2:2†	18	0	1

* Genotypes were inferred from the observed linkage of *cry1* and *MAT*.
† One tetrad from clone #5 segregated 3 *HO*:1 *ho* products. Hence, *ho* can undergo gene conversion to *HO*.

*cry1 MATα/CRY MAT***a** and *cry1 MAT***a***/CRY MATα* cells. These results may be accommodated by the following sequence of events:



We presume that the *MATα/mat***a***-2* cell is switched by *HO* to *MAT***a***/mat***a***-2*, which in turn can switch both loci in a single event to produce a *MATα/MATα* cell. We and others showed previously that *HO* can direct the double switch of *MAT***a***/MAT***a** to *MATα/MATα*, and *vice versa* (KLAR and FOGEL 1977; HICKS, STRATHERN and HERSKOWITZ 1977a; TAKANO *et al.* 1977). The *MATα/MATα* cells can switch one or the other *MAT* locus to yield a mixture of cells where the *cry1* allele is in coupling with either *MAT***a** or *MATα*. We did not recover any tetraploids from this zygotic clone. Tetraploids are expected if *MAT***a***/mat***a***-2* and *MATα/MATα* cells existed in the mitotic progeny of a zygote. However, in other similar crosses involving D4, we have frequently observed a mixture of diploid and tetraploid cells in the progeny of a single zygote.

The low number of tetrads in classes III, VI, VII and VIII in Table 2 is expected, since these classes require a crossover event between the closely linked markers *MAT* and *cry1*. Since *HO* segregates indeoendently of *MAT* and *cry1*, a high frequency of tetrads in classes IX and X is expected.

Since the wild-type *CRY* allele was coupled with both *MAT***a** and *MATα* among the tetrads reported in Table 2, we conclude that the *mat***a***-2* mutant allele is switched to functional *MATα* and functional *MAT***a** in the presence of *MATα* and *HO*. The *MATα/MAT***a** cells do not exhibit switching by *HO* (WINGE and ROBERTS 1949; HAWTHORNE 1963b). However, because *MATα/mat***a***-2* is switched efficiently, we suggest that the *mat***a***-2* allele has lost its capacity to turn off or repress the *HO*-directed switching in diploids.

Can mat**a**-2 *alone manifest* HO *mediated switches?*

The results of the preceding section clearly demonstrate that the *mat***a***-2* allele is switched by *HO* in the presence of *MATα* allele, *i.e.*, in *MATα/mat***a***-2* diploids. Since the *mat***a***-2* mutant lost its capacity to turn off or repress the *HO*-directed switching in *MATα/mat***a***-2* diploids, we question whether this allele alone is competent to turn on or derepress the process required for its own switching. To resolve this question, a strain with *mat***a***-2 HO* genotype is required. Strain D4 carries *mat***a***-2 ho* genotype. Any attempt to transfer *HO* into *mat***a***-2 ho* cell involves hybridization with a *MATα HO* cell. As is clear from the preceding section *MATα/mat***a***-2 HO/ho* cells readily switch to yield *MAT***a***/MATα* products. Hence, *mat***a***-2 HO* strains are not derivable as meiotic segregants from the above hybrid. However, we can construct a sporulatable *CRY MATα/cry1 mat***a***-2/cry1 mat***a***-2/CRY MAT***a** *ho/ho/ho/HO* tetraploid. Since *cry1* is a recessive drug-resistance marker closely linked to *MAT*, resistant segregants should frequently be *cry1 mat***a***-2/cry1 mat***a***-2*. Further, because the tetraploid carries a single copy of *HO* segregating independently of *MAT* and *cry1*, half of the *cry1* segregants should contain a copy of *HO* and the other half *ho*. If

*mat*a*-2* alone can express *HO*-mediated switching, then the *cry1 HO* segregant clones are expected to lose their mating phenotype and be capable of sporulation. The above prediction requires that *HO* exhibit dominance over *ho* (HOPPER and HALL 1975; KLAR and FOGEL 1977; HICKS, STRATHERN and HERSKOWITZ 1977a; TAKANO *et al.* 1977).

Strain BZ5276-3-1295D (*CRY MAT*α *ho*) was mated to K17 (*cry1 mat*a*-2 ho*) and the cells from the zygotic clone were again mated to K17. The resulting progeny of the triploid zygote *CRY MAT*α*/cry1 mat*a*-2/cry1 mat*a*-2* was hybridized to strain T-1023-23B-1A (*CRY MAT*a *HO HM*α *hma*). The resulting tetraploid progeny, strain J33, was sporulated and dissected. Among 80 tetrads analyzed, 25 segregants were observed to be cryptopleurine resistant. About half (12/25) the resistant segregants displayed the *mat*a*-2/mat*a*-2* phenotype. The rest (13/25) were nonmaters and capable of sporulation. Two such sporulation positive segregants (-16A and -23C) were dissected to assess their ploidy level. The results displayed in Table 4 demonstrate that these segregants generated a mixture of diploid and tetraploid cells, a result expected from switching of *mat*a*-2/mat*a*-2* cells by *HO*.

We conclude that the *mat*a*-2* allele can manifest switching by *HO* since *mat*a*-2/mat*a*-2 Ho/ho* cells yield sporulation proficient, nonmater products.

Recovery of functional MATα *and* MATa *from* mata-2 *in heterothallic* (ho) *strains*

Heterothallic strains can effect switches at the *MAT* locus with a low frequency (about 10^{-6}) (HAWTHORNE 1963a; RABIN 1970). These rare events are recovered by "rare mating" strains of the same mating type containing multiple complementary auxotrophic markers. The rare mated clones are identified as prototrophs on selective media. The resulting diploids are sporulated and the spontaneous switches of *MAT* are recovered and identified by tetrad analysis. We employed this approach for switching the *mat*a*-2* allele to *MAT*α and subsequently to *MAT*a. The newly derived alleles are designated *MAT*α' and *MAT*a$'$ by analogy with the notation of TAKANO and OSHIMA (1970). We may inquire whether the defective *mat*a*-2* and a standard wild-type *MAT*a allele differ in their *ho*-directed switching.

TABLE 5

Ploidy levels and meiotic segregation pattern among tetrads obtained from mata-2/mata-2 HO/ho *segregants of strain J33* (CRY MATα/cry1 mata-2/cry1 mata-2/CRY MATa ho/ho/ho/HO)

Segregant	Tetrads tested	Heterozygous marker analyzed	Segregation pattern ++++	+++−	++−−	Tetraploid:diploid*
J33-16A	24	*lys2*	7	6	11	13:11
J33-23C	8	*leu2*	3	1	4	4:4

* Tetraploid:diploid ratios were ascertained from segregation patterns for heterozygous markers. These ratios are approximate since tetraploids can also produce 2+:2− pattern. Here 2+:2− pattern is attributed only to diploids.

Switch of mata-2 *to MATα′*: Strain D4 (*mat***a***-2*) was mixed with strain K14 (*Mat***a**) carrying complementary nutritional requirements and clones derived from rare matings were selected as described above. The presence of the *cry1* marker in strain K14 allowed us to monitor which parental allele was switched in the rare mated cell clones. We isolated 43 such clones. Fourteen exhibited a *Mat***a** phenotype and were sporulation deficient, 28 were nonmaters, but sporulation proficient, and one clone had *Matα* phenotype and was sporulation deficient. The "*Mat***a**" clones probably resulted from rare fusions between *mat***a***-2* and *Mat***a** cells. Only a low frequency of prototrophs possessing the *Matα* phenotype is expected since such cells would fuse with the *Mat***a** cells abundantly present in the mating mixture. Five apparent diploid, sporulation proficient prototrophs were dissected to assess which parental allele had switched. The data presented in Table 5 shows that all clones tested yielded 2 *Mat***a**:2 *Matα* segregants. Other heterozygous markers, *ura1*, *trp4*, *leu1*, *met13*, segregated 2+:2− in 44 tetrads analyzed. The coupling of *CRY* to *MATα′* demonstrates that the *mat***a***-2* allele switched to *MATα*. Thus, we conclude that the *mat***a***-2* allele can be interconverted to a functional *MATα* by *ho*-mediated switching. A recombined *cry1 MATα* segregant (strain K18) from a tetratype ascus of clone #5 was selected for use in a study of secondary switching of *MATα′* to *MAT***a′**.

Switching of MATα′ (derived from mata-2) *to* MAT**a**: Single colony isolates of the K18 strain (*cry1 MATα′* obtained as meiotic segregants of clone #5 from the preceding experiment) were rare-mated with strain BZ5276–3–1295D (*CRY*, *MATα*). To characterize the genetic constitution of the resulting diploids at *MAT*, we exploited the occurrence of reciprocal mitotic recombination, which leads to the appearance of resistant papillae on cryptopleurine-containing media (Klar and Fogel 1977). Rare mated diploids are expected to be cryptopleurine sensitive because the dominant wild-type allele (*CRY*) confers sensitivity. Since *cry1* is closely linked to *MAT*, mitotic recombination events between the centromere and the *cry1* locus could produce cells homozygous for *cry1* and the associated *MAT* allele. This protocol allows us to ascertain which of the parental alleles had experienced an interconversion at *MAT* in each clone without resorting to tetrad analysis. A total of 213 rare-mated cell clones were picked to plates of GNA media. After 24 hours of growth, these master plates were replica-plated

TABLE 5

Coupling of CRY *and* MAT *in "rare-mated"* CRY mata-2 × cry1 MAT**a** *(D4 × K14) clones*

Clone no.	Genotype*	Ascus types PD	NPD	TT
5	*CRY MATα′/cry1 MAT***a**	7	0	3
8	*CRY MATα′/cry1 MAT***a**	7	0	2
9	*CRY MATα′/cry1 MAT***a**	9	0	0
11	*CRY MATα′/cry1 MAT***a**	7	0	0
13	*CRY MATα′/cry1 MAT***a**	8	0	1

* Genotype was inferred from the *cry1* and *MAT* linkage.

to media containing cryptopleurine and the resistant papillae were induced with UV, as described in the MATERIALS AND METHODS. Mating types of the rare mated diploids and the *cry* papillae were tested by standard complementation assays. The data in Table 6 show that switches of *MATα'* to *MAT***a'** (class I) occur nearly as often as switches of wild-type *MATα* to *MAT***a** (class II). The validity of the *cry* papillae method was established by tetrad analysis of two rare mated cell clones from each of classes I and II. Class I clones carried *cry1* in coupling with *MAT***a**, and in class II *cry1* was coupled with *MATα* (data not shown). Classes III, IV and V were not analyzed further. However, we suggest that class III reflects the presence of *MATα/MATα'* diploids, *i.e.*, illegitimate matings without a coincident switch. Such illegitimate matings between cells of the same mating type have been reported previously (RABIN 1970). Class IV might represent *cry1 MATα/O* since the recessive *cry1* marker is expressed in the diploid. The symbol *O* signifies a chromosome *III* loss or deletion of region covering *CRY* and *MATα*, which allows a cell to mate as *Mat***a**. Also class V could be *CRY MATα/O*; it seems to have lost the *cry1* marker since no *cry* papillae were observed. Classes IV and V might have originated by the mating of *Matα* cells with the other parental cells that lost the *MATα* locus either by deletion or chromosome *III* loss due to nondisjunction. We propose that cells deleted for *MAT* region are capable of mating as *Mat***a** cells.

We conclude that *MATα'* can switch to functional *MAT***a** at the low rates characteristic of heterothallic strains.

Recovery of mat**a**-2 *from MATα'*? One rare mated cell clone in Table 6 (class VI) produced *cry* papillae of both mating types. This clone was not a mixture of two cell types since single-colony isolates from this clone also exhibited this property. About 20 to 30% of the papillae were of "*Mat***a**" and the rest were *Matα* type. Several "*Mat***a**" papillae were mated to standard *Matα* strain; the resulting triploids had *Matα* phenotype. Hence, we suppose that these "*Mat***a**" papillae were phenotypically similar to the *Mat***a**-*2* strains. We designate the recovered allele as *mat-1* since we cannot assert whether it is an allele of *MAT***a** or *MATα*. STRATHERN (1977) has shown that some *MATα* mutants behave in a

TABLE 6

Characterization of diploids from cry1 MATα' × CRY MATα (*K18 × BZ5276-3-1295D*) *forced matings*

Class	Diploid mating phenotype	Sporulation	Growth on *cry* media	Papillae on *cry* media	Mating type of *cry* papillae	Number observed	Postulated switch
I	Nonmater	+	—	+	*Mat***a**	31	*MATα'* → *MAT***a**
II	Nonmater	+	—	+	*Matα*	20	*MATα* → *MAT***a**
III	*MATα*	—	—	+	*Matα*	19	none
IV	*MATα*	—	+	NA*	NA	20	none
V	*MATα*	—	—	—	NA	122	none
VI	*MATα*	—	—	+	*Mat***a** and *Matα*	1	*MATα'* → *mat-1*

* NA signifies not applicable.

manner similar to *mat***a**-*2* strains. We postulate that a parent with *cry1 MATα′* has changed to *cry1 mat-1*, which after mating with the *CRY Matα* parent produced a *CRY MATα/cry1 mat-1* diploid.

How can we account for *cry* papillae of both mating types arising from such a rare mated clone? Apparently, the *mat-1/mat-1* papillae are generated by mitotic recombination events occurring between the *cry1* locus and the centromere of chromosome *III*. The *Matα* papillae could be produced by *CRY* to *cry1* gene conversions or by matings between *cry1 mat-1* cells present in the same clone. A recent report by MEADE, RILEY and MANNEY (1977) established that *cry* resistance is controlled by the ratio of *cry1:CRY* alleles in the hybrid.

The presumptive *MATα/mat-1* clone was crossed to a *MAT***a** strain and the resulting triploid was sporulated and dissected. The hybrid yielded a typical triploid spore-survival frequency of about 15% when 60 tetrads were dissected. Eight segregants with *mat-1* phenotype were recovered for further characterization. Thus, one among 32 switches of *MATα′* to *MAT***a** gave rise to an allele with *mat***a**-*2* phenotype.

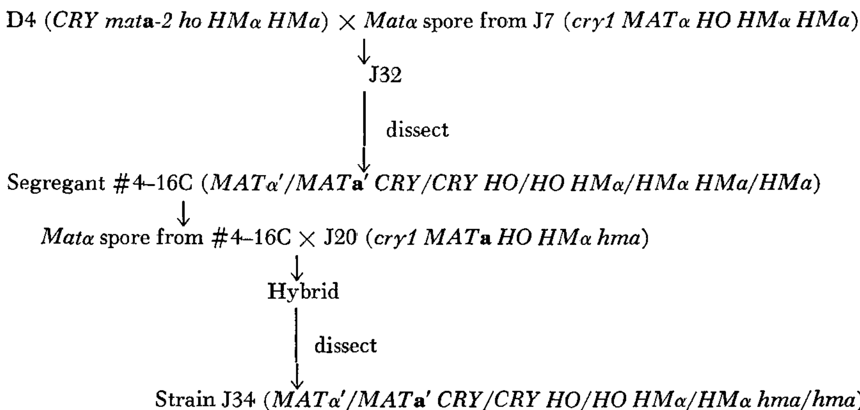
Features of mat-1: The *mat-1* allele might result from spontaneous mutation of *MATα′* during the *MATα′* × *MATα* rare matings. To establish the relationship between the recovered *mat-1* and the parental *mat***a**-*2*, all previous experiments conducted on the *mat***a**-*2* allele were repeated with *mat-1*. The findings may be summarized as follows: Both alleles display indistinguishable properties with respect to *MAT* switches, pheromone response, barrier effect, suppressibility and map position. However, unlike the parental allele, the recovered *mat-1* allele complements the *matα ste1–5* mutation, demonstrating that these alleles are not identical. The *matα ste1–5/mat-1* hybrids exhibit *Matα* phenotype and are incapable of sporulation (data not shown).

Failure to recover mat**a**-2 *from* MATα′ *by* HO *switching*

Specifically, we wished to determine whether the *mat***a**-*2* allele can be recovered by *HO*-induced switching of *MATα′*, where the *Matα′* was previously derived from the *mat***a**-*2* by *HO*-mediated interconversions. To test this, we required a strain with genotype *MAT***a**′/*MATα′ HO/HO HMα/HMα hma/hma*, where the *MAT***a**′ and *MATα′* alleles are the switched derivatives of the *mat***a**-*2* parental allele. Tetrads from this strain should produce 2 diploid (*MAT***a**′/*MATα′*):2 haploid (*MAT***a**′) segregants. This expectation arises because during germination the *Matα* spore progeny undergo a switch at their mating-type locus frequently, as early as the four-cell stage. Then, the sister cells of opposite mating type fuse to yield *MAT***a**′/*MATα′* diploids (HAWTHORNE 1963b; HICKS and HERSKOWITZ 1976a). However, since the strain is homozygous *hma*, the *Mat***a** spores are stable and yield haploid colonies. If the early vegetative cell progenies of *Matα′* spores switch to *mat***a**-*2*, then the resulting fusion products or zygotes should be *MATα′/mat***a**-*2*. The *MATα′/mat***a**-*2* diploid exhibits switching and therefore we expect the zygotes or their immediate progenies to switch to *MAT***a**′/*mat***a**-*2*. But the resultant *MAT***a**′/*mat***a**-*2* cells may not switch, since the parental strain is *hma/hma*. The *MAT***a**′/*mat***a**-*2* cells can then be hybridized

with *Matα ho* cells carrying complementary auxotrophic markers to yield triploids from which segregants containing the *mat***a**-*2* mutation can be recovered.

The construction of the required strain was undertaken as follows. A segregant (#4–16c) of genotype *CRY Matα′/CRY MAT***a**′ *HO/HO* was obtained from hybrid J32. This diploidized segregant must have arisen from a spore that contained a switched derivative of the *mat***a**-*2* parental allele. It was taken from a tetrad that yielded 2 *cry1 MATα/CRY MAT***a** products. A cell from strain J20 (*cry1 MAT***a** *HO HMα hma*) was mated with a *MATα* spore from #4–16C, and the hybrid was subjected to tetrad analysis. Both *CRY* segregants from a tetrad that yielded four diploid segregants must have the required genotype, *i.e.*, *MAT***a**′*/MATα′ CRY/CRY HO/HO HMα/HMα hma/hma*. One of these segregants, strain J34, was used for further studies. We may display the construction of J34:



Strain J34 was sporulated and the dissected tetrads were positioned adjacent to (3 mm) a streak of *Matα* cells grown overnight on GNA media. The *Matα* cells excrete the sex pheromone that blocks the growth of *Mat***a** spores. As expected, two of four spores in each tetrad "shmooed", a response characteristic of *Mat***a** cells to the presence of α-pheromone (DUNTZE, MACKAY and MANNEY 1970). The tetrads were dissected in the presence of α-pheromone simply to differentiate *Matα* spores from *Mat***a** spores, since we were interested only in the switching of *Matα* spore progenies. In most instances, among *Matα* spores progenies, "twin zygotes" were observed at the four-cell stage. This is obtained because two of four cells switch their mating type from *MATα* to *MAT***a** or to *mat***a**-*2* (?) and then fused with the unswitched cells to produce zygotes. These zygotes were separated and removed from the pheromone source. If some of these zygotes had a *MATα′/mat***a**-*2* constitution, we expect these zygotes or their immediate progenies to switch to *MAT***a**′*/mat***a**-*2*, which can be mated to the *Matα ho* tester strain. A segergant with *mat***a**-*2* allele can then be recovered from this triploid. About ten to 20 cells of strain 3628–6D (*Matα ho*) were placed adjacent to each zygote. Strain 3628–6D carries nutritional requirements complementary to that of J34. After three days of growth on rich complex media, the colonies were

replicated onto selective media to test if the zygotes or their progenies have mated with the *Matα ho* tester cells. Only three of 220 zygotes tested supported growth on selective media. These three hybrids were sporulated and dissected to assess the presence of the *mat***a***-2* allele. All these hybrids yielded 2+:2− segregations for *ade1*, *ura3*, *leu1*, *leu2*, *met2* and *lys2*, heterozygous markers carried in ten tetrads analyzed from each hybrid (data not shown). These data demonstrate that the hybrids were diploids and were produced by fusions of *Mat***a** cells obtained from the J34 *Matα* spore progenies, and *Matα* cells from 3628–6D strain. Spore survival of these hybrids was about 90%. The *Mat***a** cells from the zygotes of *Matα* spore progenies might arise by the production of haploid buds from the zygotes at low frequencies (FOWELL 1969).

In summary, 217 switches of *MATα'* by *HO* tested failed to produce the *mat***a***-2* allele. From these results we conclude that the *mat***a***-2* is not recoverable, or the recovery frequency is less than 0.5%.

DISCUSSION

Regulation of the mating-type locus in *Saccharomyces cerevisiae* seems to be complex; a haploid cell can express one or the other mating type. The cell must carry both *MAT***a** and *MATα* information, though at any moment only a single *MAT* is expressed. This notion is best supported by the *MAT* switches wherein cells can interconvert their mating phenotype. The experiments reported in this communication were aimed at investigating the switching of a *MAT***a** mutant allele, *mat***a***-2*. The results demonstrate that the *mat***a***-2* allele can be readily switched to a functional *MATα* and subsequently to a functional *MAT***a** in homothallic and heterothallic strains. These results are similar to those of HICKS and HERSKOWITZ (1977) and HAWTHORNE (personal communication), who recovered functional alleles of *MAT***a** and *MATα* from defective alleles of *MATα*. Hence defective alleles of both mating types can be "healed" by switching. It is not known whether the molecular change in the *mat***a***-2* mutant is located in the coding or the regulatory sequence. However, HAWTHORNE (personal communication) observed the healing of an ochre-suppressible *MATα* allele. His finding strongly demonstrates that a lesion in the coding sequence, at least in *MATα*, can be repaired or removed by switching.

Recovery of mat-1 *by switching of* mat**a**-2 *allele and its relevance to switching models*

In the introduction, we suggested that among the proposed models only the cassette and the recombination models need be considered further. Clearly, the cassette model predicts that defective *MAT* alleles can be repaired, while the recombination model allows for frequent recovery of the mutant allele. During the heterothallic switching of *MATα'* to *MAT***a**', where the *MATα'* was previously obtained from *mat***a***-2* by *ho*-induced switching, one switch (?) to *mat-1* among 32 tested interconversions was observed. The *mat-1* was indistinguishable from the parental *mat***a***-2* with respect to switching, mating response, barrier

effect etc. However, the recovered and the parental *mat***a**-*2* alleles are clearly distinguishable, since only the recovered allele complements the *mat*α *ste1–5* mutation. The new allele might well have arisen by a spontaneous mutation of *MAT*α′ during rare matings of *Mat*α′ with *Mat*α strains. If we consider the *ho* switching frequency to be approximately 0.5×10^{-6} (HAWTHORNE 1963b; RABIN 1970), 1/32nd of this frequency is certainly within the range of the spontaneous mutation frequency. RABIN (1970) and STRATHERN, RINE and HERSKOWITZ (personal communication) also observed the appearance of "*mat***a**-*2*"-type mutations in rare matings between two normal *MAT*α strains.

Our uncertainty concerning the recovery of parental *mat***a**-*2* would be diminished considerably had this allele been recovered by *HO*-directed switching, because no selective forces are operative for isolating new *MAT* mutations during *HO*-operated interconversions. Since we did not recover the *mat***a**-*2* allele by *HO*-mediated switching of *MAT*α′ among 217 switches tested, we suggest that the *mat***a**-*2* is not recoverable, or is recoverable with a probability of less than one in 217. However the precise switching mechanism might operate, it circumvents recovery of the mutant *mat***a**-*2* information. These observations are consistent with the cassette model. In this communication, the cassette model is treated as a specific version of the controlling-element model. In a separate set of recent experiments, we isolated mutations of *HM*α and *HMa* loci. When a mutant *HM*α allele is used for switching *MAT*α to *MAT***a**, the recovered *MAT***a** allele is defective (KLAR and FOGEL, unpublished observations). These experiments may provide genetic evidence supportive of the cassette model.

Expression of the mating phenotype and HO *manifestations*

It is interesting to compare the switching in diploids heterozygous for a mutant and a wild-type allele at the mating-type locus. Standard *MAT*α/*MAT***a** cells do not exhibit switching. However, we and others (KLAR and FOGEL 1977; HICKS, STRATHERN and HERSKOWITZ 1977a; TAKANO *et al.* 1977) have observed single and double switching of *MAT***a**/*MAT***a** and *MAT*α/*MAT*α diploids to yield *MAT*α/*MAT***a** diploids or *MAT*α/*MAT*α/*MAT***a**/*MAT***a** tetraploids, respectively. This observation leads us to suggest that the phenotype relative to mating capacity rather than ploidy controls the manifestations of the *HO*-induced switching mechanism (KLAR and FOGEL 1977). *HO* must somehow "sense" the status at *MAT* and induce changes only if a single mating-type phenotype is expressed, and fails to do so if both functional mating types are present. Studies on *MAT*α/*mat***a**-*2* cells, which exhibit the *Mat*α phenotype and are readily switched by *HO*, reinforce our view. It may be hypothesized that *MAT* somehow turns on or derepresses the *HO* system. Consequently, it should be possible to isolate *MAT* mutants defective in this function. Such a defect might be represented by the *MAT*α-inconvertible (*Mat*α-*inc*) allele, a naturally occurring variant, which switches to *MAT***a** with low frequency of about 1/1000 (TAKANO, KUSUMI and OSHIMA 1973). Alternatively, *MAT*α-*inc* might represent a structural change, *e.g.*, small deletion or inversion that somehow results in reducing the efficiency of switching.

Diploid *MATα/MAT***a** cells are patently stable to switching; perhaps both *MAT* alleles reciprocally inactivate each other's *HO*-inducing capacity. Equally plausible is the hypothesis that the *MATα/MAT***a** state represses *HO* function, while *MAT***a** or *MATα* states do not. Our analysis of *MATα/mat***a**-*2/mat***a**-*2/ MAT***a** *ho/ho/ho/Ho* tetraploids are consistent with this notion, since *mat***a**-*2/ mat***a**-*2* segregants were recovered from this hybrid. It would appear as if once a cell acquires an active copy of the *MAT***a** and *MATα* loci, the *HO* functions are blocked. Again, if the *MATα/MAT***a** state represses the *HO* function, one should be able to isolate mutants or natural variants of *HO* that are not repressible. This suggestion might apply to "super-homothallism" variants (TAKANO, personal communication) that are supposedly effective in switching *MATα/MAT***a** cells. Such a situation would inevitably lead to increasing polyploidy.

Homothallism as a model system for differentiation

It is increasingly clear that the mating-type locus constitutes a central component in a complex regulatory system. MACKAY and MANNEY (1974) have suggested that the mating-type alleles are themselves regulatory in function. These alleles govern various steps of conjugation, ability to produce and respond to mating-type specific pheromones and agglutination in the early stages of conjugation. In *MATα/MAT***a** diploids, the genetic constitution at the mating-type locus controls mating inhibition, meiosis and sporulation, X-ray survival, UV-induced mitotic recombination and budding pattern (reviewed by CRANDEL, EGEL and MACKAY 1976). The *MAT***a** and *MATα* cells vary in so many functions that one can consider them as different cell types. Elegant studies by HICKS and HERSKOWITZ (1976a) and STRATHERN (1977) on the pattern of mating-type switching in the cell lineages reveal several analogies between *MAT* interconversion and clonal differentiation in higher organisms. Since clonal differentiation is a central feature in the development of higher organisms, the study of *HO*-mediated interconversions of *MAT* represents an important model system for differentiation. Recently, HERSKOWITZ *et al.* (1977) extended the cassette model to account for development. Since yeasts possess a smple life cycle and also because they are amenable to sophisticated genetic analysis, their mating-type locus may comprise the most readily analyzable control system yet identified in eukaryotes.

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