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Genetics

Healing of *mat* mutations and control of mating type interconversion by the mating type locus in *Saccharomyces cerevisiae*

(yeast/cell type determination/homothallism/cassette model)

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ABSTRACT **Homothallic yeasts switch cell types (mating types a and α) at high frequency by changing the alleles of the mating type locus, *MAT*a and *MAT*α. We have proposed in the cassette model that yeast cells contain silent *MAT*a and *MAT*α blocs ("cassettes"), copies of which can be substituted at the mating type locus for the resident information. The existence of silent cassettes was originally proposed to explain efficient switching of a defective *MAT*α locus (*mat*α) to a functional *MAT*α locus. We report here that this "healing" of *mat* mutations is a general property of the mating type interconversion system and is not specific to the class of *mat*α mutations studied earlier: a defective *MAT*a (*mat a1*) switches readily to *MAT*a and various *mat*α loci switch readily to *MAT*α. These observations satisfy the prediction of the cassette model that all mutations within *MAT*a and *MAT*α be healed. These studies also identify *MAT* functions that control the switching process: the same functions known to promote sporulation and prevent mating in a/α cells also inhibit the switching system in a/α cells. Finally, we present additional characterization of a natural variant of *MAT*α, *MAT*α-inc [Takano, I., Kusumi, T. & Oshima, Y. (1973) *Mol. Gen. Genet.* 126, 19–28] that is insensitive to switching. Our observation that *MAT*α-inc acts in *cis* suggests that it may be altered in a site concerned with excision of *MAT*α-inc or its replacement by another cassette.**

Cell type in the yeast *Saccharomyces cerevisiae* is controlled by the mating type locus (*MAT*), which has two alleles, *MAT*a, determining the a cell type, and *MAT*α, determining the α cell type. Cells of one mating type switch to the other mating type at low frequency (approximately 10^{-6}) in strains carrying the *ho* allele (heterothallic strains) and as often as every cell division in strains carrying the *HO* allele and appropriate accessory genes (homothallic strains) (refs. 1–3 and unpublished data). These changes in cell type are due to changes at the mating type locus itself. To explain these observations and others suggesting the existence of cryptic copies of the mating type loci (described below), we have proposed the "cassette model," in which cell type is determined by insertion of one of two cassettes of regulatory information into the mating type locus (4, 5). Yeast cells are proposed to contain silent *MAT*a and *MAT*α information, copies of which become expressed when inserted into the mating type locus (Fig. 1). Mating type interconversion thus is explained as the substitution of one cassette by another, catalyzed by the *HO* gene or some function under its control. Genetic studies by Naumov and Tolstorukov (8) and by Harashima *et al.* (9) have identified the loci that may be the silent cassettes: *HM*a, on the left arm of chromosome III, and *HM*α, on the right arm of chromosome III (Fig. 1). Because *HM*a is required for switching from a to α and *HM*α is required for switching from α to a, we have proposed that *HM*a is the silent *MAT*α cassette and that *HM*α is the silent *MAT*a cassette.

The mating type locus controls mating, sporulation, and activity of the mating type interconversion system: *MAT*a (and *MAT*a/*MAT*a) and *MAT*α (and *MAT*α/*MAT*α) cells mate and do not sporulate, whereas *MAT*a/*MAT*α cells sporulate and do not mate. The mating type locus also controls mating type interconversion in that interconversion occurs in *MAT*a and *MAT*α *HO* haploids and in *MAT*a/*MAT*a and *MAT*α/*MAT*α diploids carrying at least one *HO* gene, but does not occur in *MAT*a/*MAT*α diploids (10–13). Although the structures of the *MAT* alleles are not known, genetic experiments suggest that *MAT*a and *MAT*α may be nonhomologous blocs of DNA (ref. 14; G. F. Sprague and J. Rine, personal communication). The α mating type locus codes for at least two functions, denoted as *MAT*α*1* and *MAT*α*2* (ref. 15 and unpublished data), and the a mating type locus for at least one function, denoted as *MAT*a*1*. *MAT*α*2* and *MAT*a*1* are necessary for sporulation because *MAT*a/*mat*α*2* and *mat*a*1*/*MAT*α strains do not sporulate (16, 17).

A key observation on mating type interconversion that led to the cassette model is that strains with a defective *MAT*α locus (carrying a *mat*α*1* mutation, which leads to defective mating) can switch to a functional *MAT*a and subsequently to a functional *MAT*α (ref. 5; and D. Hawthorne, personal communication, cited in ref. 5). The efficient restoration of function to a defective α mating type locus in homothallic cells was explained by proposing that these cells contain an additional copy or copies of *MAT*α information and, by extension, of *MAT*a information. If silent *MAT*α and *MAT*a information exists in the yeast genome, then the mating type interconversion process should restore a functional mating type locus to strains carrying *mat*α*2* and *mat*a*1* defects. These predictions are confirmed here. We also present experiments on the manner in which the mating type locus controls mating type interconversion in a/α cells and in cells with a variant α mating type locus, *MAT*α-inc (18).

MATERIALS AND METHODS

Strains. *S. diastaticus* (source of *MAT*α-inc) was kindly provided by O. L. Rudakov (Institute of Microbiology, USSR Academy of Sciences, Moscow) and was observed to behave like the *S. diastaticus* strain of ref. 18. The *mat*α*2-2* mutation was obtained by selection for cells with a mating ability from a *mat*α*1-5* strain (15). *mat*α*2-2* is linked to *CRY1* and does not complement *mat*α*2-1* (which was originally called *ste73* in ref. 14).

Media and Genetic Methods. These are described in ref. 3.

Scoring of Mating Type and *HO*. Mating type was scored by a complementation assay (3) in which cells to be tested were mated with strains of known mating type and tested for prototrophy. For some experiments, mating type of individual cells was scored microscopically by the response of cells to the mating pheromone, α-factor, which causes arrest and morphological

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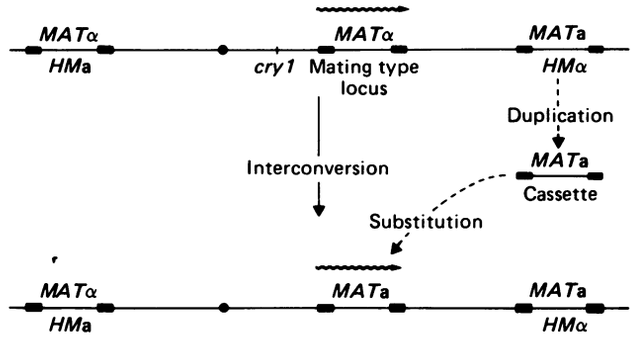

FIG. 1. Cassette model of mating type interconversion. Chromosome III, not drawn to physical or genetic scale, is shown for an α cell (top line) and for an **a** cell (bottom line), according to the cassette model. *MAT***a** and *MAT*α are blocs (cassettes) of regulatory information that determine cell types **a** and α, respectively. Wavy arrow indicates that the cassette at the mating type locus is expressed; those at *HM***a** and *HM*α are not. Interconversion from α to **a** occurs by substitution of a replica of *HM*α into the mating type locus. Rectangles indicate hypothetical sites involved in mating type interconversion (see text). The positions of *HM***a** and *HM*α are from ref. 6. The *cry1* locus is approximately 4 cM (centimorgans) from the mating type locus (7). Filled circle indicates the centromere.

change in **a** cells but not in α or **a**/α cells (19). α-Factor was supplied by a streak of α cells (strain 70) placed within 1 mm of the cells to be tested. *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. 3.

Budding Pattern. a, a/a, α, and α/α cells exhibit "medial budding," in which the first bud of a cell is near the junction of this cell and its parent (ref. 20; see Fig. 2). In contrast, **a**/α cells exhibit "polar budding," in which the first bud of a cell is opposite the junction of the cell and its parent. Differences in budding pattern (and timing of bud emergence; see ref. 11) can thus be used to follow changes in cell type from **a/a** and α/α to **a**/α due to mating type interconversion (11).

RESULTS

Interaction of *HO* and *mat*a*1*. The **a*** mutation (here called *mat***a***1*) is a recessive mutation of *MAT***a** that affects mating little if at all but that is unable to promote sporulation in *mat***a***1*/*MAT*α cells (17). *mat***a***1*/*MAT*α strains are also defective in turning off mating: unlike *MAT***a**/*MAT*α strains, which are nonmaters, *mat***a***1*/*MAT*α strains behave as α in all respects—mating ability (17), production of and response to mating type-specific pheromones, and budding pattern (unpublished observations). We have analyzed the *mat***a***1* mutation for two properties, its ability to turn off mating type interconversion and its ability to be healed as a result of mating type interconversion.

Cells carrying *mat***a***1* (strain 17-15) were mated to *MAT*α *HO* spores (strain X10-1B) to form *mat***a***1*/*MAT*α *ho*/*HO* zygotes. Activity of the mating type interconversion system was monitored by microscopic observation of the clones for (*i*) the presence of zygotes formed beween siblings, (*ii*) the presence of cells that respond to the pheromone α-factor, and (*iii*) a change in budding pattern (see *Materials and Methods*). Of six *mat***a***1*/*MAT*α *ho*/*HO* zygotes analyzed, four produced clones containing zygotes formed between siblings. In contrast, no zygotes were observed in progeny of *MAT***a**/*MAT*α *ho*/*HO* zygotes. The mating type interconversion system thus appears to be active in *mat***a***1*/*MAT*α *ho*/*HO* cells.

In order to determine whether the *mat***a***1* mutation can be healed (that is, switched to *MAT***a**), we have genetically marked the mating type loci in a *mat***a***1*/*MAT*α *ho*/*HO* strain with the closely linked mutation, *cryl-3*, which is approximately 4 cM (centimorgans) from the mating type locus (7). Progeny of *CRY1 mat***a***1*/*cryl-3 MAT*α *ho*/*HO* zygotes were observed microscopically as before and subsequently analyzed for the constitution of the mating type loci. As shown below, diploids of the form *CRY1 MAT***a**/*cryl-3 MAT*α were produced, indicating that *mat***a***1* can be healed. Eight *CRY1 mat***a***1*/*cryl-3 MAT*α *ho*/*HO* zygotes were allowed to grow in the presence of α-factor, with cells separated by micromanipulation after cell division. Seven clones exhibited signs of mating type interconversion by the third generation, producing zygotes between siblings, α-factor-sensitive cells, and polar budding subclones. A representative pedigree of one clone is shown in Fig. 2, in which the zygote (Z) and its second daughter (D2) gave rise only to cells with the polar budding pattern after the first cell division. The stability and budding pattern of these cells is consistent with their having become *MAT***a**/*MAT*α. This clone (XJ123-IP) and another stable subclone (XJ123-VP) derived from a similar pedigree were analyzed further. In both cases the colonies exhibited a nonmating response and sporulated efficiently, as expected for *MAT***a**/*MAT*α clones, and showed 2:2 segregation for cryptopleurine resistance and nutritional markers, indicating that they are diploid. From analysis shown in Table 1, the constitution of subclone IP is inferred to be *CRY1 MAT***a**/*cryl-3 MAT*α *ho*/*HO* and that of subclone VP, *cryl-3 MAT***a**/*CRY1 MAT*α. The coupling of *CRY* and *MAT***a** indicates that XJ123-IP cells have sustained a switch from *mat***a***1* to *MAT***a**.

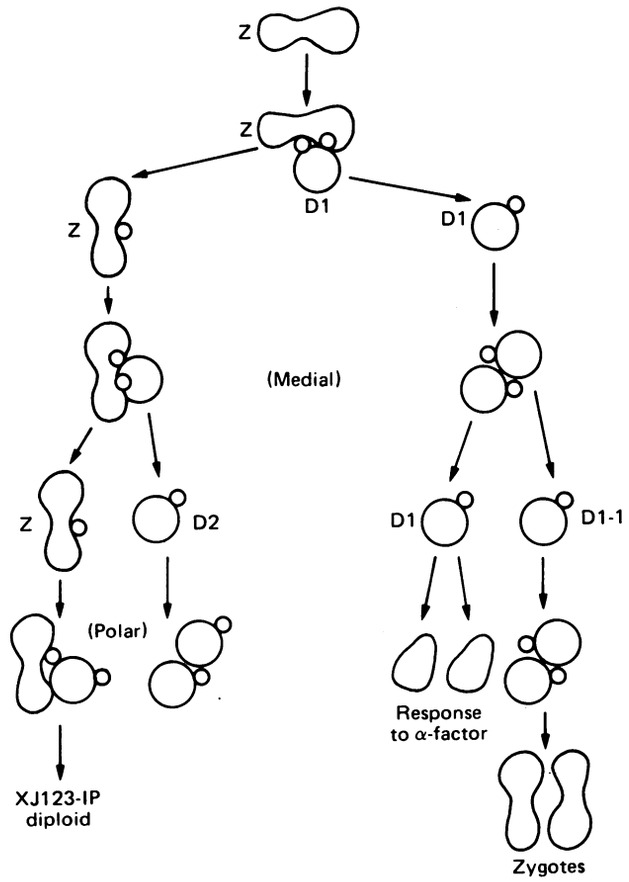

FIG. 2. Pedigree analysis of a *mat***a***1*/*MAT*α *ho*/*HO* zygote. The first few cell divisions of a *mat***a***1*/*MAT*α *ho*/*HO* diploid zygote in the presence of α-factor are shown. This pedigree demonstrates changes in cell type by three different assays: changes in budding pattern, response to α-factor, and mating type (seen by mating between siblings). Cell symbols: Z, the original zygote formed by cell-to-spore mating; D1, the first daughter of Z; D1-1, the first daughter of D1, etc. XJ123-IP is analyzed further in Table 2.

Table 1. Analysis of stable polar budding subclones from XJ123 (*mat***a***1/MAT*α *ho/HO*) and 73HZA (*MAT***a**/*mat*α*2 ho/HO*)

Subclone	Genotype of segregants*		
	*ho MAT***a**, *cry:CRY*	*ho MAT*α, *cry:CRY*	*HO*, *cry:CRY*
XJ123-IP	1:18†	20:1	19:21
	Interpretation: *CRY mat***a***1/cry MAT*α → *CRY MAT***a**/*cry MAT*α		
XJ123-VP	22:1	0:15	16:22
	Interpretation: *CRY mat***a***1/cry MAT*α → *CRY MAT*α/*cry MAT***a**		
73HZA-P	22:0	0:18‡	18:22
	Interpretation: *cry MAT***a**/*CRY mat*α*2* → *cry MAT***a**/*CRY MAT*α		

* Data are pooled from complete tetrads in each case. Numbers indicate the ratio of *cry* (cryptopleurine resistant) to *CRY* (cryptopleurine sensitive) segregants of each type. *HO* and mating type were scored as described in *Materials and Methods*.

† All of these *CRY* segregants promoted sporulation after mating with α *ho* strain 70.

‡ All of these *cry* segregants promoted sporulation after mating with **a** *ho* strain 227. XJ123 was formed by mating *cry1-3 MAT*α *HO* spores (strain 292) and *CRY1 mat***a***1 ho* cells (strain 17-15). 73HZA was formed by mating *cry1-3 MAT***a** *HO* spores (strain CRHO) and *CRY1 mat*α*2-1 ho sir1-1* cells (strain XJ104-25A).

All 18 **a** *ho CRY* segregants from XJ123-IP were able to support sporulation when crossed with *MAT*α *ho* strains, which confirms that these **a** cells have a functional *MAT***a** locus. The coupling of both *cry* and *MAT***a** in subclone VP indicates that both mating type loci have switched: *mat***a***1* to *MAT*α and *MAT*α to *MAT***a**. VP may thus have arisen via a *cry MAT*α/*CRY MAT*α or *cry MAT***a**/*CRY mat***a***1* intermediate (see also refs. 11, 21, and 22). In conclusion, the *mat***a***1* mutation is defective in turning off mating type interconversion in *mat***a***1*/*MAT*α diploids and can be healed to *MAT***a**.

Interaction of *HO* and *mat*α*2-1.* MacKay and Manney (14, 16) isolated strain VC73 as a mating-deficient mutation derived from an α strain and deduced that it has a mutation in *MAT*α (which has been confirmed by genetic analysis; refs. 15 and 23). The mutation in this strain, *mat*α*2-1*, also affects sporulation and turn off of mating: *MAT***a**/*mat*α*2-1* strains cannot be induced to sporulate and mate weakly as **a**. *mat*α*2-1* is clearly different from the other *mat*α mutations, *mat*α*1-2* and *mat*α*1-5*, shown to be healable by Hicks and Herskowitz (5), in that *mat*α*1* mutants are able to promote sporulation when mated with an **a** cell. The observation that *mat*α*2-1* complements *mat*α*1* mutants further indicates that *mat*α*2-1* is different from the other *mat* mutations (15). We have analyzed *mat*α*2-1* as we analyzed *mat***a***1* above to determine whether *mat*α*2-1* promotes turn off of mating type interconversion in *MAT***a**/*mat*α*2-1 ho/HO* cells and whether it can be healed by mating type interconversion.

Because *mat*α*2-1* leads to a defect in mating, we have used a *mat*α*2-1* strain that carries the *sir1-1* mutation, a recessive suppressor of the mating defect of *mat*α*2-1* (refs. 15, 23, and 24; unpublished data). It was thus possible to form zygotes between a *CRY1 mat*α*2-1 ho* strain and a *cry1-3 MAT***a** *HO* spore by cell-to-spore mating. Two zygotes, 73HZB and 73HZA, were analyzed. 73HZB grew into a colony in which a large fraction of cells were able to sporulate, producing asci of two different sizes. Segregation of drug resistance and nutritional markers indicated that 11 of the smaller asci were diploid and that 7 of the larger asci were tetraploid (15). Because *MAT***a**/*mat*α*2 ho/ho* cells do not sporulate, these results indicate that progeny of 73HZB acquired a functional α mating type locus and, hence, that *mat*α*2-1* is defective in turning off *HO*. These contentions were affirmed by microscopic and segregation analysis of zygote 73HZA.

The first cell divisions of 73HZA were similar to those shown in Fig. 2 except that two zygotes were formed between the progeny of D1 and D1-1. One of these zygotes was analyzed further and sporulated efficiently, giving segregation indicating that it was tetraploid (15). The zygote and its second daughter (D2) gave rise to stable polar budding subclones. The subclone derived from the zygote after its second division (73HZA-P) sporulated efficiently and gave segregation indicating that it was diploid. As before, the ability of progeny from 73HZA to sporulate indicates that these cells have a functional α mating type locus. This was confirmed directly by segregation analysis of 73HZA-P (Table 1), which shows that this clone is indeed composed of **a**/α diploids in which the functional *MAT*α locus is coupled to *CRY1*. 73HZA-P thus has sustained a change of the mating type locus from *mat*α*2-1* to *MAT*α. (The observation that α *ho* spores from 73HZA-P promote sporulation when mated with **a** *ho SIR* strain 227 indicates that *sir1-1* is not responsible for the *MAT*α*2*$^+$ phenotype of the segregants.) In conclusion, these results show that *mat*α*2-1* is defective in turning off mating type interconversion in *MAT***a**/*mat*α*2-1* cells and that the *mat*α*2-1* mutation can be healed.

Interaction of *HO* and *mat*α*1 mat*α*2.* We have recently constructed *mat*α*1 mat*α*2* double mutants by recombination between known *mat*α mutants and by mutation derived from *mat*α*1* mutants. As described elsewhere (ref. 15 and unpublished observations), the double mutants have the mating phenotype of **a** cells. Furthermore, the diploid formed upon mating with an α cell, *mat*α*1 mat*α*2*/*MAT*α, has the mating phenotype of an α cell and does not sporulate. Two double mutants, *mat*α*1-5 mat*α*2-1*, derived by recombination, and *mat*α*1-5 mat*α*2-2*, derived by mutation (see *Materials and Methods*), have been tested for their ability to turn off mating type interconversion and for their ability to be healed.

Zygotes were formed between *CRY MAT*α *HO* spores (strain X10-1B) and *cry mat*α*1-5 mat*α*2-2 ho* cells (strain XS2B-15A) by cell-to-spore mating and were observed microscopically. Five of six zygotes exhibited signs of activity of the mating type interconversion system within a few cell divisions—zygotes between siblings, response to α-factor, and polar budding clones. Thus, as expected for a zygote initially with the α phenotype, the mating type interconversion system is active.

A stable polar budding subclone from one zygote was analyzed further and shown to be a *cry MAT***a**/*CRY MAT*α diploid, indicating that *mat*α*1 mat*α*2* had switched to *MAT***a**. Sporulation of this diploid (which is heterozygous for *HO*) led to production of *cry* segregants that formed *MAT***a**/*MAT*α colonies. The *MAT***a** locus derived from *mat*α*1 mat*α*2* thus is readily switched to *MAT*α. In summary, the *mat*α*1-5 mat*α*2-2* double mutant behaves like *mat***a***1*—it is healable and, as expected, does not turn off mating type interconversion in *mat*α*1 mat*α*2*/*MAT*α diploids.

Table 2. Mating type interconversion in diploid strains containing *MAT*α-inc

ho/HO zygotes formed*	Clones with zygotes or responders to α-factor	Stable diploids *CRY MAT***a** / *cry MAT*α	Stable diploids *cry MAT***a** / *CRY MAT*α
XBA *cry* α-inc/*CRY* **a***1*	3/44†	10	0
XBB *cry* α-inc/*CRY* α*1* α*2*	0/37	10	0
XBC *cry* α$^+$/*CRY* **a***1*	47/48	1‡	2‡
XBD *cry* α$^+$/*CRY* α*1* α*2*	37/39	4‡	1‡

* Zygotes were formed by cell-to-cell or cell-to-spore mating between the following strains: XBA, XHB44-18d and 17-15; XBB, XHB44-18d and DC65; XBC, XHB6-14d and 17-15; XBD, XHB6-14d and DC65. Gene symbols are: *cry, cry1-3*; α-inc, *MAT*α-inc; α$^+$, *MAT*α; α*1*, *mat*α*1-5*; α*2*, *mat*α*2-1*; **a***1*, *mat***a***1*.

† Two responders were haploid buds from the zygote; the third burst.

‡ Two clones from XBC and one from XBD gave segregations indicating that they are mixtures of both type of diploid.

Studies on *MAT*α-inc. Takano *et al.* (18) characterized a variant of the α mating type locus, *MAT*α-inc ("inconvertible"), present in strains of *S. diastaticus*, which is insensitive to the mating type interconversion system: *MAT*α-inc *HO HM***a** *HM*α spores grow into clones that have α phenotype and that contain few (approximately 0.1%) **a**/α cells. The *MAT*α-inc locus thus controls mating type interconversion in some manner. Two kinds of explanations for its action are that *MAT*α-inc is defective in a function necessary for promoting mating type interconversion or that it is a defective substrate for the mating type interconversion system. To learn more about the nature of *MAT*α-inc, we have determined whether its insensitivity to switching can be overcome by the presence of another mating type locus or whether its action is *cis* or *trans* dominant. We have thus analyzed switching behavior of zygotes that are *ho/HO* and *mat***a***1/MAT*α-inc or *mat*α*1-5 mat*α*2-1/MAT*α-inc and, as controls, *mat***a***1/MAT*α or *mat*α*1-5 mat*α*2-1/MAT*α. These zygotes, which should initially have α phenotype, have been analyzed for the ability of progeny from the zygote to respond to α-factor or to form zygotes among siblings. Either response requires that the original *MAT*α locus be replaced with *MAT***a**, since only cells of the **a** phenotype respond to α-factor (19) or mate to α cells. The *MAT*α loci are marked with *cry1-3* in these zygotes, enabling us to determine which mating type loci have switched after formation of stable (presumably **a**/α) cells.

In support of earlier results, mating type interconversion occurs efficiently in *mat***a***1/MAT*α *ho/HO* zygotes (XBC; Table 2): 47 of 48 zygotes produced progeny within a few generations that responded to α-factor or that formed zygotes. Similar results were obtained with *mat*α*1-5 mat*α*2-1/MAT*α *ho/HO* zygotes (XBD), in which 37 of 39 produced progeny that responded to α-factor or that formed zygotes. These results show that *MAT*α was efficiently removed in progeny of these zygotes. Stable diploids from XBC and XBD were also grown into colonies and analyzed for the structure of their mating type loci; both *cry MAT***a**/*CRY MAT*α and *CRY MAT***a**/*cry MAT*α diploids were produced (Table 2). Subsequent analysis from XBD showed that *mat*α*1-5 mat*α*2-1* can switch to *MAT*α and thus is healed. In contrast, zygotes between *MAT*α-inc and *mat***a***1* (XBA) or *MAT*α-inc and *mat*α*1 mat*α*2* (XBB) did not produce any diploid progeny that responded to α-factor or that formed zygotes between siblings. These results indicate either that *MAT*α-inc cannot be switched to *MAT***a** or that mating type interconversion does not occur in these diploids. To determine whether mating type interconversion occurs, we grew 10 independent XBA and XBB zygotes into colonies after their third division. In all cases, the colony gave a nonmating response and contained a high fraction of cells able to sporulate. Dissection of asci from these 20 clones (20 asci from each) demonstrated that all were diploid and that in all cases the *MAT*α-inc allele was still present and coupled to *cry1* (data not shown). Fifty haploid *CRY* segregants with the **a** phenotype were confirmed to be *MAT***a** by their ability to promote sporulation after mating with α *ho* cells. These results demonstrate that mating type interconversion does occur in *mat***a***1/MAT*α-inc *ho/HO* and *mat*α*1 mat*α*2/MAT*α-inc diploids since *mat***a***1* and *mat*α*1 mat*α*2* are readily switched to *MAT***a**. The α mating type locus, however, does not switch in these cells, although both mating type loci are able to switch in *mat***a***1/MAT*α and *mat*α*1 mat*α*2/MAT*α *ho/HO* cells. Thus, the switching defect of *MAT*α-inc in the presence of *mat***a***1* or *mat*α*1 mat*α*2* mating type loci is *cis* dominant, suggesting that this mating type locus may not be an efficient substrate for the mating type interconversion machinery.

DISCUSSION

Mating type interconversion in homothallic *S. cerevisiae* is a controlled differentiation of cell type. During mitotic growth of homothallic cells, cells of one type give rise to cells of the other type in a specific pattern (ref. 3; unpublished data). Cell type is governed by the mating type locus, which is hypothesized to control expression of unlinked genes, and the mating type locus is itself controlled by action of the mating type interconversion system. The results described here bear both on the nature of the mating type interconversion event and on how this process is regulated.

A model for mating type interconversion must account for the following observations: (*i*) Interconversion between **a** and α involves a heritable change of the mating type locus. (*ii*) Because *MAT***a** and *MAT*α are codominant and recessive mutations exist for each, one mating type locus is not simply an inactive form of the other. (*iii*) As originally noted by Hicks and Herskowitz (5) and Hawthorne (cited in ref. 5) and extended here, the mating type interconversion process can heal mutations of *MAT***a** and *MAT*α.

From the observation that *mat*α*1* mutations are healed efficiently to *MAT*α, it was proposed that yeast cells contain an unexpressed copy (or copies) of *MAT*α and, by extension, of *MAT***a** which are the sources of the functional *MAT* information (4, 5). If this is true, then mutations within *MAT***a** and *MAT*α in addition to *mat*α*1* should also be efficiently healed. We have found this to be the case for the single *MAT***a** mutation, *mat***a***1*, that we have tested. Klar *et al.* (21) have recently made similar observations on an independently isolated mutation of *MAT***a**. We have also shown that the *mat*α*2-1* mutation and double mutations *mat*α*1-5 mat*α*2-1* and *mat*α*1-5 mat*α*2-2* are efficiently healed to *MAT*α. Thus, all mutations within *MAT***a** and *MAT*α analyzed so far are healed. Oshima and colleagues (9, 25), from analysis of genes required for mating type interconversion, proposed that *HM***a** and *HM*α are controlling elements whose association with a

site at the mating type locus leads to an α or an **a** cell, respectively. Our observations on healing of *MAT* mutations lead us to favor a specific version of this hypothesis, in which *HM***a** is equivalent to *MAT*α information itself and *HM*α is equivalent to *MAT***a** information itself. Additional evidence for this equivalence comes from analysis of mutations (such as *sir1*) that allow expression of *HM***a** and *HM*α *in situ* (unpublished data) and from mutations in *HM***a** and *HM*α (unpublished data; A. Klar, personal communication). Mating type interconversion is thus proposed to occur by replacement of the information at the mating type locus by a copy of the *MAT***a** or *MAT*α information from *HM*α or *HM***a**. Other models (discussed in refs. 4 and 5), notably a "flip-flop" model, do not readily account for healing of *MAT* mutations. The observation that *mat*α*1* and *mat*α*2* mutations can be healed simultaneously is consistent with the view that *MAT*α is a single cassette rather than being composed of separate "mini-cassettes" for each complementation group.

The mating type locus controls mating type interconversion in at least two respects: (*i*) mating type interconversion is turned off in **a**/α diploids (11–13); and (*ii*) the switching process has directionality—**a** cells switch preferentially to α and α cells switch to **a** (unpublished data; see below). Turn off of mating type interconversion in **a**/α diploids is clearly due to the constitution of the mating type loci and not to diploidy *per se* since mating type switches occur in *MAT***a**/*MAT***a** and *MAT*α/*MAT*α diploids (11–13) and, as shown here, in *MAT***a**/*mat*α*2*, *MAT***a**/*mat*α*1 mat*α*2*, and *mat***a***1*/*MAT*α diploids. The same functions of the mating type locus known to control sporulation and mating are thus responsible for turn off of mating type interconversion. This finding makes teleological sense according to the view that the role of mating type interconversion is to produce diploids capable of sporulation, rather than to produce cells with ever-increasing ploidy.

How do *MAT***a***1* and *MAT*α*2* regulate mating type interconversion? MacKay and Manney (14) have suggested that the mating type loci code for regulators that control expression of other genes necessary for mating and sporulation. Together these regulators (designated pMAT**a**1 and pMATα2) may also induce a negative regulator of the mating type interconversion machinery. Another possibility is that pMAT**a**1 and pMATα2 play a more direct inhibitory role. For example, pMAT**a**1 might inhibit transposition of the *MAT***a** cassette from *HM*α, and pMATα2 might inhibit transposition of the *MAT*α cassette from *HM***a**. Mating type interconversion thus would not occur in **a**/α diploids. This model, though untested, has the appealing feature that it can account for the directionality of mating type interconversion. Homothallic cells that are competent to switch, having experienced at least one cell cycle, change to the other mating type 73% of the time (unpublished data). Competent cells thus do not insert a *MAT***a** or *MAT*α cassette at random, but must have a mechanism for sensing which information is at the mating type locus. For example, functions coded by the mating type loci might determine which cassette is selected for insertion into the mating type locus. As noted above, pMAT**a**1 function coded by a *MAT***a** and pMATα2 coded by a *MAT*α might inhibit replacement of these mating type loci by homologous cassettes.

MATα-inc is a novel allele of the mating type locus in that it is stable even in the presence of *HO*, *HM***a**, and *HM*α (19). Another interesting property of this variant is that *MAT*α-inc cells that have switched (at low frequency) to *MAT***a** subsequently interconvert between *MAT***a** and *MAT*α at normal frequency (18). This behavior could be explained if *MAT*α-inc were defective in a function coded by the mating type locus necessary to promote its own removal, in which case the mutation would be healable in the same manner as other mutations within *MAT*α. *MAT*α-inc, however, does not appear to be deficient in such a function, since it is stable in cells in which mating type interconversion occurs—in *mat***a***1*/*MAT*α-inc and *mat*α*1 mat*α*2*/*MAT*α-inc diploids and, as shown by Takano and Arima (26), in *MAT*α/*MAT*α-inc diploids formed by protoplast fusion. Possible explanations for the behavior of *MAT*α-inc are that it is a cassette that has been misinserted (18, 26), analogous to a λ prophage integrated at a secondary insertion site (27), or that *MAT*α-inc is altered in a structural region necessary for interconversion (see Fig. 1). In the latter case, the "healing" of *MAT*α-inc might occur by excision of the defective recognition region from the mating type locus during the interconversion process or by recombination of the defective site with other sites in the genome.

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