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Genetics

Control of yeast cell types by mobile genes: A test

(cell type differentiation/cassette model/eukaryotic gene control/gene rearrangement/diploidization)

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ABSTRACT **The yeast *Saccharomyces cerevisiae* changes cell types by switching the alleles of the mating type locus (*MAT*) from a to α and vice versa. In the cassette model, these switches—e.g., from a to α—occur when a replica of silent α information (an α "cassette") replaces the resident a cassette at the mating type locus and is thereby expressed. We have identified a mutation in the locus postulated to be the silent α information (*HMLα*) and find that a mutation is introduced into the mating type locus as a result of interconversion: *HMLα*⁻ *MATα* cells switch to *MAT*a and then to *MATα*⁻. The *MATα*⁻ mutation leads to defective mating and behaves like some previously identified *MATα*⁻ mutations. These observations satisfy the prediction of the cassette and controlling element models that genetic information is transmitted from *HMLα* to the mating type locus.**

Cell type in yeast is controlled by the mating type locus (*MAT*), on the right arm of chromosome III, which has two alleles, a and α (Fig. 1; see ref. 1). Cells with the α allele have 'α' mating type and can mate efficiently (i.e., form zygotes) with 'a' cells and vice versa, whereas cells of like mating type do not mate. The a/α diploid formed upon mating is a third cell type, which does not mate but which can be induced to undergo meiosis, yielding a tetrad of two a and two α spores. Analysis of mutations at the mating type locus suggests that the α and a alleles may be nonhomologous blocs (2, 3) coding for regulatory genes that control other genes more directly involved in mating and sporulation (2).

The mating type of homothallic strains, which carry *HO* and appropriate accessory genes (4, 5), is unstable; switches between the a and α alleles occur as often as every cell division (6). Homothallic cells thus give rise to colonies containing stable a/α diploids formed by matings between siblings, a process termed "diploidization" (7). In strains with *ho* ("heterothallic" strains), mating type is stable, although rare switches occur at a frequency of approximately 10^{-6} (8–10). A remarkable property of mating type interconversion is that cells unable to mate because of a mutant α allele at *MAT* switch efficiently to a and subsequently to functional *MATα* (10). The *MATα*⁻ mutation is thus "healed" by the interconversion process. Other mutations that prevent mating but that are not at the mating type locus are not healed (10).

To explain healing of *MAT* mutations and other aspects of mating type interconversion, it has been proposed in the cassette model (10, 11) that yeast cells contain two cryptic loci, one with silent α information and one with silent a information, in addition to the *MAT* locus where mating information is expressed (e.g., because it is adjacent to an active promoter site; see Fig. 1). Switching, for example from a to α, occurs when a replica of silent α information (an α "cassette") replaces the resident a cassette at the *MAT* locus where α is then expressed. The *HO* gene codes for or controls the enzymatic machinery for this process. The silent α information in *Saccharomyces cerevisiae* is proposed to be at the previously identified *HMLα* locus, on the left arm of chromosome III, which is required for the a to α switch (see Fig. 1) (1, 5). Likewise, the *HMR*a locus, on the right arm of chromosome III, which is required for the α to a switch, is postulated to be the silent a information. Thus *HMLα* and *HMR*a are, according to the cassette model, the ultimate sources of information expressed at the mating type locus. Oshima and Takano (5, 12, 13) have independently proposed a model of interconversion in which *HMLα* and *HMR*a produce controlling elements that associate with an affinity site at the mating type locus, thereby composing the α and a mating type alleles, respectively.

How can the cassette model be tested? A central feature of this hypothesis is that genetic information is transmitted from *HMLα* and *HMR*a to the mating type locus. Thus, healing of mutations at the mating type locus should be a general phenomenon. Indeed, mutations both of *MAT*a and in the two known complementation groups of *MATα* (*MATα1* and *MATα2*) are healed by interconversion (10, 14, 15). Similarly, mutations of *HMLα* and *HMR*a should be moved to the mating type locus. We describe here the isolation of a mutant of *HMLα* and find that this prediction is satisfied—*HMLα*⁻ *MATα* cells switch to *MAT*a, and these *MAT*a cells switch to *MATα*⁻.

MATERIALS AND METHODS

Strains are laboratory stocks except for strain 277 (*HO MAT*a *HML*a *HMR*a, T-1059-18B in ref. 1), kindly provided by Y. Oshima (Osaka University, Osaka, Japan).

Media and genetic methods are described in ref. 9. Mating type was scored by complementation assay or by microscopic observation of the response of cells to the pheromone, α-factor, which causes growth arrest and morphological change in *MAT*a cells but not in *MATα*, *MATα1-5*, or *MAT*a/*MATα* cells (2, 16). In the isolation of *HMLα* mutants, production of 'α' cells from segregants of *MAT*a/*MATα1-5 HO/ho* diploids was detected by replica plating sporulated colonies of the diploids onto a cell lawns, in which case 'α' cells in the spore clones mate (form prototrophs) and 'α⁻' cells do not. *HO* was scored in colonies by testing for mating proficiency (weak, variable mating with both mating type testers) and ability to sporulate, as in ref. 9. In some cases, the presence of *HO* was scored in 'a' segregants by mating to *ho MATα2-1 sir1-1* (strain XJ104-25a; ref. 17). The *sir1-1* mutation allows efficient mating by *MATα2-1* mutants (17, 18). Presence of *HO* was indicated by the ability of the diploid to switch to *MAT*a/*MATα* and thus sporulate (14).

Nomenclature is that adopted by the Ninth International Conference on Yeast Genetics and Molecular Biology, Rochester, NY, 1978, and corresponds to that in earlier work as follows: *HMLα* = *HM*a; *HMR*a = *HMα*; *HML*a = *hm*a; *HMRα* = *hmα*.

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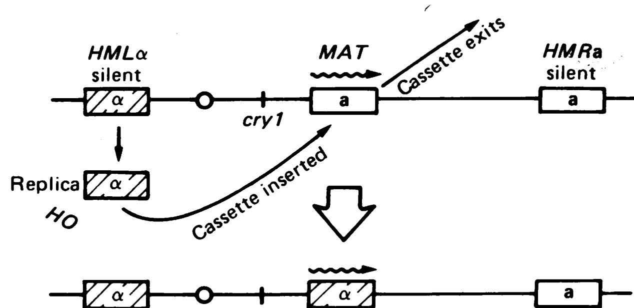

FIG. 1. The cassette model of mating type interconversion. Chromosome III, not drawn to scale, is depicted. α and **a** denote blocs of regulatory information (cassettes) that determine cell type. Wavy arrow indicates that the bloc of information at the *MAT* locus is expressed; those at *HMLα* and *HMR***a** are not. A *MAT***a** cell (top line) switches to *MATα* (bottom line) when a replica of silent α information at *HMLα* replaces the resident cassette at *MAT*.

RESULTS

Isolation of a Putative *HMLα* Mutant. The *HMLα* gene was identified from analysis of the natural variant allele, *HML***a**, introduced into *S. cerevisiae* from *Saccharomyces oviformis* (19). Strains that are *HO HML***a** *HMR***a** switch from α to **a** but not from **a** to α. Consequently, *HO HML***a** *HMR***a** *MAT***a** spores give rise to colonies that mate as '**a**', whereas *HO HMLα HMR***a** *MAT***a** spores give rise to colonies containing **a**/α diploids. The *HML***a** allele is not simply an inactive *HMLα* allele, because it has been shown that *HML***a** is functionally equivalent to *HMR***a** (20, 21). According to the cassette model, *HML***a** is silent **a** information where silent α information, the *HMLα* allele, is ordinarily found. We have utilized the *HML***a** allele in our isolation scheme for a mutation within *HMLα*. The parental strain from which the mutant was derived (strain 251; XJ46-10d in ref. 18) is *ho HMLα HMR***a** and carries the *MATα1-5* mutation, which reduces mating ability 1:10^5 (2). Diploids formed with a strain of genotype *HO HML***a** *HMR***a** *MAT***a** (by selective methods) segregate *HO HMLα* spores, which give rise to cells with a functional 'α' mating type. In contrast, it is expected that a mutant defective in *HMLα* would form a diploid with the *HO HML***a** *HMR***a** *MAT***a** strain that should segregate only *HMLα*$^-$ or *HML***a** spores and which would thus not give rise to cells with 'α' mating ability. Among 14,000 colonies grown from an ethyl methanesulfonate-treated culture of strain 251, one strain (E66) exhibited the mutant phenotype and was studied further.

dip66* Interferes with the Switch from a to α.** If the mutation in E66, here called *dip66*, is indeed in *HMLα*, then it should interfere with switching from **a** to functional α but not with switching from α to **a**. To determine the effect of *dip66* on switching, E66 (*ho cry1-3 MATα1-5 dip66*) was rare mated by prototroph selection to spores of strain X10-1B (*HO/HO HMLα CRY1 MATa** *HMR***a**/*HMLα CRY1 MATα HMR***a**) to form diploid strain H1. As expected for a diploid containing both *HO* and a mutation interfering with the switch from **a** to functional α, diploid H1 yielded meiotic segregants that grew into colonies exhibiting an '**a**' mating phenotype and that failed to diploidize despite carrying *HO*. One such segregant (H1-19B) bearing *HO, dip66*, and *cry1-3* (see below) was chosen for analysis and was backcrossed to strain X10-1B by cell-to-spore mating. This diploid (H2, Table 1) is homozygous for *HO* and heterozygous for *dip66* and for the *cry1-3* mutation, which confers resistance to the drug cryptopleurine and is tightly linked to the mating type locus (approximately 4 centimorgans; ref. 22). Because *cry1-3* and *MAT***a** are coupled in this diploid, it is possible to infer from the segregation of cryptopleurine resistance (CryR) whether a spore was initially *MAT***a** or *MATα*. Of the segregants from H2, approximately half of the CryR spores (most of which were initially *MAT***a**) were unable to diploidize, whereas almost all of the CryS spores (most of which were initially *MATα*) diploidized normally (Table 1). (The two CryS segregants that did not diploidize may be *CRY1 MAT***a** recombinants.) These results indicate that strain H2 is heterozygous for a mutation, presumably *dip66*, that blocks switching from *MAT***a** to functional *MATα* but does not block switching from *MATα* to *MAT***a**.

A diploid such as H2 should yield *HO dip66 HMR***a** *MATα* spores, which can diploidize to form strains of genotype *HO/HO dip66/dip66 HMR***a**/*HMR***a** *MAT***a**/*MATα*. Such diploids were identified as segregants from tetrads of H2 in which all four spores had diploidized. These diploids should give a distinctive segregation pattern in which the *MATα* but not the *MAT***a** spores are able to diploidize. Several such segregants were found, and data for one, H2-22c, are shown in Table 1 (diploid H3). The initial mating type of spores from H3 was inferred from the response of the spores to α-factor. Twenty-

Table 1. Genetic analysis of the *dip66* mutation

Diploid	Genotype	Phenotype of spore clones			
		'**a**'	**a**/α	'**a**'	**a**/α
		CryR		CryS	
H2	*HO dip66 cry1 MAT***a** *HMR***a** *HO HMLα CRY1 MATα HMR***a**	25	27	2	50
		α-Factor resistant		α-Factor sensitive	
H3	*HO dip66 MAT***a** *HMR***a** *HO dip66 MATα HMR***a**	1	28	29	0
		CryR		CryS	
H4	*HO dip66 cry1 MAT***a** *HMR***a** *HO HML***a** *CRY1 MATα HMR***a**	43	1	2	42

H2, H1-19b × X10-1B; H3, H2-22c (a segregant from H2); H4, H1-19b × XHB71-19c. All diploids were formed by cell-to-cell or cell-to-spore matings. Data are from four-spored tetrads except for H3, which includes 10 three-spored tetrads. The *HMR***a** allele in XHB71-19c was ultimately derived from strain 277. Clones grown from spores were analyzed for cryptopleurine resistance (CryR, *cry1-3*) or sensitivity (CryS, *CRY1*) and for mating and sporulation behavior ('**a**'—diploidization-deficient, mates as '**a**' and sporulates weakly or not at all; **a**/α—diploidization-proficient, sporulates, mates weakly). For analysis of H3, spores were tested for response to α-factor and were then removed from α-factor and grown into colonies.

eight of 29 spores which were initially *MAT*α (insensitive to α-factor) diploidized, whereas all 29 of the *MAT***a** spores (sensitive to α-factor) failed to diploidize and grew into colonies giving '**a**' mating behavior. We conclude from this segregation pattern that *dip66* blocks the switch from *MAT***a** to functional *MAT*α but does not affect the switch from *MAT*α to *MAT***a**. The exceptional α segregant might be a colony in which the α cells died soon after germination—e.g., at the two-cell stage.

dip66* Maps in or near *HML*α.** The segregation pattern of H2 (Table 1) indicates that *dip66* is unlinked to the mating type locus because only half of the CryR spores (most of which are *MATa**) show the mutant phenotype. To determine whether *dip66* maps to the *HML*α locus, H1-19B (*HO dip66 HMR***a** *cry1-3 MAT***a**) was crossed to an *HO HML***a** *HMR***a** *CRY1 MAT*α spore from strain XHB71-19c (H4, Table 1). If *dip66* is in *HML*α, then H4 should fail to segregate $HML\alpha^+$ recombinants. As in the case of H3, the α but not the **a** segregants should diploidize. All 22 tetrads from H4 showed two diploidization-proficient and two diploidization-deficient segregants in each tetrad. From the coupling of *MAT***a** to *cry1-3*, it can be inferred that spores initially *MAT***a** were unable to diploidize, whereas spores initially *MAT*α were able to diploidize. These results indicate that $HML\alpha^+$ recombinants were not obtained and hence that *dip66* is in or near *HML*α.

The results in this and the preceding section indicate that the *dip66* mutation (hereafter called *HML*α*-66*) is a lesion in the *HML*α locus.

Switching of *HO HML*α*-66* Cells to 'a**' and 'α^-'.** What is the nature of the *HML*α*-66* genetic alteration, and how does it disrupt diploidization? *HML*α*-66* might be a "mutation" to *HML***a**, as has been reported by Blair *et al.* (23) and Kozhina (24), or it might be a mutation of the *HML*α information itself. If *HML*α*-66* is a mutation within *HML*α and, as proposed in the cassette model, *HML*α is a source of α genetic information, then *MAT***a** *HO HML*α*-66 HMR***a** cells should interconvert between mating-deficient $MAT\alpha^-$ cells and mating-proficient *MAT***a** cells. If *HML*α*-66* is defective in a function of *HML*α necessary for switching from **a** to α—for example, in an enzyme required for this process—or if *HML*α*-66* is a change to *HML***a**, then *MAT***a** *HO HML*α*-66 HMR***a** cells should simply be "stuck" as *MAT***a**. To distinguish between these possibilities we have analyzed the pattern of cell type changes in the progeny of single *MAT***a** and *MAT*α *HO HML*α*-66 HMR***a** spores by pedigree analysis (see Fig. 2), in which mother and daughter cells are separated by micromanipulation in the presence of α-factor. Strain H3 (*HO/HO HML*α*-66/HML*α*-66 MAT***a**/*MAT*α *HMR***a**/*HMR***a**) was the source of *MAT***a** and *MAT*α spores bearing *HO* and *HML*α*-66*.

(*i*) *Switching by* HO HMLα-66 MATa HMRa. In each of 10 cases, *HO HML*α*-66 MAT***a** spores gave rise to α-factor-resistant cells within two or three cell divisions (Fig. 2 *left*). The same kind of behavior is seen for $HO\ HML\alpha^+$ *MAT***a** spores

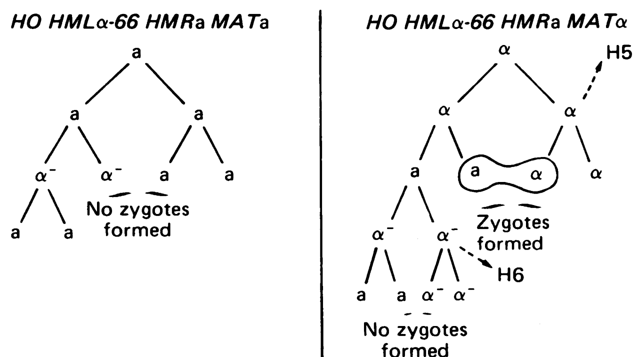

FIG. 2. Lineage of *HO HML*α*-66 HMR***a** spores. (*Left*) First few cell divisions of **a** spores. (*Right*) First few cell divisions of α spores. **a** cells were distinguished from α cells by their response to α-factor. α^- cells do not form zygotes. H5 is a diploid between the indicated cell and a *MAT***a** *ho* cell. H6 is a rare diploid between the indicated cell or one of its α^- progeny and the same *MAT***a** *ho* strain (segregation analyzed in Table 2).

(6, 9). However, the *HML*α*-66* cells grew into colonies that gave an '**a**' mating phenotype and were composed of approximately equal numbers of α-factor-resistant and α-factor-sensitive cells, whereas $HML\alpha^+$ cells grow into colonies containing **a**/α diploids formed by mating between '**a**' and 'α' siblings. A separate analysis of 29 *HO HML*α*-66 MAT***a** spores showed that none grew into colonies containing zygotes when examined after 3 or 4 and 8–10 generations after germination and that each of six colonies examined contained both α-factor-resistant and α-factor-sensitive cells. *HO HML*α*-66 MAT***a** cells thus are able to switch mating types; they switch to α-factor resistance, but they do not acquire the ability to mate as 'α'. These results are inconsistent with *HML*α*-66* being defective in switching *per se*. Because there exist known mutations of *MAT*α that have a nonmating, α-factor-resistant phenotype (2), these results suggest that *HO HML*α*-66 MAT***a** cells switch to $MAT\alpha^-$.

(*ii*) *Switching by* HO HMLα-66 MATα HMRa. In 8 of 13 cases, the α-factor-resistant spores from H3 (presumed to be *MAT*α) gave rise to zygotes formed by mating between sibling cells at the four-cell stage (Fig. 2 *right*). This observation confirms that these spores intially have a functional α mating type locus and that they readily switch to *MAT***a** [further confirmation that these spores have a functional *MAT*α allele is given below (Table 2)]. The behavior of these *HML*α*-66* spores at the four-cell stage is like that of $HML\alpha^+$ spores. In a separate analysis, 32 *MAT***a** (α-factor sensitive) cells derived from 16 *MAT*α *HML*α*-66 HO* spores were removed from α-factor and subsequently examined for zygotes after 3 or 4 and 8–10 more cell divisions. No zygotes were observed in any of the 32 clones; each of these clones contained both α-factor-sensitive and α-factor-resistant cells. In the four cases examined, the cells in

Table 2. Genetic analysis of cells derived from an *HO HML*α*-66 MAT*α spore

			Phenotype of spore clones				
			HO		*ho*		
Diploid	Genotype		'**a**'	**a**/α	'**a**'	'α'	NM
H5	*ho HMLα cry1 MAT***a** *HMR***a**	CryR:	29	24	55	4	0
	*HO HMLα-66 CRY1 MATα HMR***a**	CryS:	1	57	1	57	0
H6	*ho HMLα cry1 MAT***a** *HMR***a**	CryR:	30	20	52	0	2
	HO HMLα-66 CRY1 $MAT\alpha^-$ *HMR***a**	CryS:	22	31	2	0	49

*ho cry1-3 MAT***a** strain XHB61-1a was used in both diploids. H5 was formed by cell-to-cell mating to the first daughter of an 'α' spore from strain H3; H6 was formed by a selected mating with cells derived from the same 'α' spore as used for H5 (see Fig. 2). Data are from four-spored tetrads. Clones grown from spores were analyzed as in Table 1. NM, nonmating, sporulation-deficient.

the colony mated efficiently as 'a' (efficiency, 0.5) but not as 'α' (efficiency, 2×10^{-4}). The 'a' cells derived from spores that were initially *MATα* thus behave like the *MAT*a *HO HMLα-66* spores described in the preceding section—they switch mating types but do not acquire the ability to mate as 'α'. The *MATα HO HMLα-66* spores have therefore switched to *MAT*a and then to the phenotype of a $MAT\alpha^-$ cell (Fig. 2 *right*).

Nonmating Cells Derived from *MATα* and *MAT*a *HO HMLα-66* Cells Have a Mutation of *MATα*. To determine whether the nonmating cells produced in lineages from *MAT*a and *MATα HO HMLα-66* spores have a mutation of the α mating type locus, colonies with such cells were mated to *MAT*a *ho* cells by selected mating to form putative $MAT\alpha^-/MAT$a *ho/HO* diploids. The segregations for each of 12 diploids of this type were similar, and, as expected for diploids of the *MAT*a/$MAT\alpha^-$ genotype, each gave rise to nonmating *ho* segregants. No mating proficient 'α' *ho* segregants occurred in 306 tetrads pooled from these 12 diploids (unpublished results).

The segregation from one of these *MAT*a/$MAT\alpha^-$ *ho/HO* diploids (H6) is compared in Table 2 with a control *MAT*a/*MATα ho/HO* diploid (H5). H5 and H6 were formed by matings between the same *cry1-3 MAT*a *ho* strain and different progeny of a *CRY1 MATα HO HMLα-66* spore: for H5, a mating-proficient 'α' first daughter cell was used; for H6, a putative 'α^-' interconvertant was used (see Fig. 2). H5 yielded *ho* segregants, half of which were 'α' mating proficient (Table 2) with the 'α' phenotype linked to CryS, as expected for a *cry1-3 MAT*a/*CRY1 MATα ho/HO* diploid. In contrast, diploid H6 did not yield mating-proficient 'α' *ho* segregants. Instead, half of the *ho* segregants were nonmating and half were 'a' mating, with the nonmating phenotype linked to CryS as expected for a *cry1-3 MAT*a/*CRY1* $MAT\alpha^-$ *ho/HO* diploid (Table 2). To analyze the mating-defective segregants further, one nonmating *ho CRY1* segregant from H6 was mated (by selective methods) to an *ho cry1-3 MAT*a strain. The resulting diploid (of putative genotype *cry1-3 MAT*a/*CRY1* $MAT\alpha^-$ *ho/ho*) was nonmating and sporulation-proficient and yielded two 'a' and two nonmating segregants in each of 54 tetrads examined (data not shown). The nonmating phenotype was linked to CryS. These results confirm that the nonmating phenotype is due to a mutation in the *MATα* allele. Because several $MAT\alpha^-$ *ho* strains derived independently from *MAT*a *HO HMLα-66* cells all exhibited a characteristic temperature-sensitive defect in mating (unpublished observations), we presume that the same mutation, designated *MATα-66*, is present at the mating type locus in these $MAT\alpha^-$ strains. *MATα-66 ho* strains mate neither as 'a' (efficiency, 10^{-5}) nor as 'α' (efficiency, 2×10^{-4}). The *MATα-66* mutation resembles previously isolated mutations in the *MATα1* gene [e.g., *MATα1-5* (14, 18)] in that it reduces mating ability without reducing the sporulation proficiency of *MAT*a/*MATα-66* diploids.

If *MATα-66* is indeed a mutation of the mating type locus, then it should be "healed" by mating type interconversion in an *HO HMLα* background. An indication that this is so comes from analysis of H6 (Table 2). Because H6 is *ho/HO cry1-3 MAT*a/*CRY1 MATα-66 HMLα-66/HMLα*, half of the *HO MATα-66* spores should be *HMLα* and be able to diploidize if *MATα-66* is healable. Approximately half (31/53) of the *HO* CryS segregants (most of which should be *MATα-66*) were able to diploidize, indicating that *MATα-66* was converted to *MAT*a and *MATα* in these clones.

In conclusion, *MAT*a and *MATα HO HMLα-66* cells switch to cells with an 'α^-' phenotype due to a mutation of the mating type locus, *MATα-66*, which can be segregated into *ho* backgrounds where it is stable.

DISCUSSION

Mating type interconversion in yeast is a change of cell type due to a change of alleles at the mating type locus (12, 25). Oshima and colleagues (5, 12, 13) proposed that *HMLα* and *HMR*a code for controlling elements whose association with a site at the mating type locus forms the α and a alleles, respectively. In the cassette model, *HMLα* is silent α information and *HMR*a is silent a information. Switches of mating type are proposed to occur when replicas of the silent information substitute for the resident information at the mating type locus. A central prediction of these models is that mating type interconversion in a strain with a mutation of *HMLα* should introduce a mutation into the mating type locus (Fig. 3). The results described here bear out this prediction. In particular, we have isolated a mutation in *HMLα* (*HMLα-66*) and have shown that *HO*-promoted mating type interconversion in strains with *HMLα-66* generates a mutation of the mating type locus. We describe elsewhere (23) the isolation of mutations in the gene postulated to be the silent a information, the *HMR*a locus, and the transfer of mutant a information of the mating type locus (in *ho* cells) as a result of mating type interconversion. A. Klar (personal communication) has isolated mutations of *HMR*a by a different method and likewise shown that mating type interconversion (in *HO* strains) results in production of a mutant a mating type locus.

The *HMLα-66* mutation (*dip66* in Table 1) has the properties expected for a mutation of *HMLα*: *MATα HO HMLα-66 HMR*a strains diploidize, whereas *MAT*a *HO HMLα-66 HMR*a strains do not. Furthermore, within the resolution of our crosses, the *HMLα-66* mutation maps to *HMLα*. Having identified a mutation in *HMLα*, we have been able to analyze the switching behavior of *HO HMLα-66* strains. We observe (Figs. 2 and 3) that (*i*) *MATα HO HMLα-66 HMR*a spores switch to *MAT*a and subsequently to cells with

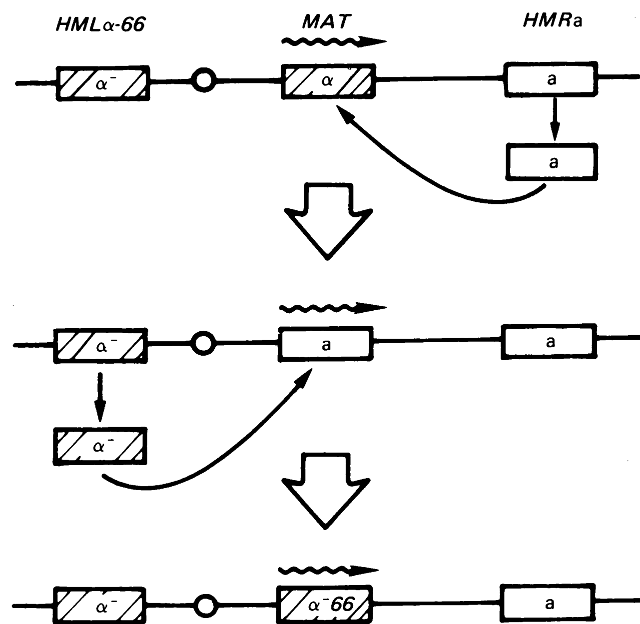

FIG. 3. Structure of chromosome III of *HMLα-66 HMR*a cells according to the cassette model as the cells undergo homothallic "wounding" depicted in Fig. 2. *MATα* cells (top line) switch to *MAT*a (middle line) which then switch to $MAT\alpha^-$ (bottom line) when a replica of the defective α^- information at *HML* is inserted at *MAT*.

the expected phenotype of *MAT*α^- mutants, resistant to α-factor but unable to mate as 'α'; (*ii*) *MAT***a** *HO HML*α*-66 HMR***a** spores switch directly to such 'α^-' cells; (*iii*) these 'α^-' cells switch readily to '**a**' cells, which switch to 'α^-', etc. That these 'α^-' cells are genotypically *MAT*α^- was confirmed by genetic analysis in which the mating type locus of these cells was segregated into *ho* backgrounds and shown to contain a mutation at the α mating type locus, *MAT*α*-66*. This mutation greatly reduces efficiency of mating and is allelic to *MAT***a** and linked to *CRY1*, as expected for a mutation in *MAT*α. Although complementation tests between *MAT*α*-66* and mutants defective in the two known *MAT*α complementation groups (*MAT*α*1* and *MAT*α*2*) (3, 18) have not yet been performed, the behavior of *MAT*α*-66* is like that of a *MAT*α*1*$^-$ mutation. As expected for a mutation of *MAT*α (either in *MAT*α*1* or *MAT*α*2*), *MAT*α*-66* can be healed to *MAT*α in *HO HML*α *HMR***a** strains. In summary, these studies show that mating type interconversion in *HO HML*α*-66 HMR***a** cells introduces what appears to be a "standard" mutation into the mating type locus and support the notion that homothallic yeast cells switch mating type from **a** to α by replacing the resident information at *MAT* with a replica of silent α information at *HML*α (Fig. 3).

The lineage of *HO HML*α*-66 HMR***a** *MAT*α spores (Figs. 2 and 3) displays a pattern of homothallic "wounding"—*MAT*α spores give rise to *MAT***a** cells which then give rise to *MAT*α^- cells. The failure of functional α information to reappear at the mating type locus indicates that when the α cassette initially at the mating type locus was removed it was lost and not stored in the cell in a retrievable form. This result supports the notion that there is a one-way flow of genetic information from *HML*α (and *HMR***a**) to the mating type locus, which had been deduced on other grounds (ref. 11; J. Rine, personal communication).

The *HML*α*-66* mutation may have arisen by recombination rather than by *de novo* mutation. Because *HML*α*-66* was isolated in a strain carrying the *MAT*α*1-5* mutation and because *HML*α appears to be silent α information (see below), recombination might have placed the *MAT*α*1-5* mutation at *HML*α. Consistent with this proposal, we have observed that both *MAT*α*1-5* and *MAT*α*-66* exhibit a characteristic temperature-sensitive mating defect (data not shown).

The results described here are consistent with *HML*α being equivalent to silent *MAT*α information but do not rigorously prove it to be so. Additional support for the equivalence of *HML*α and *MAT*α and of *HMR***a** and *MAT***a** comes from mutations that appear to allow expression of the information at *HML*α and *HMR***a** *in situ* in *ho* strains (17, 26, 27) and from chromosomal rearrangements (deletion or circle formation) that appear to allow expression of the information at *HML*α and *HMR***a** by fusion to an essential site at the mating type locus (3, 28).

The confirmation of a central feature of the cassette model for differentiation of *S. cerevisiae* cell types raises the question as to the generality of such a mechanism. Can a cassette mechanism account for mating type interconversion in other yeasts, such as *Schizosaccharomyces pombe*, for which a "flip-flop" model has been proposed (29)? Do cells of higher eukaryotes become differentiated by activation of regulatory genes through cassette transposition? Elsewhere we discuss an extension of the cassette model to account for orderly production of multiple cell types in higher eukaryotic development by a process of sequential cassette insertion (3, 6).

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