INTERCONVERSION OF YEAST CELL TYPES BY TRANSPOSABLE GENES

AMAR J. S. KLAR

Cold Spring Harbor Laboratory, P. O. Box 100, Cold Spring Harbor, N. Y. 11724

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ABSTRACT

The **a** and α cell types of budding yeast *Saccharomyces cerevisiae* are controlled by alternate alleles of the mating-type locus (*MAT*), *MAT***a** and *MAT*α. The cell types can be interconverted by switching alleles of *MAT*. The loci *HMR***a** and *HML*α, which are loosely linked to *MAT*, are involved in mating-type switching. Experimental evidence for their role in *MAT* interconversion is presented. As a result of switching, the homothallic and heterothallic strains containing the amber and ochre mutations within the *HMR***a** locus yield corresponding amber and ochre mutant *mat***a** loci. Similarly, the *hml*α mutant strain generates *mat*α mutant alleles. That is, specific mutations from *HMR***a** *and HML*α are transmitted to *MAT*. A replica of the mating-type coding information originating from these loci is transposed to *MAT*, where it replaces the existing information. Furthermore, "Hawthorne deletions" in strains containing *hmr***a**-amber/ochre result in production of *mat***a**-amber/ochre alleles. Therefore, genetic information for *MAT***a** resides at *HMR***a**. The switches occur in a defined set of clonally related cells. Thus, the efficient interconversion of yeast cell types is mediated by an unidirectional transfer of genetic information between nonallelic sites in a nonrandom and programmed fashion. The results are inconsistent with the "flip-flop" models, but satisfy a key prediction of the general controlling element and the specific cassette models proposed for mating-type interchange.

THE mating behavior of *Saccharomyces cerevisiae* is controlled by two alleles of the mating type-locus (*MAT*), *MAT***a** and *MAT*α (LINDEGREN and LINDEGREN 1943). The corresponding **a** and α cell types can be interconverted by reversible genetic changes at *MAT*. In heterothallic (*ho*) strains, the cell types change with a frequency of only about 10^{-6} (HAWTHORNE 1963a; RABIN 1970), while the homothallic (*HO*) strains may change frequently as often as every cell generation (WINGE and ROBERTS 1949; HAWTHORNE 1963b; OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1977; STRATHERN and HERSKOWITZ 1979). These switches represent heritable changes at *MAT* and the continued presence of the homothallism genes is not required for the maintenance of the altered allele. The mitotic products of a single haploid *HO* cell may express opposite mating types and therefore fuse to produce *MAT***a**/*MAT*α diploids. *MAT***a**/*MAT*α diploids define a third cell type: they are unable to mate, do not exhibit further switching, but are capable of meiosis and sporulation.

MAT interconversion is promoted by genes *HO*, *HML*α (alternate allele *HML***a**; see footnote to Table 1 for the new nomenclature used to designate the homothallism loci) and *HMR***a** (alternate allele *HMR*α). Either *HML*α or *HMR*α is required for switching *MAT***a** to *MAT*α; likewise, either *HMR***a** or *HML***a** is needed for switching *MAT*α to *MAT***a** (TAKANO and OSHIMA 1970; NAUMOV and TOLSTORUKOV 1973; HARASHIMA, NOGI and OSHIMA 1974; KLAR and FOGEL 1977). *HML* and *HMR* are located, respectively, on the left and right arms of chromsome *III* and are only loosely linked to *MAT* (HARASHIMA and OSHIMA 1976; KLAR and FOGEL 1977). *MAT* is situated about 25 centiMorgans away from the centromere on the right arm of the same linkage group (MORTIMER and HAWTHORNE 1969). *HO* has been mapped to chromosome *IV* (G. KAWASAKI, personal communication). Most heterothallic laboratory strains have the genotype *HML*α *HMR***a** *ho* (HAWTHORNE, quoted in HICKS and HERSKOWITZ 1977).

Several molecular models have been proposed to explain *MAT* interconversion. According to the "flip-flop" models, both *MAT***a** and *MAT*α alleles reside at *MAT* and they share a common regulatory site, *e.g.*, promoter, operator. In these models, it is postulated that the switches are mediated by inverting the regulatory site by DNA sequence modification (HAWTHORNE, quoted in HOLLIDAY and PUGH 1975) or by recombination (BROWN 1976; HICKS and HERSKOWITZ 1977). OSHIMA and TAKANO (1971; see also HARASHIMA, NOGI and OSHIMA 1974) proposed the "controlling element" model. According to this model, *HML*α and *HMR***a** and their alternate alleles (HARSHIMA, NOGI and OSHIMA 1974) code for mating-type specific controlling elements and the *MAT* acts as their affinity site. The attachment of an *HMR***a** or *HML***a** element differentiates the *MAT* locus to an **a** allele and the attachment of an *HML*α or *HMR*α element forms an α allele. The gene product of *HO* is hypothesized to catalyze the insertion and removal of these elements at *MAT*. HICKS, STRATHERN and HERSKOWITZ (1977) proposed a similar but more specific scheme, the cassette model. Here, the *HML*α and *HMR*α loci are suggested to be sites of unexpressed α information and *HMR***a** *and HML***a** are sites of silent **a** information. *MAT* interconversion is proposed to occur by transposition of DNA copies of silent **a** and α information into *MAT* with the concomitant removal of the resident information previously expressed at that locus. Since the silent loci remain unaltered, only a copy of the information is transposed.

A key prediction of the more general controlling element model and the specific cassette model is that, as a result of switching events, strains with mutations in *HML* and *HMR* can generate corresponding mutant *MAT* alleles. We and others have recently described results that satisfied this prediction (KLAR and FOGEL 1979; BLAIR, KUSHNER and HERSKOWITZ 1979; KUSHNER, BLAIR and HERSKOWITZ 1979). However, these data can also be explained by the modification model proposed by HAWTHORNE (D. HAWTHORNE, quoted in HOLLIDAY and PUGH 1975). His model proposes that heritable (but reversible) sequence modifications of the regulatory site (*e.g.*, promoter, operator) result in the alternate expression (*e.g.*, by inverting the regulatory site) of *MAT***a** and *MAT*α

alleles, both of which are present at the *MAT* locus. Modifications may be due to changes in the base sequences (D. HAWTHORNE, quoted in HOLLIDAY and PUGH 1975) or methylation and demethylation of specific bases (HOLLIDAY and PUGH 1975). In this model, gene products of the *HML* and *HMR* loci are proposed to code for the hypothesized modification functions. Thus, the *hmlα* and *hmr***a** mutants may be predicted to catalyze the imprecise modifications such that a particular *MAT* allele will receive a defective regulatory element. As a result of switching, such a cell would alternate between *MATα* and *mat***a** in *hmr***a** mutants and between *MAT***a** and *matα* in *hmlα* mutants—precisely the result obtained by KLAR and FOGEL (1979) and BLAIR, KUSHNER and HERSKOWITZ (1979).

To differentiate between the transposition and the modification models, it is essential to correlate the mutational defect within the *hmlα* and *hmr***a** loci with that observed in the *MAT* alleles generated by switches in the mutant strains. The controlling element and the cassette models predict that the mutant information should be *faithfully* copied and substituted into *MAT*, where it should be expressed as a mutant allele. In the studies conducted by KLAR and FOGEL (1979) and BLAIR, KUSHNER and HERSKOWITZ (1979), the identity between mutations in *hmr***a** and *hmlα* and those in the *MAT* alleles was not established. The present studies were undertaken to that end. I demonstrate that strains possessing nonsense mutations in *HMR***a** yield defective *mat***a** alleles carrying the corresponding nonsense mutations. Similarly, switches in the *hmlα* mutant strain yield *matα* alleles with phenotypic properties identical to those exhibited in the *hmlα* allele when that is allowed to be expressed *in situ.*

MATERIALS AND METHODS

Strains: All strains of *Saccharomyces cerevisiae* are listed in Table 1.

Media and techniques: All media for growth and sporulation and techniques for micromanipulation and tetrad analysis have been described by MORTIMER and HAWTHORNE (1969), Sensitivity to cryptopleurine was tested on media as described by GRANT, SANCHEZ and JIMENEZ (1974). Diploids were generated by cell-to-cell, cell-to-spore or rare-matings, as detailed earlier (KLAR and FOGEL 1977).

Isolation of hmra *and* hmlα *mutations:* Mutations of these loci were isolated and mapped by the procedure of KLAR, FOGEL and MACLEOD (1979). The rationale for isolating these mutations is briefly outlined here. Analysis of the proposed silent mating-type loci, *HMR***a** and *HMLα*, is made difficult by their cryptic nature. We have described a mutation, *mar1* (*ma*ting type *r*egulator), that is proposed to permit the expression of the normally silent loci. A strain of genotype *HMLαMATα HMR***a** (cassette designation [α] α [**a**]) *mar1* is sterile, a phenotype similar to those of the *MAT***a**/*MATα* cells, since α and **a** information located at *HMLα*, *HMR***a** and *MAT* is expressed. Such a strain was mutagenized with ethyl methanesulfonate as described earlier (KLAR, FOGEL and RADIN 1979). Mutants that expressed the α phenotype were screened. A predominant class of these mutants is produced by mutations in the *HMR***a** locus. The α phenotype is contributed by the expression of α information at *MAT* and *HMLα*. To avoid isolation of clonally related mutants, cells from 11 independent clones were mutagenized and screened for the mutant phenotype. A total of 48 putative *HMR***a** mutants from 90,000 cells that survived mutagenesis were isolated. Whether the mutants carried an amber or ochre lesion was determined by their co-suppression with the known amber and ochre markers present in the strain. Four mutants lost the α phenotype and regained sterility when

TABLE 1

Strain list

Strain	Genotype*	Source
K81	*MATα HMLα hmr***a**-ochre *ho arg4–17 thr1 met13 ura1 ilv3 trp1–1 lys1–1 SUP16*	This study
K82	*MATα HMLα HMR***a** *ho cry1 aro7 trp1–1 trpx arg4–17 met13 ade6*	This study
K83	*MATα HMLα hmr***a**-amber *ho aro7 trp1–1 ade8–10 lys1–1 thr1 leu2 SUPB*	This study
K84	*MATα HMLα HMR***a** *ho cry1 aro7 trp1–1 lys1–1 met13 ilv3 ade6*	This study
K85	*MAT***a***/MATα HMLα/HMLα hmr***a**-ochre/*hmr***a**-ochre *HO/HO met13/met13 trp1–1/trp1–1 leu2–1/leu2–1 his2/his2*	This study
K86	*MAT***a** *HMLα HMR***a** *ho cry1 lys2 leu2 his2*	This study
K74	*MAT***a***/MATα HMLα/HMLα hmr***a**-amber/*hmr***a**-amber *HO/HO trp1–1/trp1–1 aro7/aro7 ade6/ade6 ilv3/ilv3 lys1–1/lys1–1*	This study
K75	*MAT***a** *HMLα HMR***a** *ho cry1 arg4–17 ilv3 thr1 ura1 aro7 trp1–1 met13*	This study
K76	*MATα HMLα HMR***a** *ho cry1 SUPA aro7 trp1–1 thr1 ade8–10*	This study
K77	*mat***a** *hmlα–1 hmr***a** *mar1 ho met13 lys1 lys1 ura3*	This study
K78	*mat***a** *HML***a** *hmr***a** *ho met13 lys1–1 trp1–1 his4 leu2 thr4 mar1*	This study
K79	*MATα hmlα–1 HMR***a** *ho aro7 leu2 trp1–1*	This study
J20	*MAT***a** *HML***a** *HMR***a** *HO his4 leu2 lys2 his2 metx*	Klar, Fogel and Radin (1979)
K80	*matα hmlα–1* or *HML***a** *HMR***a** *ho cry1 metx his4 his2 aro7 leu2*	This study
DC5	*MAT***a** *HMLα/HMR***a** *ho leu2 his3*	J. Strathern
S41	*MAT***a***/MATα HMLα/HMLα HMR***a***/HMR***a** *HO/HO arg4/arg4*	R. & M. Esposito
K87	*MAT***a***/MATα HMLα/HMLα hmr***a**-ochre/*hmr***a**-ochre *HO/HO ade6/ade6 lys1–1/lys1–1*	This study
A3060– 3B	*MATα HMLα HMR***a** *ho his4–260 ade2–1 SUP11–1*	G. Fink
4A	*MATα HMLα HMR***a** *ho cry1 SUP16 arg4–17 met13 thr1 ura1 trp1*	This study

* The genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (Plischke *et al.* 1976), except a new terminology for the homothallism genes is used. The new terminology was proposed and accepted by investigators in the field at the International Congress in Yeast Genetics in 1978. Results, particularly those presented in this paper, lead to a simpler nomenclature for the homothallism genes. The loci *HM***a** and its allele *hm***a** are designated as *HMLα* (homothallism locus located on the left arm of chromosome *III*) and *HML***a**, respectively. The loci *HMα* and *hmα* are correspondingly designated *HMR***a** (homothallism locus situated on the right arm of chromosome *III*) and *HMRα*. It should be noted that the new designation is independent of any model proposed for mating-type interconversion. Either *HMLα* or *HMRα* is required for the activation of the *MATα* allele at the mating-type locus. Similarly, either *HML***a** or *HMR***a** is required for the activation of the *MAT***a** allele at the mating-type locus. The mutant alleles of these loci are assigned small letters, for example, *hmr***a**, *hmlα*, etc. In the text, unless otherwise indicated, strains carry the genotype *HMLα HMR***a** *ho*. Mutant *MATα* and *MAT***a** loci are symbolized as *matα* and *mat***a**, respectively. *SUPA* and *SUPB* are uncharacterized, while *SUPIV* is an amber suppressor that acts on the UAG alleles, *trp1–1* and *aro7–1*. *SUPA* and *SUPB* are also amber suppressors.

the amber suppressors were introduced, and two became sterile in the presence of ochre suppressors. One amber (designated *hmr***a**-amber) and one ochre (designated *hmr***a**-ochre) mutation of *HMR***a** were used in the present study. Both of these mutations map close to or at *HMR***a** (data not shown) and were suppressible by the corresponding known amber and ochre suppressors.

Similarly, from a sterile *HML*α *MAT***a** *HMR***a** ([α] **a** [**a**]) *mar1* strain, a mutant that expressed an **a** phenotype was isolated. This mutation maps close to or at *HML*α (this paper) and is designated as *hml*α*-1*.

Analysis of MAT**a** *alleles generated by switching* MATα *in heterothallic* (ho) *strains:* Heterothallic strains can effect switches at *MAT* with a low frequency of about 10^{-6} (HAWTHORNE 1963a; RABIN 1970). These infrequent events are recovered by "rare-mating" strains that are otherwise incapable of mating (*e.g.*, **a** × **a**, $\alpha \times \alpha$). Strains to be mated in this fashion carry complementary auxotrophic markers. The rare-mated hybrid 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 technique to recover cells in which *MAT*α switched to *MAT***a** (or *mat***a**) in strains carrying amber and ochre mutations in *HMR***a**. Strains with the mutant *mat***a** allele mate as **a** cells (KASSIR and SIMCHEN 1976).

RESULTS

*Switching in the hmr***a***-ochre strain:* A total of 96 hybrid clones between strains K81 (*CRY1 MAT*α *hmr***a**-ochre *SUP16*; see Table 1 for complete genotype) and K82 (*cry1 MAT*α) were selected by the rare-mating technique (see MATERIALS AND METHODS). In each case, the parent that had undergone the switch could be determined by the linkage of the switched allele to the closely linked marker, *cry1* (GRANT, SANCHEZ and JIMENEZ 1974). *SUP16* is a translational ochre suppressor.

The 96 hybrids were classified according to their mating type, sporulation proficiency, parent that experienced the switch and spore viability. As presented in Table 2A, five classes of hybrids were observed. At least 10 asci from each hybrid that was capable of sporulation were analyzed by tetrad analysis (data not shown). Based on that analysis, the hybrids of classes I and II were judged to possess the genotype *CRY1 MAT*α*/ cry1 MAT***a**. The newly generated *MAT***a** allele was in coupling with the closely linked *cry1* allele, indicating that these hybrids were generated by switching in the K82 parent, which carried the wild-type *HMR***a** allele. Class I hybrids yielded 2**a**:2α segregants, while the single Class II hybrid yielded 2α:2**a**-lethal segregant clones. The class II hybrid will be discussed below.

Hybrids of classes III and IV exhibited an α phenotype but were capable of sporulation. These hybrids were judged to possess the genetic constitution *CRY1 mat***a***/cry1 MAT*α as determined by ascus dissection and tetrad analysis (data not shown). (The hybrids expressed an α phenotype and were capable of sporulation because of suppression of the *mat***a** allele by *SUP16*, as demonstrated below. Suppressors in the crosses were monitored by the suppression of auxotrophic markers). The *mat***a** allele signifies the defective *MAT***a** locus. The cells carrying the *mat***a** allele express an **a** mating type, but the diploids constructed by mating them to the α cells (*i.e.*, *mat***a***/MAT*α) express an α phenotype. Such *mat***a** mutations have been previously described (KASSIR and SIMCHEN 1976;

TABLE 2

Analysis of hybrids selected by rare-mating MATα hmr**a**-*amber/ochre* ho *with* MATα HMR**a** ho *strains*

Class	No. observed	Mating type	Sporulation	Deduced genotype	Switched parent	Postulated switch of *MATα* to:
(A) K81 (*MATα CRY1 hmr***a**-ochre *ho SUP16*) × K82 (*MATα cry1 HMR***a** *ho*) hybrids						
I	19	nonmater	+	*MATα/MAT***a**	K82	*MAT***a**
II	1	nonmater	+	*MATα/MAT***a**-lethal	K82	*MAT***a**-lethal
III	17	α	+	*MATα/mat***a**	K81	*mat***a**
IV	3	α	+	*MATα/mat***a**-lethal	K81	*mat***a**-lethal
V	56	α	—	*MATα/MATα*	none	none
(B) K83 (*MATα CRY1 hmr***a**-amber *ho SUPB*) × K84 (*MATα cry1 HMR***a** *ho*) hybrids						
I	20	nonmater	+	*MATα/MAT***a**	K84	*MAT***a**
II	1	nonmater	+	*MATα/MAT***a**-lethal	K84	*MAT***a**-lethal
III	10	α	+	*MATα/mat***a**	K83	*mat***a**
IV	2	α	+	*MATα/mat***a**-lethal	K83	*mat***a**-lethal
V	183	α	—	*MATα/MATα*	none	none

KLAR, FOGEL and RADIN 1979). The *mat***a** alleles were uniformly found to be linked to *CRY1*, indicating that they arose by switching in the parent containing the *hmr***a**-ochre mutation (K81). Hybrids from class II yielded 2α:2**a** segregants, while those from class IV yielded 2α:2**a**-lethal meiotic products. Diploids of the latter class will be discussed in a subsequent section. The *mat***a** alleles were then tested further to determine whether they contained the ochre mutation.

Diploids of the genotype *MATα/MAT***a** are unable to mate and can undergo meiosis and sporulation. But the *MATα/mat***a** strains express an α phenotype and are incapable of meiosis and sporulation. The hybrids in class III express an α phenotype and are able to sporulate. These hybrids carry an ochre suppressor, *SUP16*, in the heterozygous state. Results presented in Table 3 clearly demonstrate that the *mat***a** defective allele carries an ochre mutation. *MATα/mat***a** hybrids lacking the suppressor failed to sporulate. It is clear that *SUP16* in the heterozygous configuration suppresses the *mat***a** allele for the sporulation functions, but does not prevent the hybrids from expressing an α phenotype. Apparently, the *MAT***a** function needed for sporulation is sufficient in the *MATα/mat***a**-ochre *SUP16/+* hybrids, but a higher level of *MAT***a** gene product is required to produce the sterile *MATα/MAT***a** phenotype. Such hybrids with

TABLE 3

Suppression of mat**a** *alleles obtained by switching* MATα *in K81* (hmr**a**-*ochre*) *strain*

Hybrid	Suppressor	Mating type	Sporulation
*MATα/mat***a**	+/+	α	—
*MATα/mat***a**	*SUP16/+*	α	+
*MATα/mat***a**	*SUP16/SUP16*	sterile	+
*MATα/mat***a**	*SUP11/+*	sterile	+
*MATα/MAT***a**	+/+	sterile	+

homozygous *SUP16* or heterozygous *SUP11* (another ochre suppressor) are sterile and sporulation proficient as is characteristic of *MATα/MAT***a** diploids. *SUP16*, which causes insertion of serine at UAA sites, is less efficient than a tyrosine inserter, *SUP11* (ONO, STEWART and SHERMAN 1979). Therefore, it is concluded that a strain with the *hmr***a**-ochre mutant allele switches *MATα* to *mat***a**-ochre by heterothallism.

Hybrids representing class V (Table 2A) may represent illegitimate matings without switching. Such a class of hybrids obtained during rare-mating selections among α × α crosses has been reported earlier (HAWTHORNE 1963a; RABIN, 1970; HICKS and HERSKOWITZ 1977; KLAR, FOGEL and RADIN 1979) and will not be discussed here.

Switching in hmr**a**-*amber strains*: Experiments similar to those presented above were conducted with a strain containing the *hmr***a**-amber mutation. Five classes of hybrids between strains K83 (*CRY1 MATα hmr***a**-amber *SUPB*) and K84 (*cry1 MATα*) were observed (Table 2B). These classes are identical to those presented in Table 2A, which were discussed in the preceding section. Hybrids in classes III and IV exhibited α mating type and were capable of sporulation. These hybrids resulted from switches in the K83 parental strain where *MATα* was switched to a defective *mat***a** allele. Class III hybrids yielded 2α:2**a** meiotic segregants, while class IV hybrids produced 2α:2**a**-lethal tetrads. Class IV hybrids will be discussed below. All the **a** segregants derived from hybrids presented in class III harbor a defective *mat***a** allele, since the diploids constructed by hybridizing these segregants with α strains expressed an α mating type, and the hybrids that lacked the suppressor were incapable of sporulation (Table 4). However, such hybrids gain the capacity to sporulate when any of the three different amber suppressors (*SUPB, SUPIV, SUPA*) are present in the heterozygous or homozygous state. Triploids *MATα/mat***a**/*mat***a** *SUPA/SUPA/SUPA* are sterile and sporulation proficient. Therefore, a strain with the *hmr***a**-amber mutant allele switches *MATα* to defective *mat***a**-amber allele heterothallically.

Activation of silent mating-type **a** *information proposed to exist at the* HMR**a** *locus by chromosomal rearrangements:* During the rare-mating experiments, several hybrids that yielded 2α:2**a**-lethal spores were obtained. Such hybrids

TABLE 4

Suppression of mat**a** *alleles obtained by switching* MATα *in K83* (hmr**a**-*amber*) *strain*

Hybrid	Suppressor	Mating type	Sporulation
*MATα/mat***a**	+/+	α	—
*MATα/mat***a**	*SUPB*/+	α	+
*MATα/mat***a**	*SUPB/SUPB*	α	+
*MATα/mat***a**	*SUPIV*/+	α	+
*MATα/mat***a**	*SUPA*/+	α	+
*MATα/mat***a**	*SUPA/SUPA*	weak α	+
*MATα/mat***a**/*mat***a**	+/+/+	α	—
*MATα/mat***a**/*mat***a**	*SUPA/SUPA/SUPA*	sterile	+

were first characterized by HAWTHORNE (1963a). He demonstrated that cells may switch from *MATα* to *MAT***a** by a deletion extended distally from *MAT* to between *thr4* and *MAL2*. The lethal lesion is tightly linked to *MAT*, and the other end of the deletion is mapped to within one cM of the *HMR***a** locus (HAWTHORNE 1963a; STRATHERN 1977; STRATHERN *et al.* 1979). Such a deletion, called "Hawthorne's deletion," can be routinely obtained from α × α rare-matings. The *MATα/MAT***a**-lethal diploids sporulate and uniformly yield 2 α and 2 inviable **a** spores in each tetrad. The inviable spores express an **a** mating type because they may be mated with α strains and thus be rescued. In terms of the cassette model, the mating-type switch associated with the deletion has been interpreted as the removal of *MATα* information originally present at *MAT* and activation of the normally silent **a** information at *HMR***a** by fusion to *MAT* (STRATHERN *et al.* 1979).

The experiments involving strains of *hmr***a**-amber and *hmra*-ochre mutations allow one to test rigorously the cassette fusion interpretation of HAWTHORNE's deletion. It is predicted that such events produced in these strains will generate defective *mat***a** alleles carrying the specific mutations present in *HMR***a**. Results presented below satisfied this prediction.

Rare-matings between standard α strains and α strains carrying the *hmr***a**-ochre or -amber mutations generated a class of hybrids that were capable of sporulation, but exhibited an α phenotype. A subclass of these hybrids yielded 2α:2**a**-lethal segregants. Three hybrids (Table 1A, class IV) between K81 (*CRY1 MATα hmr***a**-ochre *SUP16*) × K82 (*cry1 MATα*) resulted from switches of *MATα* to *mat***a** in the K81 parent with the subsequent matings of those cells with K82 cells. These hybrids were sporulated and subjected to tetrad analysis. A total of 10 tetrads from each hybrid was analyzed. Each hybrid produced tetrads containing 2 *cry1* α and 2 inviable spores. The linkage of *CRY1* allele with lethality suggests that the *mat***a** allele was produced by an event in the K81 strain. As discovered by HAWTHORNE (1963a), the inviable spores exhibited an **a** phenotype because early during the spore germination it was possible to rescue them by mating with the α strains. The **a** spores possessing the lethal mutation were mated to cells from strain K82 by the spore-to-cell mating method (see MATERIALS AND METHODS). Thirteen zygotic clones were constructed and their mating type and capacity to sporulate were determined. All exhibited α mating phenotype. Since the hybrids from which the **a** spores with the lethal mutation were derived contained *SUP16* in the heterozygous state, half of the **a** segregants were expected to inherit the suppressor. Four such zygotic clones between **a**-lethal spores with K82 carried *SUP16* in the heterozygous condition. They were able to sporulate. The balance of nine zygotic clones lacked the suppressor and were sporulation-deficient. Furthermore, the **a**-lethal spores were hybridized to cells from A 3060-3B (*MATα SUP11*). All six zygotic clones tested were nonmaters and capable of sporulation. Therefore, each spore with the lethal mutation carried the *mat***a**-ochre allele since the sporulation defect of that allele was suppressible with the ochre suppressors.

Similarly, two hybrids between K83 (*CRY1 MAT*α *hmr***a**-amber *SUPB*) and K84 (*cry1 MAT*α), when subjected to ascus dissection and tetrad analysis, yielded 2 *cry1* α and 2 inviable **a** spores in each of 10 tetrads analyzed (Table 1B, class IV). Spores with the lethal mutation exhibited an **a** phenotype since it was possible to rescue them by mating with the cells from strain K82. Four hybrids, all exhibiting an α phenotype, were constructed. Two hybrids containing an amber suppressor (*SUPB*) in the heterozygous configuration were competent to sporulate, while the other two that lacked the suppressor were sporulation-deficient. These results demonstrate that a strain possessing the *hmr***a**-amber mutation may switch *MAT*α to *mat***a** by HAWTHORNE's deletion and that *mat***a** allele carries an amber lesion.

Hybrids in classes II (Table 1, A and B) represent *MAT*α→*MAT***a** interconversions associated with HAWTHORNE's deletion in strains containing the functional *HMR***a** loci.

In summary, strains carrying the *hmr***a**-amber/ochre mutant forms generate setrains with the corresponding *mat***a**-amber/ochre alleles due to mating-type interconversions associated with HAWTHORNE's deletions. Thus, these results support the notion that HAWTHORNE's deletions remove *MAT*α information originally present at *MAT* and activate normally silent **a** information by fusion at *HMR***a** (STRATHERN *et al.* 1979).

Analysis of MAT *alleles generated by switching* MATα *in homothallic* (HO) *strains:* Recently we have documented that diploid strains of the general genotype *HML*α/*HML*α *MAT***a**/*MAT*α *hmr***a**/*hmr***a** *HO/HO* produce asci containing 2**a**:2α spores (KLAR and FOGEL 1979). The **a** spores grow to establish MATα/MAT**a** clones due to switches and subsequent mating between cells of the opposite mating type early during the spore generation. The α spores yield dual-mater segregant clones that are incapable of sporulation. These results were interpreted to mean that *MAT*α switches to defective *mat***a** in *hmr***a** strains. Such α and **a** (cells with *mat***a** mate as **a** cells) cells mate to produce *MAT*α/*mat***a** diploids, which grow, switch and mate continuously to produce cells of higher ploidy. Unlike *MAT*α/*MAT***a** cells, *MAT*α/*mat***a** cells switch readily (STRATHERN, BLAIR and HERSKOWITZ 1979; KLAR, FOGEL and RADIN 1979). The continuous switching cycle yield cells of both mating types resulting in the dual-mater behavior of the spore clones obtained from α segregants (KLAR and FOGEL 1979). The *mat***a** alleles generated by switching strains containing *hmr***a**-amber and -ochre alleles were recovered and analyzed as discussed below.

Homothallic strains with hmr**a**-*ochre mutation switch* MATα *to* mat**a**-*ochre:* Strain K85 (*MAT*α/*MAT***a** *hmr***a**-ochre/*hmr***a**-ochre *HO/HO*) was subjected to ascus dissection and tetrad analysis. Several α spores were allowed to grow and switch, and the zygotes resulting from mating between each spore's progeny were placed adjacent to cells from a heterothallic strain K86 (*MAT***a** *ho*). If the α spore progeny switch *MAT*α to *mat***a**, then the resulting zygotic *MAT*α/*mat***a** cells should express an α phenotype. The matings between zygotes or their immediate progeny with K86 could be obtained easily by selecting for complementation of several auxotrophic markers. Because the triploids *MAT*α/*mat***a**/

*MAT***a** *HO/HO/ho* are not expected to switch further, the *mat***a** *ho* segregants can be derived by ascus dissection and tetrad analysis. Such a triploid hybrid was selected and analyzed to assess the presence of *mat***a** allele. The hybrid yielded low spore viability (about 15%) a result typical of triploids (MORTIMER and HAWTHORNE 1969). A total of 86 meiotic segregants were tested for their mating type; 53 were either nonmater or dual maters, 14 had an α phenotype and 19 had an **a** phenotype. Among the **a** segregants, four were judged to carry an altered *mat***a** allele, since, after mating with α cells, the resulting hybrids exhibited an α phenotype. The *MATα/mat***a** hybrids were sporulation-deficient. However, hybrids between putative *mat***a** segregants with strain 4A (*MATα SUP16 ho*), which contains an ochre suppressor, were able to undergo meiosis and sporulation.

To map the newly recovered defective *mat***a** allele, the hybrid *MATα/mat***a** *SUP16/+*, constructed as described above, was subjected to tetrad analysis. Each tetrad yielded 2α:2**a** segregants (Table 5, Hybrid A). All the **a** segregants possessed *mat***a**, since after mating with the α cells, the hybrids exhibited the α phenotype. Furthermore, the mutant allele showed close linkage to *CRY1* marker, about 3.0 cM (Table 5, Hybrid A). Since the met*a allele* is suppressible with an ochre suppressor, maps at *MAT* and is closely linked to *CRY1*, we conclude that strain K85, which contains the *hmr***a**-ochre mutant form, yields defective *mat***a**-ochre alleles by switching *MATα*.

Homothallic strain with hmr**a**-*amber mutant allele switches* MATα *to* mat**a**-*amber:* Experiments similar to those presented above were conducted with strain K74 (*HMLα/HMLα MATα/MAT***a** *hmr***a**-amber/*hmr***a**-amber *HO/HO*), which possesses the hmr**a**-amber mutation. It was sporulated, and zygotes obtained by matings between the α spore progeny were placed adjacent to cells from a heterothallic strain K75 (*cry1 MAT***a** *ho*). If most of the zygotes were *MATα/mat***a** cells, they should express an α phenotype. Matings between zygotes or their immediate progeny with cells from strain K75 were obtained by selecting for complementation of various auxotrophic markers. A hybrid constructed in this fashion was characterized by ascus dissection and tetrad analysis. The hybrid yielded a low spore viability of about 17%, characteristic of triploid strains (MORTIMER and HAWTHORNE 1969). Of 61 surviving spore clones tested, 41 exhibited dual-mater or nonmater phenotypes, 10 were α and 10 exhibited an

TABLE 5

Mapping of the mat**a**-*ochre/amber alleles obtained by switches in the* HO *strains*

Hybrid	*MAT*(α:a)	Marker pair	Ascus types* PD	NPD	TT	Map distance (cM)
(A) *MATα/mat***a**-ochre	2:2	*MAT-cry1*	16	0	1	3.0
(B) *MATα/mat***a**-amber	2:2	*MAT-cry1*	25	0	2	4.0

* Entries in the table correspond to the numbers of asci that displayed various segregation patterns (details of the hybrid construction in the text). PD, NPD and TT represent parental ditype, nonparental ditype and tetratype asci, respectively. Only one of the segregants carrying *mat***a**-ochre and one carrying *mat***a**-amber allele were used for mapping studies.

a mating phenotype. Two of the **a** cells were judged to carry the *mat***a** allele, since the hybrids constructed by their mating with the α cells (*i.e.*, *mat***a**/*MAT*α) exhibited an α phenotype. Such diploids were incapable of sporulation. However, when the putative *mat***a** segregants were crossed with strain K76 (*cry1 MAT*α *ho SUPA*), which contains an amber suppressor, the resulting hybrids were capable of sporulation. When such a hybrid (*MAT*α/*mat***a** *SUPA*/+) was subjected to tetrad analysis, all 27 tetrads yielded 2α:2**a** segregants (Table 5, Hybrid B). All of the **a** segregants possessed the *mat***a** allele, since after mating with the α cells, the hybrids exhibited an α phenotype. Furthermroe, based on the data presented in Table 5, we calculate that the *mat***a** allele is linked to *CRY1* by about 4 cM. Since the *mat***a** allele is suppressible by an amber suppressor, we conclude that strain K74, which contains the *hmr***a** *amber* mutation, switches *MAT*α to *mat***a**-amber form by homothallism.

Analysis of MAT *alleles generated by switching* MAT**a** *in the* hmlα *mutant*

Mapping and phenotypic properties of the hmlα *mutation:* As briefly outlined in MATERIALS AND METHODS (see KLAR, FOGEL and MACLEOD 1979 for details), the *mar1* mutants allow expression of the silent mating-type information proposed to exist at the *HML* and *HMR* loci. Therefore, it is possible to map and determine the phenotypic properties of mutant *HML* and *HMR* loci. Strain K77 (*hml*α*-1 mat***a** *hmr***a**-amber *mar1 ho*) exhibits a strong **a** but weak α phenotype. This phenotype will be designated as **a** >> α. Strain K78 (*HML***a** *mat***a** *hmr***a**-amber *mar1 ho*) exhibits **a** phenotype. Procedures to construct such strains will be presented elsewhere (KLAR, in preparation). A hybrid between K77 and K78 was constructed. The hybrid exhibited an **a** phenotype and was capable of sporulation. When subjected to tetrad analysis, it segregated 2**a** >> α:2**a** meiotic products. The mating-type phenotype was linked to *his4* by 32.4 cM and to *leu2* by 42.1 cM (Table 6). Thus, in this hybrid the mating type maps to the left of *his4*, where *HML* has been mapped (HARASHIMA and OSHIMA 1976). I presume that the **a** >> α phenotype is contributed by the *hml*α*-1* allele, which can be expressed in the *mar1* strains. Also, the *hml*α allele provides the α function necessary for sporulation. The expression of the *HML***a** allele presumably turns off the weak α mating phenotype contributed by the *hml*α allele in the

TABLE 6

Mapping of the hmlα *mutation-Asci obtained from K77* × *K78*
(hmlα/HMLa mata/mata hmra/hmra mar1/mar1)

Marker pair	Ascus types* PD	NPD	TT	Map distance (cM)
MAT–his4	13	0	24	32.4
MAT–leu2	6	0	32	42.1
his4–leu2	29	0	8	10.8

* Entries in the table correspond to the numbers of asci that displayed the various segregation patterns.

K77 × K78 hybrid, thus conferring an **a** phenotype, *i.e.*, *HML***a** is dominant to *hml*α*-1*.

Strain with hmlα-1 *mutant allele switches* MAT**a** *to defective* matα *by homothallism:* When the *hml*α mutant was used for *MAT* interconversion, defective *mat*α alleles with phenotypic properties identical to those of the *hml*α mutation were obtained. A hybrid between strains K79 (*hml*α *CRY1 MAT*α *HMR***a** *ho MAR1*) and J20 (*HML***a** *cry1 MAT***a** *HMR***a** *HO MAR1*) was constructed. It is sensitive to cryptopleurine because the wild-type *CRY1*-sensitive allele is dominant to the mutant *cry1* resistant allele (GRANT, SANCHEZ and JIMENEZ 1974). Spontaneous mitotic recombinants capable of growing on media containing cryptopleurine (*i.e.*, *cry1/cry1*) were selected (KLAR and FOGEL 1977). Because *cry1* is in coupling and closely linked to the *MAT***a** allele, most of the *cry1-cry1* recombinants are presumably *hml*α*/HML***a** *cry1/cry1 MAT***a***/MAT***a** *HMR***a***/HMR***a** *HO/ho, i.e.*, homozygous for *cry1* and the tightly linked *MAT***a** allele. These recombinants may switch one or both *MAT***a** alleles to *MAT*α (or *mat*α), provided that the single *hml*α mutant locus is able to perform its function, namely, switching *MAT***a** to *MAT*α. The cryptopleurine-resistant recombinants, when tested for mating type, exhibited the **a** >> α phenotype. Also, they were able to sporulate and could be subjected to tetrad analysis. If, as predicted by the cassette model, a copy of the defective α information from *hml*α is transferred to *MAT* during switching, then we expect to recover segregants with the **a** >> α phenotype. Since the hybrid is *HO/ho,* half of these segregants will inherit the *ho* allele and thus be stable at *MAT*. Several such segregants were recovered (data not shown). Hybrids constructed by mating such a segregant [strain K80, *cry1 mat*α (?) *ho*] with cells from DC5 (*CRY1 MAT***a** *ho*) exhibit an **a** phenotype, but are able to sporulate. Tetrad analysis of these hybrids produced 2**a** >> α:2**a** segregants in each of 20 asci tested. From a pairwise combination of the mating type and the *cry1* markers, we obtained a 19:0:1 ratio of PD: NPD:TT tetrads, respectively. Thus, the **a** >> α behavior is allelic to *MAT* and linked to *cry1* by about 2.5 cM. Also, the *mat*α allele provided the α functions needed for sporulation, because the hybrid K80 × DC5 (*mat*α*/MAT***a**) was sporulation proficient. Furthermore, that hybrid expresses an **a** phenotype. Thus, the *mat*α obtained is recessive to *MAT***a**. The *mat*α allele apparently represents an authentic mutation of *MAT*α since it complements the *mat*α*1–5* mutation described by MACKAY and MANNEY (1974).

Thus, the identical phenotypic properties of the *hml*α*–1* allele, as assayed in the *mar1* strain, and the *mat*α locus obtained by switching, as assayed in the *MAR1* strain, supports the conclusion that *HML*α carries the unexpressed *MAT*α information and that a replica of this information is transposed to *MAT* during switching.

Pedigree analysis of hmr**a** *mutants*: The transposition models for the mating-type interconversion predict that strains with mutations within *HMR***a** may switch **a** (*MAT***a**) to α(*MAT*α) and the α cells should switch to **a**⁻ (*mat***a**) cells. This prediction was tested by following the cell lineage of **a** and α spores produced by strain K87 (*HML*α*/HML*α *MAT***a***/MAT*α *hmr***a**-ochre/*hmr***a**-ochre

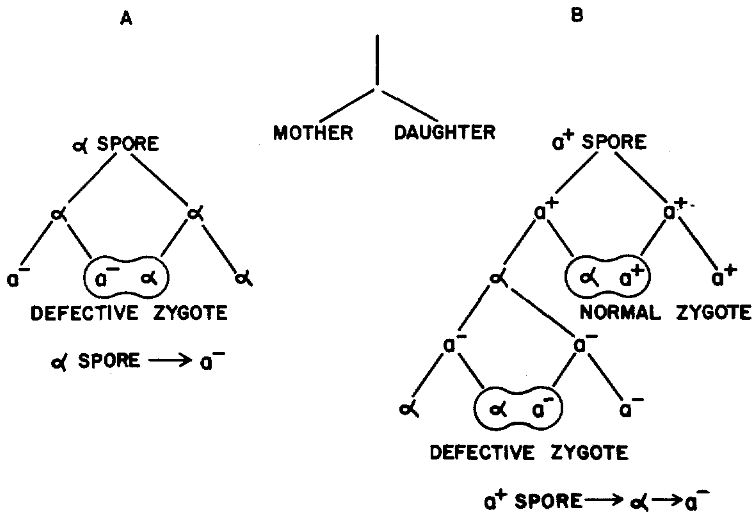

FIGURE 1.—Lineage of K87 (*HMLα/HMLα MAT***a***/MATα hmr***a**-ochre/*hmr***a**-ochre *HO/HO*) spores. (A) The α (*MATα*) spore progenies switch to $\mathbf{a}^-$ (*mat***a**). (B) The $\mathbf{a}^+$ (*MAT***a**) spore progenies switch to α (*MATα*) and those α cells switch to $\mathbf{a}^-$ (*mat***a**) cells. (See text for details.)

HO/HO) according to the procedures described by HICKS and HERSKOWITZ (1977) and STRATHERN and HERSKOWITZ (1979). Six α spores obtained from this strain switched half of their progeny at the 4-cell stage (Figure 1A) and the other six at the 8-cell stage. The **a** cells carry the *mat***a** allele since the zygotes constructed by matings between the α cells and the newly generated **a** cells produced clones that were dual maters and incapable of sporulation. Thus, the α cells switch to $\mathbf{a}^-$—*i.e.*, *MATα*→*mat***a** interconversion. Further, the **a** spore progeny produced functional α (*MATα*) cells since the zygote clone resulting from mating between the α cell and the **a** cell at the 4-cell stage exhibited a sterile, sporulation-procient phenotype (Figure 1B). The other α cell produced **a** cells within the next generation, but the resulting zygotic clones were bimaters and incapable of sporulation. Thus, the α cells produced $\mathbf{a}^-$ (*mat***a**) cells. In no case did an α cell produce functional $\mathbf{a}^+$ cells. Similarly, we observed the $\mathbf{a}\rightarrow\alpha\rightarrow\mathbf{a}^-\rightarrow\alpha\rightarrow\mathbf{a}^-$ pattern in strains containing the *hmr***a**-amber mutation (data not shown). Therefore, *hmr***a** mutants efficiently switch *MAT***a** to *MATα*, but *MATα* is switched to a mutant *mat***a** allele.

DISCUSSION

Mating-type interconversion is a change of cell type due to a change of alleles at the mating-type locus. The cassette model was proposed to account for the remarkable observation that the defective *matα* (and a natural variant of *MATα*) can switch to *MAT*$\mathbf{a}^+$ and then to functional wild-type *MATα*$^+$ (HICKS and HERSKOWITZ 1977; D. HAWTHORNE, quoted in HICKS and HERSKOWITZ 1977; TAKANO, KUSUMI and OSHIMA 1973; STRATHERN, BLAIR and HERSKOWITZ 1979).

Similar "healing" occurs with *mat*a mutations (KLAR, FOGEL and RADIN 1979; STRATHERN, BLAIR and HERSKOWITZ 1979; MASCIOLI and HABER 1980). Based on this observation, HICKS, STRATHERN and HERSKOWITZ (1977) argued that the cells must have silent copies of *MAT* located elsewhere in the genome to provide genetic information during switching. However, the healing was also explainable by the flip-flop model (see BROWN 1976; HICKS and HERSKOWITZ 1977). HAWTHORNE's modification model can also account for this phenomenon if one assumes that the mutations tested lie in the sequence that experiences the modification.

The experiments presented in this paper were designed to test a key prediction of the controlling element (OSHIMA and TAKANO 1971) and the cassette models (HICKS, STRATHERN and HERSKOWITZ 1977) for mating-type interconversion. We have demonstrated that strains possessing the *hmr*a-amber or -ochre alleles yield only defective *mat*a alleles subsequent to switching *MAT*α in heterothallic and homothallic strains. The *mat*a alleles carry the corresponding amber or ochre mutational defect originally present at *HMR*a. Therefore, the nonsense mutations are transmitted from *HMR*a to the mating-type locus. Subsequent to switching, the amber or ochre mutation is retained at *HMR*a and that locus could be used repeatedly (data not shown); thus, only a copy of the unexpressed mating-type information existing at *HMR*a is transferred to *MAT*, where it substitutes for the resident information previously expressed at that locus (Figure 2). Similarly, a strain carrying the *hml*α mutation switches *MAT*a to defective *mat*α. The *mat*α alleles generated exhibit phenotypic properties identical to those of the *hml*α allele when that is allowed to be expressed *in situ*.

Another mechanism for activating the silent **a** information existing at *HMR*a by chromosome *III* rearrangements has been proposed by STRATHERN, BLAIR and HERSKOWITZ (1979). We have obtained evidence consistent with their interpretation of "HAWTHORNE's deletions" that are routinely obtained from $\alpha \times \alpha$ rare-matings. These deletions are proposed to remove *MAT*α information originally present at *MAT* and concomitantly to activate the normally silent **a** information at *HMR*a by fusion to *MAT*. We have documented that strains possessing *hmr*a-amber/ochre mutant alleles, subsequent to events involving

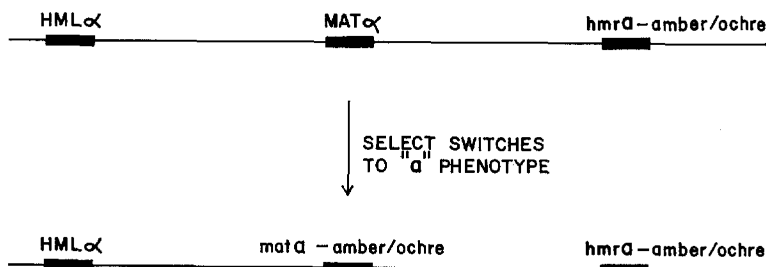

FIGURE 2.—Selection of mating-type switches from α to **a** in strains containing *hmr*a-amber/ochre mutant loci. The *MAT* alleles produced carry the corresponding amber/ochre defect originally present at *HMR*a. The source locus, *HMR*a, is unaltered and retains the amber/ochre lesion.

HAWTHORNE's deletion, generate *mat***a** alleles carrying the corresponding amber/ochre mutations (Figure 3).

It has been proposed that information residing at *HMLα* and *HMR***a** is kept silent by a negative control (KLAR, FOGEL and MACLEOD 1979; RINE *et al.* 1979; HABER and GEORGE 1979). Thus, transposition of copies of that information to *MAT*, as well as chromosome *III* rearrangements involving *MAT* and the *HM* loci, relieve that information from repression.

HML and *HMR* mutations used in this study were isolated in the *mar1* mutants. KLAR, FOGEL and MACLEOD (1979) proposed that the *MAR1* gene product(s) regulates the *HML* and *HMR* loci by a negative control, and that removal of the control by the *mar1* mutation allows the expression of silent **a** and *α* information. Also, they noted that silent mating-type information may reside at *HM* loci or elsewhere in the genome. In the latter model, the *HM* loci were assumed to act as positive regulatory genes that are needed for the expression of silent mating-type information. The results presented here demonstrate that structural information for the **a** and *α* genes indeed reside at the *HM* loci. Therefore, the *MAR1* hypothesis is also verified by these experiments. It should be stated that the presence of the **a** and *α* coding sequences at *HM* loci and transposition of their replicas to *MAT* during switching was specifically proposed in the cassette model (HICKS, STRATHERN and HERSKOWITZ 1977). Results are also in accord with the controlling element model (OSHIMA and TAKANO 1971) in which it is proposed that the attachment of controlling elements coded by the *HM* loci with the mating-type locus gives rise to *MAT***a** and *MATα* alleles. The cassette model is treated here as a specific version of the controlling element model. The data rule out the flip-flop and the modification models.

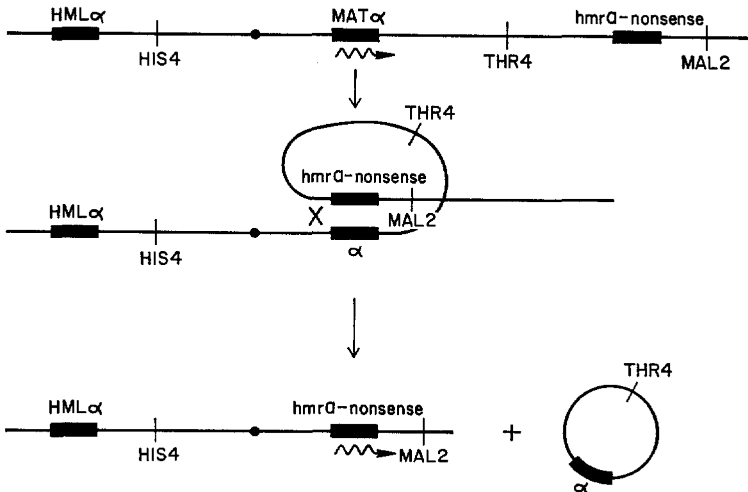

FIGURE 3.—Production of HAWTHORNE deletions in *hmra*-amber/ochre strains. An intra chromosomal recombination event fuses *HMR* to *MAT*, and thus deleting the sequences between *MAT* and *HMR*. The event activates the *HMR* locus to generate *mata*-amber/ochre allele. The deleted sequences present on the acentric ring are lost. The centromere is indicated by a dot and the arrow indicates the expressed locus. (Chromosome *III* map is not drawn to scale.)

It may be suggested that the *HML* and *HMR* loci carry multiple copies of *MAT*α and *MAT***a** information. Switching may constitute reciprocal translocation of the information between *MAT* and the storage loci. This possibility seems unlikely because we can isolate spontaneous mutations at *HMR* at a frequency of about 10^{-7} (KLAR, FOGEL and MACLEOD 1979). Furthermore, when the *hmr***a**-amber/ochre mutants are used for switching, only defective *mat***a**-amber/ochre alleles are recovered.

Some limited speculations concerning the molecular mechanism of the switching process are appropriate at this point. It is interesting to note that the "storage" loci *HMR* and *HML* map on the same chromosome where *MAT* resides. KLAR and FOGEL (1977) provided evidence that the *HML***a**/*HML*α *MAT*α/*MAT*α *HMR*α/*HMR*α (cassette terminology, [**a**] α [α]/[α] α [α]) diploid can switch one or the other or both α loci at *MAT* to *MAT***a** in a single cell cycle. Since this hybrid carries only a single *MAT***a** storage locus (*i.e.*, *HML***a**), a particular donor locus could be used more than once in a single cell division cycle. Furthermore, the cryptic loci can provide information to be used to switch a *MAT* allele in the same chromosome or in its homologue. The cells may synthesize multiple replicas of the diffusible cassettes during switching or the chromosome *III* arms may swing around and insert a copy at *MAT* by a concerted replication-substitution reaction. Since there is nonreciprocal transfer of information, a mechanism such as directed (but unidirectional) gene conversion is likely. Gene conversion as a possible mechanism for mating-type interconversion has also been proposed by HICKS and STRATHERN (1977). To elucidate the molecular details of the transposition process, we have cloned the mating-type locus (HICKS, STRATHERN and KLAR 1979). A clone containing DNA corresponding to *MAT*α shows physical homology to DNA sequences closely linked to or at *MAT*, *HML*, and *HMR* loci. The homology may allow for the alignment of these sequences during transposition. Mating-type interconversion occurs in a defined subset of clonally related cells in an orderly fashion (HICKS and HERSKOWITZ 1976; STRATHERN and HERSKOWITZ 1979). Therefore, *MAT* switches are mediated by unidirectional transfer of genetic information between nonallelic sites in a nonrandom and programmed fashion.

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Corresponding editor: F. SHERMAN