A MUTATION THAT PERMITS THE EXPRESSION OF NORMALLY SILENT COPIES OF MATING-TYPE INFORMATION IN *SACCHAROMYCES CEREVISIAE*

JAMES E. HABER AND JEANNE P. GEORGE

Rosenstiel Basic Medical Sciences Research Center
and
Department of Biology, Brandeis University, Waltham, Massachusetts 02254

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ABSTRACT

Studies of heterothallic and homothallic strains of *Saccharomyces cerevisiae* have led to the suggestion that mating-type information is located at three distinct sites on chromosome 3, although only information at the mating-type (*MAT*) locus is expressed (HICKS, STRATHERN and HERSKOWITZ, 1977). We have found that the recessive mutation *cmt* permits expression of the normally silent copies of mating-type information at the *HM***a** and *HM*α loci. In haploid strains carrying *HM***a** and *HM*α, the *cmt* mutation allows the simultaneous expression of both **a** and α information, leading to a nonmating ("*MAT***a**/*MAT*α") phenotype. The effects of *cmt* can be masked by changing the mating-type information at *HM***a** or *HM*α. For example, a cell of genotype *MAT***a** *hm***a** *HM*α *cmt* has an **a** mating type, while a *MAT*α *hm***a** *HM*α *cmt* strain is nonmating. Expression of mating-type information at the *HM* loci can correct the mating and sporulation defects of the *mat***a*** and *mat*α*10* alleles. Meiotic segregants recovered from *cmt*/*cmt* diploids carrying the *mat* mutations demonstrate that these mutants are not "healed" to normal *MAT* alleles, as is the case in parallel studies using the homothallism gene *HO*.——All of the results are consistent with the notion that the *HM***a** and *hm*α alleles both code for α information, while *HM*α and *hm***a** both code for **a** information. The *cmt* mutation demonstrates that these normally silent copies of mating-type and sporulation information can be expressed and that the information at these loci is functionally equivalent to that found at *MAT*. The *cmt* mutation does not cause interconversions of mating-type alleles at *MAT*, and it is not genetically linked to *MAT*, *HM***a**, *HM*α or *HO*. In *cmt* heterozygotes, *cmt* becomes homozygous at a frequency greater than 1% when the genotype at the *MAT* locus is *mat***a***/*MAT*α or *mat*α*10*/*MAT***a**.

HAPLOID strains of *Saccharomyces cerevisiae* may exist in one of two mating types, designated **a** and α. Mating type is determined by variants of a single locus (*MAT*) on chromosome 3. The exact relationship between **a** and α mating information remains unknown, although it seems certain that the *MAT***a** allele is not simply an inactive form of *MAT*α or *vice versa*. This can be clearly seen in a comparison of the behavior of *MAT***a**/*MAT*α, *MAT***a**/*MAT***a** and *MAT*α/*MAT*α diploids. The *MAT***a**/*MAT*α diploid is nonmating and able to undergo

meiosis and sporulation, while diploids homozygous for *MAT***a** or *MAT*α mate with cells of opposite mating type, but are unable to sporulate. Thus, it seems that *MAT***a** and *MAT*α are, in fact, co-dominant alleles of a single locus, affecting both mating and sporulation.

Recently, studies of both homothallic and heterothallic strains of *S. cerevisiae* have led to the hypothesis that there are at least three copies of mating-type information at different chromosomal locations in a haploid strain, but that only the information at the *MAT* locus is expressed (HICKS, STRATHERN and HERSKOWITZ 1977). In homothallic strains of yeast, haploid cells can undergo an interconversion of mating type from *MAT***a** to *MAT*α or from *MAT*α to *MAT***a** as frequently as every cell generation (HICKS and HERSKOWITZ 1976). Such mating-type interconversions are apparently dependent on the presence of unexpressed copies of opposite mating-type information. A decisive experiment that led to this idea was the observation by HICKS and HERSKOWITZ (1977) that a mutant *MAT*α allele could be converted to a normal *MAT***a** and then "healed" back to a normal copy of *MAT*α information. A similar experiment has also been carried out beginning with a mutant *MAT***a** allele (WYGAL and HABER 1977; KLAR, FOGEL and RADIN 1979). In order for mutant mating-type information to be "healed," it has been postulated that there are additional unexpressed copies of both **a** and α mating type information in a haploid cell.

HICKS, STRATHERN and HERSKOWITZ (1977) suggested that these unexpressed copies of mating-type information are probably located at two other loci, designated *HM***a** and *HM*α, located on chromosome 3 (Table 1). These loci have been shown to be involved in the homothallic interconversion of mating-type information at the *MAT* locus (TAKANO and OSHIMA 1970; HARASHIMA and OSHIMA 1976; HARASHIMA, NOGI and OSHIMA 1977). OSHIMA and his colleagues, along with NAUMOV and TOLSTORUKOV (1973), demonstrated that the ability of a haploid homothallic (*HO*) spore to switch from *MAT***a** to *MAT*α depended on the presence of *HM***a**, while the ability of a cell to switch from *MAT*α to *MAT***a** depended on the presence of *HM*α. A cell containing *hm***a** *HM*α is unable to switch from *MAT***a** to *MAT*α, but switches efficiently from *MAT*α to *MAT***a**. Similarly, a cell of genotype *HM***a** *hm*α is unable to switch from *MAT*α to *MAT***a**, but switches from *MAT***a** to *MAT*α. The unexpected striking observation that a cell of genotype *hm***a** *hm*α can interconvert mating type in either direction led NAUMOV and TOLSTORUKOV (1973) to suggest that *HM***a** was equivalent to *hm*α and *hm***a** was equivalent to *HM*α, a proposal that has been verified by the experiments of KLAR and FOGEL (1977) and HARASHIMA and OSHIMA (1978). Based on this information, HICKS, STRATHERN and HERSKOWITZ (1977) suggested that *HM***a** and *hm*α each represented copies of α information, while *HM*α and *hm***a** each represented copies of **a** information. By this "cassette" model, a cell of genotype *hm***a** *HM*α contains only silent copies of ***a*** information and is unable to provide a source of α information necessary to interconvert *MAT***a** to *MAT*α; nevertheless, interconversion from *MAT*α to *MAT***a** is not impaired as there are two sources of *MAT***a** information. Despite the current nomenclature, *HM***a** and *hm***a** are both co-dominant alleles, as are *HM*α and

TABLE 1

Mating phenotypes and ability to switch mating type with different combinations of alleles at MAT, HM**a** and HMα

Alleles*	Mating phenotype when only *MAT* allele expressed	Ability to switch *MAT* to opposite mating type†	Predicted phenotype if all copies of mating-type information are expressed
*HM***a** *MAT***a** *HM*α (α) **a** (α)	**a**	yes	nonmating
*HM***a** *MAT*α *HM*α (α) α (α)	α	yes	nonmating
*hm***a** *MAT***a** *HM*α (**a**) **a** (α)	**a**	no	**a**
*hm***a** *MAT*α *HM*α (**a**) α (α)	α	yes	nonmating
*HM***a** *MAT***a** *hm*α (α) **a** (α)	**a**	yes	nonmating
*HM***a** *MAT*α *hm*α (α) α (α)	α	no	α
*hm***a** *MAT***a** *hm*α (**a**) **a** (α)	**a**	yes	nonmating
*hm***a** *MAT*α *hm*α (**a**) α (α)	α	yes	nonmating

*Genotypes of different arrangements of *HM***a**, *MAT* and *HM*α alleles on chromosome 3 are shown. Below the line are indicated the mating-type information presumed to be at each locus, based on the proposal of HICKS, STRATHERN and HERSKOWITZ (1977). The mating-type information in parentheses represents the fact that this information is normally not expressed.

†Mating-type interconversion may occur very frequently in homothallic strains and rarely in heterothallic strains (HICKS and HERSKOWITZ 1977).

hmα. The rules for mating-type interconversion according to the cassette model are summarized in Table 1.

The role of the *HM***a** and *HM*α genes in the interconversion of mating type does not prove that there are silent copies of mating-type information at these loci. Indeed, HARASHIMA, NOGI and OSHIMA (1974) proposed that the *HM* loci may represent controlling elements important in activating mating-type information located elsewhere. However, a more complete proof could be constructed if it were possible to demonstrate directly that *HM***a** and *HM*α loci contain mating-type information by causing the expression of this normally silent information. Several experiments have pointed to the fact that mating-type information at the *HM* loci can, in fact, be expressed under certain circumstances. For ex-

ample, STRATHERN (1977) re-examined a class of large recessive lethal deletions of chromosome 3 that result in the change from an α to **a** mating type described by HAWTHORNE (1963). STRATHERN suggested that the deletion actually represented a fusion of a site adjacent to the mating-type locus *MAT* with *HM*α. This fusion would result in the deletion of the original α mating-type information at MAT and the activation of normally silent **a** information located at *HM*α. STRATHERN also found other rearrangements for chromosome 3 that appear to result in the deletion of *MAT* and the joining of the site close to *MAT* with the *HM***a** locus on the other side of the centromere.

An alternative way to examine the location of additional copies of mating-type information in yeast would be to find mutations that would permit the expression of the normally silent copies of **a** and α information. The consequences of expressing mating-type information at *MAT*, along with the information located at or controlled by *HM***a** and *HM*α, are also included in Table 1. A haploid strain in which both **a** and α information are expressed would be expected to be nonmating, similar to a *MAT***a**/*MAT*α diploid; however, since it is haploid, it would not be expected to sporulate. On the other hand, if the silent copies of mating-type information all had the same information as the *MAT* allele (for example, *MAT*α *HM***a** *hm*α), such a strain would continue to exhibit a single mating type even if all copies were expressed.

One mutation that appears to turn on silent copies of mating-type information is the *mar1* mutant found by KLAR, FOGEL and MACLEOD (1979). A second, distinct mutation that appears to have a similar phenotype is the recessive *cmt* (change of mating type) mutation isolated by HOPPER and HALL (1975b). This mutation converts diploids cells of *MAT*α/*MAT*α genotype from asporogenous, α maters to nonmaters with excellent sporulation. *MAT***a**/*MAT***a** diploids homozygous for *cmt* are similarly nonmating and able to sporulate. All of the *cmt* haploid segregants are nonmating; in some cases a few cells able to sporulate have been found. HOPPER and HALL (1975b) suggested that *cmt* might act in heterothallic (*ho*) strains in a manner similar to that of the *HO* allele in homothallic strains.

In this paper, we show that *cmt* does not act as a homothallism gene to promote mating-type interconversions, but instead permits the expression of normally silent copies of mating-type information. The experiments described below demonstrate that these copies of mating-type information are either *HM***a** and *HM*α or directly depend on the alleles of these loci for their expression.

MATERIALS AND METHODS

Strains: The strains of *S. cerevisiae* used frequently in this study are listed in Table 2. All strains were heterothallic (*ho*) unless otherwise noted; and all strains carried the *HM* alleles *HM***a** and *HM*α unless other alleles are specified.

Genetic techniques: All of the methods for growth, sporulation and genetic analysis have been described by MORTIMER and HAWTHORNE (1969). Generally, cells were grown and sporulated at 30°. Asci from sporulated strains were dissected by micromanipulation and the colonies grown on YEPD plates. Tetrad analysis of the segregation of nutritional requirements was carried out by replica plating colonies to appropriate selective media. Mating

TABLE 2

Strains used in this study

Strain	Genotype†	Source
D135a	*MATα/MATα cmt/cmt gal1+ lys2/+ tyr1/+ his7/+ leu1/+*	HOPPER
17–15	*mata* ade2 leu1 ura3 CMT*	SIMCHEN
XT1172–S245C	*MATα ade6 his6 leu1 trp5 gal2 can1 CMT*	MACKAY
VC73	*matα10 ade6 his6 leu1 trp5 gal2 can1 CMT*	MACKAY
VN33	*matα1–5 ade6 his6 leu1 trp5 gal2 can1 CMT*	MACKAY
6D131–15A	*mata* HMa hmα his4 leu1 leu2 ura–*	GRUENSPAN
T1059–18B	*MATa HO hma HMα ade1 his4 leu2 thr4 gal1 CMT*	OSHIMA
JH95–9C	*MATa hma HMα arg4 his4 tyr7 CMT*	this study
BW105–9A	*MATα hma HMα leu1 lys1 arg4 ade1 CMT*	this study
BW105–9C	*MATα hma HMα his4 arg4 tyr7 ade1 CMT*	this study
JH110–1A	*MATa HMa hmα his4 leu2*	this study
Y55–4	*MATa/MATα HO/HO lys/lys trp/trp can1/can1 CMT/CMT*	this study
JH90–3B	*MATα cmt ade1 ade2 arg4 leu1 lys1*	this study
JH112–7A	*MATα cmt his5 his7 lys1*	this study
JH112–1B	*MATa cmt hma HMα ade1 leu1 lys1 arg4*	this study
JH117–9A	*MATα cmt HMa hmα his5 lys1*	this study
JH121–3C	*mata* HMa hmα cmt ade1 ade2 ura3 leu1*	this study

†Unless otherwise designated, all strains are *ho* and carry *HMa* and *HMα*.

phenotypes were determined by complementation tests after cross-streaking colonies with haploid *MAT***a** or *MATα* strains (HABER 1974).

RESULTS

When strain D135a (*MATα/MATα cmt/cmt*) was sporulated and dissected, all four segregants exhibited very weak mating with *MAT***a** strains to form diploids able to sporulate. When asci from one of these diploids (JH90) were dissected, the tetrads exhibited two mating and two essentially nonmating segregants, as described for the recessive *cmt* mutation by HOPPER and HALL (1975b). In some segregants a very weak capacity to mate with *MAT***a** strains was again found. This weak mating facilitated subsequent genetic analysis, but appeared to depend on the genetic background of the original strain D135a; in further crosses this character was not recovered, and all *cmt* segregants were essentially nonmating. The nonmating segregants sporulated very poorly, if at all.

The pattern of mating:nonmating in tetrads from the diploid JH90 was compared with the segregation of other genetic markers. There was no apparent linkage of *cmt* to mating type, nor is *cmt* centromere-linked, as evidenced by its segregation with the centromere-linked marker *trp1* (parental ditypes:nonparental ditypes:tetratypes = 2:3:14).

Interaction between cmt *and* hm**a**: If the *cmt* phenotype depends on the expression of **a** and *α* information from *HM***a** and *HMα*, it should be possible to show this by substituting different alleles of the *HM* genes. First, we examined the effect on the *cmt* phenotype of the *hm***a** allele (presumably **a** information

instead of the α information associated with *HM***a**). When a weakly mating *cmt* segregant (strain JH90-3B) was crossed with a heterothallic strain carrying *hm***a** (strain JH95-9C; *MAT***a** *hm***a** *HM*α *ho*) and the resulting diploid JH112 sporulated, a new pattern of mating phenotypes was found among the tetrads analyzed. Instead of obtaining two mating and two nonmating colonies, as occurs in diploids heterozygous for *cmt*, some tetrads contained three or four mating colonies (Table 3). There was a distinct excess of **a** mating colonies over α maters in these tetrads, but no tetrad contained more than two **a** segregants. Assuming that each of the 30 tetrads in Table 3 contained two *MAT***a** and two *MAT*α segregants, we found that half (30/60) of the *MAT*α segregants were nonmating and the other half α mating, as expected from a diploid heterozygous for *cmt*. Among the *MAT***a** segregants, however, only one-fourth (14/60) were nonmating, while 46/60 were **a** mating. Thus, half of the *MAT***a** *cmt* segregants must have been **a** mating, instead of nonmating. These data suggest the presence of a suppressor of the nonmating *cmt* phenotype that has no effect on *MAT*α cells, but does permit the expression of **a** mating in a *MAT***a** *cmt* cell. From these data and from the experiments described below, we concluded that the *MAT***a**-specific suppressor of *cmt* was the allele *hm***a**. By this interpretation, a *MAT***a** *HM***a** *HM*α *cmt* strain would be nonmating, while a *MAT***a** *hm***a** *HM*α *cmt* strain would be **a** mating. On the other hand, both *MAT*α *HM***a** *HM*α *cmt* and *MAT*α *hm***a** *HM*α *cmt* cells would be nonmating.

If the suppressor of *cmt* in *MAT***a** strains were *hm***a**, then strains carrying the suppressor should also prevent homothallic mating-type switching in *HO MAT***a** strains. That is, a cross between a *MAT***a** *hm***a** *HM*α *cmt ho* strain and a *MAT***a** *HM***a** *HM*α *CMT HO* cell should yield segregants that are **a** mating because they are *MAT***a** *hm***a** *HM*α *HO*. We therefore analyzed all four members of one tetrad of JH112 (*cf.* Table 3) in which there were two α and two **a** mating colonies. We assumed that the two **a** maters should have the genotype *MAT***a**

TABLE 3

Mating phenotype segregation in diploid JH112 heterozygous for MATa/MATα, cmt/CMT *and* hma/HMa*

Tetrad type†	Number of tetrads
—/—/**a**/**a**	5
—/—/**a**/α	11
—/—/α/α	1
—/**a**/**a**/α	9
—/**a**/α/α	1
a/**a**/α/α	3

*Strain JH112 had the genotype:

$$\frac{MAT\alpha\ cmt\ HM\mathbf{a}\ HM\alpha\ +\ +\ ade1\ ade2\ leu1\ lys1\ arg4}{MAT\mathbf{a}\ +\ hm\mathbf{a}\ HM\alpha\ his4\ tyr7\ +\ +\ +\ +\ arg4}$$

†Nonmating colonies are designated by —; **a** and α mating colonies are designated by **a** and α, respectively.

*hm***a** *HM*α *cmt ho*, while the two α maters were *MAT*α *HM***a** *HM*α *CMT ho*. Two sets of crosses were carried out: one with *ho CMT* haploids to demonstrate the presence of *cmt* and one with *HO CMT* cells to demonstrate the presence of *hm***a**.

When the two α mating segregants from the 2α:2**a** tetrad JH112-1 were mated with a heterothallic *MAT***a** *HM***a** *HM*α *ho CMT trp1* strain, all of the tetrads from these crosses contained 2α and 2**a** maters. There was no evidence of *cmt*. On the other hand, when the two **a** maters (JH112-1B and 1C) were crossed with a *MAT*α *HM***a** *HM*α *ho CMT trp1* strain, the pattern of mating phenotype among the tetrads again resembled that shown in Table 3; that is, the nonmating *cmt* phenotype reappeared in half of the *MAT*α segregants, but in only one-fourth of the *MAT***a** segregants.

The presence of *hm***a** was tested by tetrad analysis from crosses made between JH112-1A, 1B, 1C and 1D with spores of the homothallic strain Y55-4 (*HM***a** *HM*α *HO CMT*). The two α mating colonies (1A and 1D), when crossed with Y55-4, gave rise to tetrads in which there were two mating and two nonmating colonies (able to sporulate). There was therefore no evidence of *hm***a** in these tetrads. On the other hand, the mating phenotype segregation in tetrads derived from crosses of the **a** mating colonies (1B and 1C) with Y55-4 was complex, ranging from some tetrads containing four nonmating segregants to others containing four mating colonies (two **a** and two α). As expected, some of the nonmating colonies were homothallic diploids able to sporulate efficiently, while others, presumably cmt segregants, exhibited virtually no sporulation. More important, in tetrads where there were two **a** and two α maters, it is evident that the nonmating phenotype characteristic of *HO* segregants had been suppressed in *MAT***a** segregants by *hm***a**. Thus, the two **a** maters (JH112-1B and 1C) must carry both *hm***a** and *cmt* and have the genotype *MAT***a** *hm***a** *HM*α *cmt ho*. The two α maters (JH112-1A and 1D) must be *MAT*α *HM***a** *HM*α *ho CMT*.

Our conclusion that *hm***a** suppresses *cmt* in *MAT***a** *hm***a** *HM*α *cmt* strains was also supported by further tetrad analysis when one of the **a** mating segregants carrying both *cmt* and *hm***a** (JH112-1C) was crossed to a different *MAT*α *hm***a** *HM*α strain, BM105-9A. Among 18 tetrads from this cross, all contained two **a** mating colonies. The other two segregants, presumably carrying *MAT*α, were either both α mating (parental type), both nonmating (nonparental type) or one α mating and one nonmating (tetratype). The ratio among these three classes (P:N:T::3:4:11) confirms that *cmt* segregates independently of *MAT*α. Moreover, all of the *MAT***a** segregants from this diploid (*MAT***a**/*MAT*α *hm***a**/*hm***a** *HM*α/*HM*α *cmt*/+) were **a** mating. The absence of any nonmating segregants attributable to *MAT***a** demonstrates that the suppressor of the nonmating phenotype in *MAT***a** *cmt* strains is very closely linked to *hm***a**. Most likely, *MAT***a** *hm***a** *HM*α *cmt* strains are **a** mating, while *MAT***a** *HM***a** *HM*α *cmt* strains are nonmating.

Interaction of cmt *with* hmα: A similar series of experiments was carried out to show that *MAT*α *cmt HM***a** *HM*α strains are nonmating, whereas MATα *cmt HM***a** *hm*α strains have an α mating type. When a weakly mating *MAT*α

TABLE 4

Mating phenotype segregation in diploid JH117 heterozygous for MAT**a**/MATα, cmt/CMT *and* hmα/HMα*

Tetrad type†	Number of tetrads
—/—/**a**/**a**	0
—/—/**a**/α	10
—/—/α/α	3
—/**a**/**a**/α	1
—/**a**/α/α	5
a/**a**/α/α	2

*Diploid JH117 had the genotype:

$$\frac{MAT\alpha\ cmt\ HM\alpha\ his5\ his7\ lys1\ +\ +}{MAT\mathbf{a}\ +\ hm\alpha\ +\ +\ +\ his4\ leu2}$$

†Nonmating colonies are designated by —.

*HM***a** *HM*α *cmt* strain (JH112-7A) was crossed with a *MAT***a** *HM***a** *hm*α *CMT* strain (JH110-1A), many tetrads had more than two mating segregants (Table 4). Here, only one-fourth of the *MAT*α segregants were nonmating (11/42), while one-half (21/42) of the *MAT***a** segregants showed the *cmt* phenotype. Thus, the diploid JH117, heterozygous for *hm*α/*HM*α, contains a suppressor that restores *MAT*α *cmt* cells to α mating.

The suppressor of nonmating in *MAT*α *cmt* cells appears to be *hm*α. First, when a tetrad from JH117 in which there were two **a** and two α maters was analyzed by crossing each segregant to *ho HM***a** *HM*α *CMT* and *HO HM***a** *HM*α *CMT* cells (as described above for *hm***a**), the two α strains could be shown to be *MAT*α *HM***a** *hm*α *cmt ho*. Further, when a *MAT*α *HM***a** *hm*α *cmt* segregant from the cross described in Table 4 was crossed with *MAT***a** *HM***a** *hm*α *CMT* strain (JH110-1A), and the resulting diploid sporulated, every tetrad contained two α mating segregants. The other two segregants, presumably *MAT***a**, were either both **a** mating (parental type), both nonmating (nonparental type) or one **a** mating and one nonmating (tetratype). The ratio of these three types (P:N:T::5:4:13) is indicative both of the independent segregation of *cmt* and *MAT* and of the lack of suppression of the nonmating phenotype among the *MAT***a** *HM***a** *hm*α *cmt* segregants of this cross. In contrast, all of the *MAT*α *HM***a** *hm*α *cmt* segregants are α mating, supporting the conclusion that *hm*α suppresses the *cmt* phenotype in *MAT*α *HM***a** *hm*α strains, but has no effect on *MAT***a** *HM***a** *hm*α strains.

Does cmt *promote mating-type interconversion?* Because initially haploid *cmt* spores grow into nonmating colonies with a few (less than 1%) of the cells able to sporulate, it is possible that *cmt* acts in heterothallic (*ho*) strains to promote mating-type interconversion and diploidization (HOPPER and HALL 1975b). We have compared the action of the homothallism allele to *HO* with *cmt* to demonstrate that the latter does not promote mating-type interconversion.

In these experiments we used recessive mutant alleles of *MAT***a** or *MATα* that are defective in sporulation and mating functions. For example, a strain carrying the *mat***a*** allele described by KASSIR and SIMCHEN (1976) can mate readily with *MATα* strains, but the resulting diploid has an α phenotype and cannot sporulate. Similarly, strain VC73 carrying the *matα10* allele (MACKAY and MANNEY 1974) mates very poorly with either *MAT***a** or *MATα* strains; the resulting diploids exhibit **a** or α mating behavior, respectively, but they do not sporulate.

In the following experiments, the action of either *HO* or *cmt* was assessed by using *mat***a***/*MATα* and both *matα10*/*MAT***a** and *MATα10*/*MATα* diploids. When these diploids carry either *HO* or *cmt*, they can become nonmating and able to sporulate, although through different mechanisms. The first section characterizes the action of *HO* on the recessive *mat***a*** and *matα10* alleles; the later sections then examine the effect of the *cmt* mutation.

Effect of HO *on* mat**a*** *and* matα10: When strain 17–15 (*mat***a***) was mated with spores of a homothallic strain (*HO HM***a** *HMα*), the resulting diploid was nonmating and sporulated efficiently. Tetrads from this mating were dissected and analyzed. Most of the tetrads were derived from diploid cells from which all heterozygous nutritional markers segregated 2+:2−. The exceptions, apparently from a tetraploid, will be discussed below. In all of the tetrads obtained from diploids, there were two nonmating homothallic colonies and two heterothallic segregants. Ten heterothallic **a** mating segregants were examined to see if they had been "healed" to a normal *MAT***a** allele. In all ten cases, when these segregants were mated with a *MATα ho* strain, the resulting colonies were nonmating and able to sporulate, unlike diploids formed by mating *mat***a*** and *MATα ho* strains.

Further evidence of mating-type interconversion in these strains came from the fact that eight of the 28 tetrads analyzed exhibited 4:0 or 3:1 segregation of heterozygous nutritional markers characteristic of tetraploids. This result is similar to the consequences of mating-type switching in a *MAT***a**/*MAT***a** *HO*/*ho* diploid described by KLAR and FOGEL (1977). Some cells of a diploid homozygous for mating type can interconvert to a diploid homozygous for the opposite mating type. The mating of two such diploids of opposite mating type will then yield a tetraploid cell.

An equivalent experiment was used to demonstrate that *HO* also promotes the interconversion of *matα10* to a functional *MATα* allele. Because strains VC73 (*matα10*) can mate weakly with strains of either mating type, it was crossed with strain T1059-18C (*MAT***a** *HO hm***a** *HMα CMT*) to ensure that *MATα* information was provided only from strain VC73. Again the resulting diploid sporulated well, unlike heterothallic crosses with VC73. When tetrads were analyzed, there was again a mixture of those derived from diploids and those from tetraploids. Of 35 α mating segregants from 32 tetrads, none exhibited the very weak bisexual mating behavior of the *matα10* allele, and all produced sporulating diploids when crossed with a heterothallic *MAT***a** strain.

These experiments show that HO enables diploids constructed with either *mat***a*** or *matα10* to sporulate by interconverting the defective *mat* allele to a functional *MAT***a** or *MATα* gene. These results are in sharp contrast to those obtained with *cmt*, in which sporulating diploids are obtained without the healing of *mat***a** or *matα10*, as discussed below.

Interaction of cmt *and* mat**a***: When strain 17–15 (*mat***a***) was crossed with the α mating *cmt* strain JH117-9A (*MATα HM***a** *hmα cmt ho*), the resulting diploid (JH121) still had an α mating phenotype as expected for a *cmt*/+ heterozygote, since *cmt* is recessive. Nevertheless, this diploid was able to sporulate about 1 to 2%, unlike strains homozygous wild-type for *CMT*, the wild-type allele. It therefore seemed that the *cmt* mutation was recessive with respect to mating phenotype, but dominant or co-dominant for sporulation. However, when tetrads were dissected and analyzed, we found that the small percentage of cells able to sporulate had become homozygous for *cmt*. This point will be discussed in detail below.

In all 18 tetrads from diploid JH121, there were two nonmating and two α mating segregants. The α mating colonies could be divided into two classes after crossing them with a *MAT***a** *HM***a** *HMα CMT ho* strain and examining mating and sporulation of the resulting diploids. Of the α mating colonies analyzed in this manner, approximately one-half (8/13) yielded nonmating diploids that sporulated between 30 and 60%. The other five diploids exhibited strong **a** mating behavior and sporulated less than 1%. The first class of α mating colonies thus appears to be *bona fide MATα* segregants, whereas the second class still carries a mutant *mat* allele and are therefore most likely of genotype *mat***a*** *HM***a** *hmα cmt*. According to the hypothesis that *cmt* permits the expression of mating-type information at *HM***a** and *hmα* and that both *HM***a** and *hmα* contain α mating type information, this strain should have an α mating type even though it carries *mat***a***, because *mat***a*** is recessive to the other mating type expression. The absence of any **a** mating colonies among the segregants further suggests that all four segregants in each tetrad contain *cmt*. Thus, in any tetrad, segregants of genotype *MATα HM***a** *HMα cmt* or *mat***a*** *HM***a** *HMα cmt* must be nonmating.

The recessive *mat***a*** allele could be followed through a second generation, in which an α mating segregant of the second type (recessive, sporulation defective) JH121-3C was crossed with the *MAT***a** strain A226. The new diploid, JH123, again sporulated less than 1%, but asci could be dissected and analyzed. In these tetrads (Table 5), there were sometimes four nonmaters, or three nonmaters and one α, or two nonmaters and two α, as might be expected if all four segregants carried *cmt* and strains of genotype *mat***a*** *HM***a** *HMα cmt* or *MAT***a** *HM***a** *HMα cmt* or *MAT***a** *HM***a** *hmα cmt* were all nonmating, whereas a strain of genotype *mat***a*** *HM***a** *hmα cmt* was an α mater. We could demonstrate that the α mating segregants contained both a mutant *mat* allele and *cmt* by further test crosses. When the α maters were crossed with a *MAT***a** *HM***a** *HMα CMT* strain, the resulting diploids were **a** mating and able to sporulate about 1%.

TABLE 5

Segregation of mating phenotypes of diploid JH123 heterozygous for mat**a***/MATα *and* hmα/HMα *and carrying* cmt

Tetrad type	Number of tetrads
—/—/α/α	10
—/—/—/α	7
—/—/—/—	1

When the α maters were crossed with a *MAT***a** *hm***a** *HM*α *cmt* strain, the resulting diploids were nonmating and able to sporulate efficiently.

Thus, it is possible to obtain sporulating, nonmating cells in *cmt/cmt* diploids carrying the *mat***a*** allele. It is clear, however, that *cmt* does not act similarly to the homothallism gene, *HO*. With *HO*, the *mat***a*** allele can no longer be detected, but is converted to a functional *MAT***a** allele. The *cmt* mutation also permits a *mat***a***/*MAT*α strains to sporulate, but there is no apparent "healing" of *mat***a***.

The mating phenotype of *mat***a*** *cmt* strains clearly depends on the different alleles of *HM***a** and *HM*α. Thus, the haploid JH121-3C of genotype *mat***a*** *HM***a** *hm*α *cmt* is α mating, as expected if *mat***a*** is recessive to the α information expressed at *HM***a** and *hm*α. Other combinations of *mat***a*** and *HM* alleles were generated by crossing JH121-3C with strain JH112-1B (*MAT***a** *hm***a** *HM*α *cmt*). Tetrads from this new diploid (Table 6) include one case of 2**a**:2α mating colonies (the parental configuration) and three cases of four nonmaters, in which all four cmt segregants must express both **a** and α information. In these latter cases, two of the segregants probably contain *HM***a** and *HM*α, while the other two carry *hm***a** and *hm*α, as these two combinations of *HM* alleles appear to carry both **a** and α information (*cf.* Table 1). Thus, it is likely that a *mat***a*** *hm***a** *hm*α *cmt* strain is nonmating. The tetrads shown in Table 6 also contain **a** mating strains, some of which carry *MAT***a**, while others carry *mat***a***. The

TABLE 6

Mating phenotype segregation in tetrads of diploid JH121–3C $\times$ *JH112–1B**

Tetrad type	Number of tetrads
a/**a**/α/α	1
a/**a**/α/—	2
a/α/—/—	7
a/—/—/—	5
—/—/—/—	3

*This diploid has the partial genotype:

$$\frac{HM\mathbf{a}\ mat\mathbf{a}^*\ hm\alpha\ cmt}{hm\mathbf{a}\ MAT\mathbf{a}\ HM\alpha\ cmt}$$

*mat***a*** strains could be identified by crossing them with the *MAT*α *HM***a** *HM*α *CMT* tester strain, A12. The *mat***a***/*MAT*α diploids sporulate less than 1%, while the *MAT***a**/*MAT*α diploids sporulate about 50%. Among 16 **a** mating segregants, eight proved to be *mat***a*** *hm***a** *HM*α *cmt* by these criteria.

As a final demonstration that sporulation of *mat***a***/*mat***a*** diploids depends on the *cmt*-controlled expression of copies of mating-type information at the *HM* genes, we crossed the α mating *mat***a*** strain JH121-3C (*mat***a*** *HM***a** *hm*α *cmt*) with a *CMT mat***a*** strain (6D131-15A) carrying *HM***a** and *hm*α. This diploid, now homozygous for *HM***a**, *mat***a*** and *hm*α, contains only functional α information at *HM***a** and *hm*α, according to the cassette model of HICKS, STRATHERN and HERSKOWITZ (1977). In the absence of any functional copies of **a** information, this diploid would not be expected to sporulate, and indeed it does not. This is true when the diploid is heterozygous for *cmt* and has an **a** mating phenotype, and when it becomes homozygous for *cmt* and has an α phenotype. Again, from subclonal analysis, we find the frequency of *cmt* becoming homozygous (as evidenced by an α mating colony in about 1% (data not shown).

Interaction between cmt *and* matα10: An essentially similar series of experiments was performed with strain VC73, carrying the sporulation-defective allele, *matα10*. Because strain VC73 can mate weakly with both *MAT***a** and *MAT*α strains, it was mated with strains JH112-1B (*MAT***a** *hm***a** *HM*α *cmt*) and JH117-9A (*MAT*α *HM***a** *hm*α *cmt*). Both new diploids sporulated poorly—less than 1%. It should be noted that in the cross of VC73 with JH117-9A (*matα10/MAT*α) the *cmt* mutation enables a diploid with no *MAT***a** allele at all to sporulate, while in the case of VC73/JH112-1B (*matα10/MAT***a**), the mutation must supply *MAT*α functions. Again these results are consistent with the hypothesis that *cmt* allows the expression of normally silent copies of mating-type information. As we will show in more detail later, those cells that are able to sporulate have become homozygous for *cmt* and also are nonmating.

When asci from the VC73/JH117-9A diploid (*matα10/MAT*α *CMT/cmt*) were dissected, we obtained tetrads containing either nonmating or α mating colonies (Table 7A). Some of the α maters were apparently *MAT*α *HM***a** *hm*α *cmt* strains similar to the parental strain JH117-9A. However, other α mating segregants could be shown to contain both *cmt* and *matα10*. For example, when these latter α mating segregants were crossed with a *MAT***a** *HM***a** *HM*α *CMT* strain, the resulting diploids had a weak **a** mating phenotype and were nearly asporogenous, as expected if the *matα10* allele was present. Also, if these α mating colonies were crossed with a *MAT***a** *hm***a** *HM*α *cmt* strain, the resulting diploids were nonmating and sporulated efficiently, indicating that the α mating strain carried *cmt* and that the new diploid was homozygous for this mutation. In none of these tetrads from VC73/JH117-9A (Table 7A) was there a segregant that exhibited the original *matα10* mating phenotype. This is not unexpected if the diploids had become homozygous for *cmt*.

There were two cases in which all four segregants were α maters. Two of the four segregants in each case carried the recessive *mat* allele defective in sporu-

TABLE 7

A. *Segregation of mating phenotype in* matα10/MATα hmα/HMα *diploid VC73/JH 117–9A carrying* cmt

Tetrad type*	Number
$-/-/\alpha/\alpha$	4
$-/\alpha/\alpha/\alpha$	9
$\alpha/\alpha/\alpha/\alpha$	2

B. *Segregation of mating phenotype in* matα10/MAT**a** hm**a**/HM**a** *diploid VC73/JH 112–1B carrying* cmt

Tetrad type	Number
$-/-/-/$**a**	3
$-/-/$**a**/**a**	1
$-/-/$**a**/α	5
$-/$**a**/**a**/α	4
$-/-/$**a**/weak **a**	2
$-/-/\alpha$/weak **a**	1
$-/$**a**/α/weak **a**	4
$-/-/$weak **a**/weak **a**	1

*Nonmaters are designated by—.

lation functions, and these two segregants also carried *cmt*. If *cmt* is homozygous but the diploid is heterozygous *HMα/hmα*, it is surprising to find four α mating segregants from one meiosis, as two of the four colonies should have genotype *MATα HM***a** *HMα cmt* or *matα10 HM***a** *HMα cmt* and are expected to be nonmating. At the end of this section, we will present evidence that these "extra" α segregants arise from the presence of a modifier of *cmt* found in VC73, as well as in a related mutant strain VN33, and in the parental strain XT1172-S245C from which both were derived. The presence of yet another factor complicates the interpretation of these data. However, since the *matα10* strain VC73 could not be outcrossed by mating and sporulation without crossing it with a *cmt* strain, we could not obtain other *matα10* strains that might lack this factor. In the crosses described here, it seems clear that this factor does not diminish the major conclusion drawn from these studies: namely that *matα10/MAT***a** and *matα10/MATα* diploids are able to sporulate when *cmt* is homozygous. A second conclusion that *matα10 HM***a** *hmα cmt* strains are normal α mating, while *matα10 hm***a** *HMα cmt* are weakly **a** mating, is supported by the remaining crosses presented in this section.

When tetrads from the *matα10/MAT***a** *HM***a***/hm***a** *HMα/HM CMT/cmt* diploid VC73/JH112-1B were dissected (Table 7B), there were nonmaters and normal **a** maters, as well as very weak **a** maters and strong α maters. No segregants exhibited the weak bisexual phenotype of the parental *matα10 CMT*

strain, presumably because all segregants carried *cmt*. Both the weak **a** maters and the strong α maters could be shown to carry *mat*α*10*.

There were nine very weak **a** mating segregants among the VC73/JH112-1B tetrads analyzed (Table 7B). Four of these were crossed with the *MAT*α *HM***a** *HM*α *CMT* tester strain A12 to assess the mating and sporulation phenotype of the resulting diploids. In all four cases, the diploids were α mating and sporulated less than 1%, as expected if the weak **a** maters carried both *mat*α*10* and *cmt*. Based on the fact that this mating phenotype appears in tetrads from a diploid heterozygous for *hm***a**/*HM***a**, but not in one heterozygous for *hm*α/*HM*α (Table 7A), we conclude that these weak **a** mating colonies have the genotype *mat*α*10 hm***a** *HM*α *cmt*. The mating phenotype of these nine haploids is essentially identical to that found in *mat*α*10*/*MAT***a** *CMT*/*CMT* diploids. This weak **a** phenotype most likely arises from the expression of mating-type **a** information at both *hm***a** and *HM*α in the *mat*α*10 cmt* haploid.

As from the diploid VC73/JH117-9A (Table 7A), there were also unexpected α maters among segregants of VC73/JH112-1B (Table 7B). These α maters carry both *mat*α*10* and *cmt*, as determined by mating and sporulation tests with *MAT***a** *HM***a** *HM*α *CMT* and *MAT***a** *hm***a** *HM*α *cmt* testers: the resulting *mat*α*10*/*MAT***a** diploids were nearly asporogenous and weakly **a** mating when the *MAT***a** *CMT* parent was used, but nonmating and readily able to sporulate when the *MAT***a** *cmt* strain was used. Because JH112-1B is an **a** mating strain *MAT***a** *hm***a** *HM*α *cmt*, the only possible introduction of an *hm*α allele in VC73/JH112-1B segregants would have to come from strain VC73; however, there is evidence from the 2 mating:2 homothallic nonmating meiotic segregants of the diploid VC73/Y55-4, described above, that VC73 must also contribute the *HM*α allele. Again these results suggest the presence of another genetic factor that makes some *mat*α*10 HM***a** *HM*α *cmt* strains α mating instead of nonmating as expected. Some *mat*α*10 HM***a** *HM*α *cmt* strains are, indeed, nonmating. For example, among the tetrads from VC73/JH112-1B (Table 7B) are some containing two normal **a** mating and two nonmating colonies. Presumably the two **a** maters are *MAT***a** *hm***a** *HM*α *cmt*, so that the two nonmaters must be *mat*α*10 HM***a** *HM*α *cmt*. Again, these complications do not obscure the major fact that *cmt* suppresses the *mat*α*10* phenotype, depending on different combinations of *HM***a** and *HM*α alleles.

Another genetic element that interacts with cmt: The unexpected observation that *mat*α*10 cmt* segregants (presumably carrying *HM***a** and *HM*α) were α mating rather than nonmating led us to examine the interaction between *cmt* and another *MAT*α allele, *mat*α*1–5*, and with the normal *MAT*α strain (XT1172-S245C) from which both mutant strains VC73 (*mat*α*10*) and VN33 (*mat*α*1–5*) were derived (MacKay and Manney 1974).

The *mat*α*1–5* allele exhibits very weak α mating, but is normal in *mat*α*1–5*/*MAT***a** diploids in that they are nonmating and sporulate well. Strain VN33 (*mat*α*1–5 HM***a** *HM*α) was mated with strain JH112-1B (*MAT***a** *hm***a** *HM*α *cmt*) and the resulting diploid JH130 was sporulated and dissected (Table 8). Because *mat*α*1–5*/*MAT***a** strains are able to sporulate, cells that sporulated re-

TABLE 8

Segregation of mating phenotypes in diploid JH130 heterozygous for matα1–5/MAT**a**, hm**a**/HMα,*and* cmt/CMT

Tetrad type*	Number
a/**a**/–/–	2
a/**a**/α/–	5
a/α/–/–	1
a/α/weak α/–	1
a/weak α/–/–	2
weak α/weak α/–/–	1

*Nonmaters are designated by –. The weak α phenotype is that of the parental strain VN33, carrying the *matα1–5* (sterile) mutation.

mained heterozygous for *cmt*, as evidenced by the fact that there were no more than two nonmating segregants per tetrad. In some cases, there was only one nonmater, in part because segregants of genotypes *MAT***a** *hm***a** *HMα cmt* exhibit **a** mating. In addition to colonies that exhibited the parental *matα1–5* sterile phenotype, there was also some normal α mating segregants. As with *matα10*, one would not expect such α mating colonies if the *matα1–5* allele were still present; strains of genotype *matα1–5 HM***a** *HMα cmt* or *matα1–5 hm***a** *HMα cmt* would all be expected to be nonmaters, based on the results shown in Tables 3 and 4 above. As in the case of the α mating segregants from diploid VC73/JH112-1B (Table 7B), we can deduce that not all *matα1–5 HM***a** *HMα cmt* strains are α mating; indeed, some must be nonmating, as evidenced by the nonmating segregants in tetrads containing two **a** mating colonies. The α mating colonies might result from the interaction of *matα1–5* and *cmt* with another genetic factor found in strain VN33.

When the parental strain XT1172-S245C was crossed with the *MAT***a** *hm***a** *HMα cmt* strain JH112-1B, again the pattern of mating among the tetrads indicates that some *MATα HM***a** *HMα cmt* strains are α mating instead of nonmating. Among the presumed *MATα* segregants in 28 tetrads examined, there were 38 α maters and 18 nonmaters, as opposed to the 28 α maters and 28 nonmaters expected if *MATα HM***a** *HMα cmt* strains were invariably nonmating. Thus, there again seemed to be a genetic element present that restored approximately half of the *MATα HM***a** *HMα cmt* strains to α mating. There was no apparent effect on *MAT***a** *HM***a** *HMα cmt* strains, as the expected number of *MAT***a** *HM***a** *HMα cmt* nonmaters and *MAT***a** *hm***a** *HMα cmt* **a** maters was found among the segregants.

These data support our conclusion that, in all of the crosses involving strains VC73, VN33 and XT1172-S245C, there was a set of α mating segregants that could be explained only in terms of a modifier of *cmt*, specific for *MATα* alleles.

The unexpected α maters were found when these strains were crossed with both *MAT***a** *hm***a** *HMα cmt* strain JH112-1B and *MATα HM***a** *hmα cmt* strain JH117-9A. When either of the two *cmt* strains was crossed to unrelated *MATα*

or *MAT***a** stains, there was no evidence of any such genetic modifier. For example, when strain JH112-1B (*MAT***a** *hm***a** *HM*α *cmt*) was crossed with BW105-9C (*MAT*α *hm***a** *HM*α *CMT*), there were eight α maters and ten nonmaters among the nine tetrads examined. As expected in this case where the diploid was homozygous for *hm***a**, all of the *MAT***a** segregants (*MAT***a** *hm***a** *HM*α) were **a** mating, whether *cmt* was present or not. Thus, we conclude that the modifier of *cmt* in crosses involving derivatives of *MAT*α strain XT1172-S245C must come from that strain.

cmt *has a tendency to become homozygous*: Strains initially heterozygous for *cmt* become homozygous at a high frequency. For example, an α mating segregant JH 121-3C (Table 5) of genotype *mat***a*** *HM***a** *hm*α *cmt ade2 leu1 lys1* was mated with strain A226 (*MAT***a** *HM***a** *HM*α *CMT ade2 leu2 tyr1 met13*). The resulting adenine-requiring diploid was strongly **a** mating, confirming that *cmt* was heterozygous in the *mat***a***/*MAT***a** diploid. About 1% of the cells sporulated. The colony was then subcloned and tested for mating and sporulation. Twelve of 120 colonies proved to be nonmating. In all 12 cases, more than 40% of the cells sporulated. The simultaneous change in both the mating and sporulation phenotypes indicates that *cmt* became homozygous in those cells able to sporulate.

Very similar results were obtained after subcloning an **a** mating colony heterozygous for *cmt* and of mating genotype *mat*α*10/MAT***a**. Four out of 98 colonies became nonmating and able to sporulate efficiently. These results confirm the conclusion drawn from tetrad analysis of *mat***a***/*MAT*α and *mat*α*10/MAT* diploids (*cf.* Table 5, 6, and 7) that a small proportion of cells originally heterozygous for *cmt* become homozygous and able to sporulate.

Interaction between cmt *and* HO: Homothallic mating-type interconversion apparently depends on the expression of a single mating phenotype, as nonmating *MAT***a***/MAT*α cells do not exhibit mating-type switching; whereas *MAT***a***/MAT***a** and *MAT*α*/MAT*α cells do show interconversion. On the other hand, homothallic switching also occurs in a haploid strain carrying the *mat*α*1–5* (sterile) allele that has a very weak α mating phenotype (HICKS and HERSKOWITZ 1977). It was therefore interesting to see how *HO* would act in a nonmating *cmt* strain.

Meiotic segregants carrying both *cmt* and *HO* were obtained by crossing a weakly α mating *MAT*α *HM***a** *HM*α *cmt* strain JH112-7A with spores of the homothallic strain, Y55-4. Some of the tetrads examined contained two heterothallic colonies that mated normally and two nonmating colonies that presumably contained both *HO* and *cmt* (Table 9). The sporulation ability of these *HO cmt* segregants was extremely variable, ranging from less than 1% to more than 50%. This variability is apparently due to the presence of the *cmt* allele, as meiotic segregants containing only HO invariably sporulated better than 40%.

Several poorly sporulating *HO cmt* segregants were subcloned, and each colony was then tested for sporulation. As seen in Table 9B, the variability in sporulation is also evident among the subclones. In some cases, the colonies remain nearly asporogenous, while in others sporulation reaches 40 to 60%.

TABLE 9

Variation in sporulation among cmt HO *strains*

A. Sporulation of meiotic segregants from the *cmt HO* strain 7A/55–14C.*

Segregant	Percent sporulation
1A	<1
1B	<1
1C	<1
1D	<1
2A	<1
2B	<1
2C	<1
2D	<1
3A	30
3B	<1
3C	<1
3D	<1
4A	2
4B	<1
4C	<1
4D	<3

B. Sporulation of different subclones of the *cmt HO* strain 7A/55–14C–4A†.

Strain	Number of colonies	Percent sporulation
7A/55–14C–4A	35	<1
	12	1–5
	11	5–20
	5	20–40
	3	40–80

*Strain 7A/55–14C was derived from a haploid carrying both *cmt* and *HO*. This colony sporulated about 30%.

†Sixty-six subcloned colonies of a *cmt HO* colony that sporulated about 2% (see part A) were analyzed for their ability to sporulate.

Although all of the *HO cmt* segregants and their subclones were essentially nonmating, those that sporulated very little did exhibit infrequent, but significant, mating with *MAT***a** or *MAT*α tester strains. From two *HO cmt* colonies, such matings with heterothallic haploids were purified, sporulated and dissected. In the four cases examined, all of the nutritional markers segregated 2:2, demonstrating that the *HO cmt* parent must have been haploid.

From these observations it seems that *HO cmt* strains have a variegated phenotype. In some colonies the majority of the cells have become diploid, and able to sporulate; in others the majority of the cells have remained haploid. Thus, in some colonies the *cmt* allele has prevented self-diploidization, either by blocking

the ability of *HO* to interconvert cells to opposite mating types or by preventing mating of these cells. However, the fact that matings with both *MAT***a** and *MAT*α strains could be uncovered suggests that *HO cmt* strains are able to switch from one mating type to the other, even though all of the haploids remain nearly nonmating.

DISCUSSION

The *cmt* mutation apparently acts by permitting the expression of normally silent copies of mating-type information found in a haploid strain of *S. cerevisiae*. In haploid strains carrying the commonly found alleles *HM***a** and *HM*α, the *cmt* mutation causes both *MAT***a** and *MAT*α cells to become nonmating, but without altering or interconverting the *MAT* alleles. The most direct interpretation of these data is that the *cmt* mutation permits the simultaneous expression of **a** information (located at or controlled by *HM*α) and α information (at or controlled by *HM***a**), and that the expression of both mating-type alleles causes the cell to become nonmating, as it is in *MAT***a**/*MAT*α diploids. In order for a cell to become nonmating, it must have at least one copy of **a** and α information, expressed, but the number of copies of each type do not have to be equal, *e.g.*, *MAT***a**/*MAT*α/*MAT*α/*MAT*α is still nonmating (GUNGE and NAKATOMI 1972). If strains are constructed with other combinations of *HM***a** and *HM*α alleles, as illustrated in Table 1, a *cmt* strain may not be nonmating, but rather expresses a single mating type. All of the data we have presented are entirely consistent with the cassette model of HICKS, STRATHERN and HERSKOWITZ (1977) that *HM***a** and *hm*α contain normally silent copies of α mating-type information, while *HM*α and *hm***a** code for **a** information. From the fact that *MAT***a** *HM***a** *hm*α *cmt* is nonmating, whereas *MAT*α *HM***a** *hm*α *cmt* is α mating, we also conclude that the *MAT* allele continues to be expressed.

The relation between *cmt* and the *HM* genes is especially apparent in strains carrying recessive *mat* alleles (Table 10). For example, when *cmt* is absent, *mat***a*** strains have an **a** mating type; but when *cmt* is present, *mat***a*** strains may be either nonmating, **a** mating, or α mating, depending on the combination of *HM* alleles. In these strains, the *mat***a*** allele is apparently completely recessive to other mating-type information that is expressed. The results with both *mat***a*** and *mat*α*10* reinforce the notion that silent copies of mating-type information are located at, or are controlled by, *HM***a** and *HM*α. The fact that both *mat***a*** *HM***a** *HM*α and *mat***a*** *hm***a** *hm*α are nonmating further emphasizes the equivalence of *HM***a** to *hm*α and of *HM*α to *hm***a**.

Not only can the mating phenotype be altered by *cmt* and alleles of *HM***a** and *HM*α, but defects in the regulation of sporulation in *mat***a*** or *mat*α*10* can also be overcome. In the course of this work, we have constructed diploids of of various genotypes: *mat***a***/*MAT*α; *mat***a***/*MAT***a**; *mat*α*10*/*MAT*α; and *mat*α*10*/*MAT***a**. All of these diploids fail to sporulate without *cmt*, and all sporulate efficiently when *cmt* is homozygous. Thus, the *cmt* mutation can supply either **a** or α sporulation functions, most probably by allowing the expression

TABLE 10

Mating phenotype of defective MAT *alleles with different combinations of* HM**a** *and* HMα *alleles*

Alleles*			Mating phenotype	
			Without *cmt*	With *cmt*
*HM***a**	*mat***a***	*HM*α	**a**	nonmating
(*α*)	**a***	(**a**)		
*HM***a**	*mat***a***	*hm*α	**a**	α
(*α*)	**a***	(*α*)		
*hm***a**	*mat***a***	*HM*α	**a**	**a**
(**a**)	**a***	(**a**)		
*hm***a**	*mat***a***	*hm*α	**a**	nonmating
(**a**)	**a***	(*α*)		
*HM***a**	*mat*α10	*HM*α	weak bisexual	nonmating or α
(*α*)	α10	(**a**)		
*HM***a**	*mat*α10	*hm*α	weak bisexual	α
(*α*)	α10	(*α*)		
*hm***a**	*mat*α10	*HM*α	weak bisexual	weak **a**
(**a**)	α10	(**a**)		
*HM***a**	*MAT*α1-5	*HM*α	weak α	nonmating or α
(*α*)	α1-5	(**a**)		

*Genotypes of different arrangements of *HM***a**, *MAT* and *HM*α alleles on chromosome 3 are shown. Below the line are indicated the mating-type information presumed to be at each locus, based on the proposal of HICKS, STRATHERN and HERSKOWITZ (1977). The mating-type information in parentheses represents the fact that this information is normally not expressed.

of that information from the *HM* alleles, as well as from *MAT*. It is even possible to construct diploids that have no functional allele at *MAT*, but are still able to sporulate efficiently—for example *mat***a***/*mat***a*** *HM***a**/*hm***a** *hm*α/*HM*α *cmt*/*cmt* (Table 6). In all of the diploids, there is no "healing" of the mutant *mat* alleles.

The apparent expression of mating and sporulation information from *HM***a** or *HM*α in *cmt* strains substantially extends and supports the evidence on which HICKS, STRATHERN and HERSKOWITZ (1977) based their model. Previous evidence had been based on the ability of homothallic strains to interconvert mating type, a process totally dependent on the combination of *HM* alleles (HARASHIMA, NOGI and OSHIMA 1974). Now we can conclude that the *HM***a** and *HM*α loci must contain normally silent copies of mating-type information that can be

either (1) transposed to *MAT* in order to be expressed or (2) expressed in place through the action of *cmt*. The same combinations of alleles of *HM***a** and *HMα* that govern homothallic mating-type switching also govern the expression of mating type controlled by *cmt*. Thus, a *mat***a*** *hm***a** *hmα cmt* strain is equivalent to a *mat***a*** *HM***a** *HMα cmt* strain. The equivalence of *HM***a** and *hmα* and of *HMα* and *hm***a** shown for homothallic switching of *MAT* alleles (KLAR and FOGEL 1977; HARASHIMA and OSHIMA 1978) also seems to be true for the expression of normally silent copies of mating type.

How does cmt *act?* We must assume that wild-type cells have the capacity to regulate the expression of mating-type information at *HM***a** and *HMα*; but the regulation clearly differs from that found at *MAT*. Generally, mating-type information at *MAT* is expressed (although we do not know whether the lack of mating in a *MAT***a**/*MATα* diploid results from the mutual repression of both *MAT***a** and *MATα* alleles). On the other hand, mating-type information at *HM***a** or *HMα* is normally unexpressed. Thus, it seems that the wild-type *CMT* gene may participate in repressing expression of *HM***a** and *HMα* and that *cmt* represents the absence of this repressor. What function the *CMT* gene might play during the life cycle of *S. cerevisiae* remains obscure. There is no obvious difference in growth, sporulation or spore viability in *MAT***a**/*MATα* diploids with or without *cmt*. The *CMT* gene is not closely linked to any of the other genes in the mating system. From the segregation of mating types in diploids heterozygous for *MAT***a**/*MATα cmt*/*CMT* and either *hm***a**/*HM***a** or *hmα*/*HMα*, we also confirmed that *cmt* is not closely linked to *MAT*, *HM***a** or *HMα*. Furthermore, *cmt* segregates independently from *HO* (HOPPER and HALL 1975b).

The nonmating character of haploids caused by *cmt* is not as absolute as that found in *MAT***a**/*MATα* diploids. Our study of the interaction of *cmt* and *HO* (Table 8) showed that some cells carrying *cmt* are nevertheless able to interconvert mating type and diploidize, whereas *MAT***a**/*MATα* diploids do not exhibit any mating-type interconversion. Furthermore, occasional matings between cells of genotype *HM***a** *MATα HMα cmt* apparently occur, because *cmt* haploids give rise to a few diploid cells able to sporulate (HOPPER and HALL 1975b).

We should point out that the capacity of *cmt* strains to diploidize seems restricted to the genetic background of strain D135a in which the mutation was found, as none of the *cmt* strains we derived exhibit this phenotype.

One unusual property of the *cmt* mutation is that it readily becomes homozygous in strains that carry defective alleles of *MAT*. As measured by the simultaneous acquisition of the ability to sporulate and loss of the ability to mate in a *mat***a***/*MATα* or *matα10*/*MAT***a** diploid, the frequency of homozygosis is sometimes greater than 1% of the cells in an initially heterozygous colony. No other heterozygous markers in these strains has been found to become homozygous at a detectable frequency (less than 0.01%). It is possible that a diploid cell carrying defective *mat* alleles is at a selective disadvantage even during growth, but that expression of functional copies of mating-type information at *HM***a** and

HMα can rectify the problem. It is also possible that *cmt* will become homozygous even in *MAT***a**/*MATα* strains but, among the relatively small number of tetrads we have analyzed so far, there is no evidence that an initially heterozygous strain has become homozygous for *cmt*.

CMT *is not alone*: The *CMT* locus is not the only gene involved in maintaining the silence of *HM***a** and *HMα*. KLAR, FOGEL and MACLEOD (1979) found an entirely different recessive mutation (*mar1*) that has essentially the same properties as *cmt* in causing haploid strains carrying *HM***a** and *HMα* to become nonmating, whether carrying *MAT***a**, *MATα* or *mat***a***. The *MAR1* locus is centromere linked, about 20 cM from *trp1*, whereas *cmt* is not linked to this marker. However, both mutations appear to have the same phenotype. It may be that the repression of *HM***a** and *HMα* involves at least two essential components, represented by the *CMT* and *MAR1* genes.

By virtue of expressing mating-type information at *HM***a** and *HMα*, the *cmt* and *mar1* mutations can compensate for defects in the sporulation functions of the *mat***a*** and *matα10* alleles. There are still other mutations that can suppress the sporulation defects of the *mat* mutations. These include the *csp* and *rme* mutations (HOPPER and HALL, 1975a; KASSIR and SIMCHEN 1976) and the *ssp515* mutation (HERSKOWITZ, *et al.* 1977). The *csp* mutations have no effect on mating phenotype, while permitting diploids homozygous for *MAT***a** or *MATα* to sporulate. The *ssp515* mutation does have some effect on mating phenotype, but it is clearly different from *cmt* or *mar1*. *ssp515* suppresses the sterile phenotype of *matα10 HM***a** *HMα* to a normal *α* mating phenotype, but does not cause diploids homozygous for *MAT***a** or *MATα* to become nonmating. Nevertheless, *ssp515* depends on the presence of the *HM***a** allele to suppress *matα1–5*. The specific suppression of the sterile phenotype of *MATα* mutations may have some relation to our finding that some *cmt matα10* and *cmt matα1* strains carrying *HM***a** and *HMα* are *α* mating instead of nonmating.

The existence of factors that modify the nonmating *cmt* phenotype in *MATα* *HM***a** *HMα* cells may be common, as the original strain isolated by HOPPER and HALL (1975b) also shows weak *α* mating. One possibility is that these strains differ from those that have a complete nonmating phenotype by allelic differences at the *HMα* locus. If *cmt* fails to turn on *HMα*—the only copy of **a** information in a *MATα HM***a** *HMα cmt* strain—such a cell would express only *α* information at *MATα* and *HM***a**.

We began to study the *cmt* mutation after AMAR KLAR communicated to us his characterization of the *mar1* mutation, which has a phenotype apparently identical to *cmt*. We are extremely grateful to A. HOPPER, V. MACKAY, F. SIMCHEN, Y. OSHIMA and H. GRUENSPAN for providing some of the strains used in this work. ANITA HOPPER has contributed not only her mutation, but also her advice and criticisms of this manuscript. BARBARA WEIFFENBACH helped construct several of the strains. We are also grateful to JOHN MCCUSKER, DEBORAH MASCIOLI, NANCY PEARSON and ELLEN KRAIG for their comments. This work was supported by United States Public Health Service grant GM20056.

Note added in proof: The mutation *SSP515* described by HERSKOWITZ *et al.* (1977) has now been redesignated *sir1* by RINE, STRATHERN, HICKS and HERSKOWITZ (Genetics, in press).

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Corresponding editor: R. E. ESPOSITO