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

(Jeffrey N. Strathern, Lindley C. Blair, and Ira Herskowitz)

Communicated by Franklin W. Stahl, April 12, 1979

ABSTRACT Homothallic yeasts switch cell types (mating types a and α) at high frequency by changing the alleles of the mating type locus, MATa and MATα. We have proposed in the cassette model that yeast cells contain silent MATa and MATα blocs ("cassettes"), copies of which can be exchanged through the mating type locus for the resident information. The existence of silent cassettes was originally proposed to explain efficient switching of a defective MATa locus (matα) to a functional MATα 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 matα mutations studied earlier: a defective MATα (matα1) switches readily to MATa and various matα loci switch readily to MATα. These observations satisfy the prediction of the cassette model that all mutations within MATa and MATα 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 MATα, MATα-inc (Takano, I., Kusumi, T. & Oshima, Y. (1973) Mol. Gen. Genet. 126, 19-28) that is insensitive to switching. Our observation that MATα-inc acts in cis suggests that it may be altered in a site concerned with excision of MATα-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, MATa, determining the a cell type, and MATα, 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cell division in strains carrying the HO allele and appropriate accessory genes (homoathallic strains) (refs. 1-3 and unpublished data).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 MATa and MATα 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: HMa, on the left arm of chromosome III, and HMc, on the right arm of chromosome III (Fig. 1). Because HM a is required for switching from a to α and HMc is required for switching from α to a, we have proposed that HM a is the silent MATα cassette and that HMc is the silent MATa cassette.

The mating type locus controls mating, sporulation, and activity of the mating type interconversion system. MATa (and MATa/MATα) and MATα (and MATα/MATa) cells mate and do not sporulate, whereas MATa/MATα cells sporulate and do not mate. The mating type locus also controls mating type interconversion in that interconversion occurs in MATa and MATα HO haploids and in MATα/MMATa and MATα/MA α diploids carrying at least one HO gene, but does not occur in MATa/MAα diploids (10-13). Although the structures of the MAT alleles are not known, genetic experiments suggest that MATα and MATα may be nonhomologous blocks 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 MATα1 and MATα2 (ref. 15 and unpublished data), and the a mating type locus for at least one function, denoted as MATal. MATα2 and MATal are necessary for sporulation because MATa/mata2 and mata1/MATα 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 MATα locus (carrying a matα1 mutation, which leads to defective mating) can switch to a functional MATα and subsequently to a functional MATα (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 MATα information and, by extension, of MATα information. If silent MATα and MATα information exists in the yeast genome, then the mating type interconversion process should restore a functional mating type locus to strains carrying mata2 and mata1 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, MATα-inc (18).

MATERIALS AND METHODS

Strains. S. diastaticus (source of MATα-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 mata2-2 mutation was obtained by selection for cells with a mating ability from a mata1-3 strain (15). mata2-2 is linked to CRYI and does not complement mata2-1 (which was originally called ste73 in ref. 14).

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

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

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

1 Present address: Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724.
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 mata1. The a* mutation (here called mata1) is a recessive mutation of MATa that affects mating little if at all but that is unable to promote sporulation in mata1/MATα cells (17). mata1/MATα strains are also defective in turning off mating; unlike MATα/MATα strains, which are nonmaters, mata1/MATα 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 mata1 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 mata1 (strain 17-15) were mated to MATα HO spores (strain X10-1B) to form mata1/MATα 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 between 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 mata1/MATα ho/HO zygotes analyzed, four produced clones containing zygotes formed between siblings. In contrast, no zygotes were observed in progeny of Mata1/MATα ho/HO zygotes. The mating type interconversion system thus appears to be active in mata1/MATα ho/HO cells.

In order to determine whether the mata1 mutation can be healed (that is, switched to MATa), we have genetically marked the mating type loci in a mata1/MATα ho/HO strain with the closely linked mutation, cry1-3, which is approximately 4 cM (centimorgans) from the mating type locus (7). Progeny of CRY1 mata1/cry1-3 MATα 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 MATα/cry1-3 MATα were produced, indicating that mata1 can be healed. Eight CRY1 mata1/cry1-3 MATα ho/HO zygotes were allowed to grow in the presence of α-factor, with cells separated by micro-manipulation 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 MATα/MATα. 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 non-mating response and sporulated efficiently, as expected for MATα/MATα clones, and showed 2:2 segregation for cry1-3 MATα/CRY1 MATα. The coupling of CRY and MATα indicates that XJ123-IP cells have sustained a switch from mata1 to MATα.

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. MATα and MATα are blocks (cassettes) of regulatory information that determine cell types α and α, respectively. Wavy arrow indicates the cassette at the mating type locus is expressed; those at HMa and HMa are not. Interconversion from α to α occurs by substitution of a replica of HMa into the mating type locus. Rectangles indicate hypothetical sites involved in mating type interconversion (see text). The positions of HMa and HMa are from ref. 6. The cry1 locus is approximately 4 cM (centimorgans) from the mating type locus (7). Filled circle indicates the centromere.

FIG. 2. Pedigree analysis of mata1/MATα ho/HO zygote. The first few cell divisions of a mata1/MATα 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-spor 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 (matα/MATα ho/ho) and 73HZA (MATα/mata2 ho/ho)

<table>
<thead>
<tr>
<th>Subclone</th>
<th>Genotype of segregants*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ho MATα, cry:CRY</td>
</tr>
<tr>
<td>XJ123-IP</td>
<td>1:18&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>XJ123-VP</td>
<td>22:1</td>
</tr>
<tr>
<td>73HZA-P</td>
<td>22:0</td>
</tr>
</tbody>
</table>

* 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.

<sup>1</sup> All of these CRY segregants promoted sporulation after mating with a ho strain 70.

<sup>2</sup> All of these cry segregants promoted sporulation after mating with a ho strain 227. XJ123 was formed by mating cry1-3 MATα ho/ho spores (strain 222) and CRY1 mata1 ho cells (strain 17-10). 73HZA was formed by mating cry1-3 MATα ho spores (strain CRHO) and CRY1 mata2-1 ho sir1-1 cells (strain XJ104-25A).

All 18 a ho CRY segregants from XJ123-IP were able to support sporulation when crossed with MATα ho strains, which confirms that these a cells have a functional MATα locus. The coupling of both cry and MATα in subclone VP indicates that both mating type loci have switched: mata1 to MATα and MATα to MATα. VP may thus have arisen via a cry MATα/CRY MATα or cry MATα/CRY mata1 intermediate (see also refs. 11, 21, and 22). In conclusion, the mata1 mutation is defective in turning off mating type interconversion in mata1/MATα diploids and can be healed to MATα.

Interaction of HO and mata2-1. 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 MATα (which has been confirmed by genetic analysis; refs. 15 and 23). The mutation in this strain, mata2-1, also affects sporulation and turn off of mating. MATα/mata2-1 cells cannot be induced to sporulate and mate weakly as a mata2-1 is clearly different from the other mata1 mutations, and mata2-1 and mata1-5, shown to be healable by Hicks and Herskowitz (5), in that mata1 mutants are able to promote sporulation when mated with an a cell. The observation that mata2-1 complements mata1 mutants further indicates that mata2-1 is different from the other mat mutations (15). We have analyzed mata2-1 as we analyzed mata1 above to determine whether mata2-1 promotes turn off of mating type interconversion in MATα/mata2-1 ho/ho cells and whether it can be healed by mating type interconversion.

Because mata2-1 leads to a defect in mating, we have used a mata2-1 strain that carries the sir1-1 mutation, a recessive suppressor of the mating defect of mata2-1 (refs. 15, 23, and 24; unpublished data). It was thus possible to form tetrads between a CRY mata2-1 ho strain and a cry1-3 MATα ho spore by cell-to-spore mating. Two zygotes, 73HZA and 73HZB, 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 7 of the larger asci were tetraploid (15). Because MATα/ mata2 ho/ho cells do not sporulate, these results indicate that progeny of 73HZB acquired a functional α mating type locus and, hence, that mata2-1 is defective in turning off HO. These contentions were affirmed by microscopic and segregation analysis of zygote 73HZA.

The first cell division of 73HZA were similar to those shown in Fig. 2 except that two zygotes were formed between the progeny of D1 and D1. 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 MATα locus is coupled to CRY1. 73HZA-P thus has sustained a change of the mating type locus from mata2-1 to MATα. (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 MATα2+ phenotype of the segregants.) In conclusion, these results show that mata2-1 is defective in turning off mating type interconversion in MATα/mata2-1 cells and that the mata2-1 mutation can be healed.

Interaction of HO and mata1 mata2. We have recently constructed mata1 mata2 double mutants by recombination between known mata mutants and by mutation derived from mata1 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 a cell, mata1 mata2/MATα, has the mating phenotype of an a cell and does not sporulate. Two double mutants, mata1-5 mata2-1, derived by recombination, and mata1-5 mata2-2, 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 MATα ho spores (strain X10-1B) and cry mata1-5 mata2-2 ho cells (strain X52B-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 MATα/a CRY MATα diploid, indicating that mata1 mata2 had switched to MATα. Sporulation of this diploid (which is heterozygous for HO) led to production of cry segregants that formed MATα/MATα colonies. The MATα locus derived from mata1 mata2 thus is readily switched to MATα. In summary, the mata1-5 mata2-2 double mutant behaves like mata1—i.e., it is healable and, as expected, does not turn off mating type interconversion in mata1 mata2/MATα diploids.
Table 2. Mating type interconversion in diploid strains containing MATα-inc

<table>
<thead>
<tr>
<th>ho/HO zygotes formed*</th>
<th>Clones with zygotes or responders to α-factor</th>
<th>Stable diploids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRY MATα</td>
<td>cry MATα</td>
</tr>
<tr>
<td>XBA cry α-inc/CRY a1</td>
<td>3/44†</td>
<td>10</td>
</tr>
<tr>
<td>XBB cry α-inc/CRY a1 a2</td>
<td>0/37</td>
<td>10</td>
</tr>
<tr>
<td>XBC cry α&quot;/CRY a1</td>
<td>47/48</td>
<td>1†</td>
</tr>
<tr>
<td>XBD cry α&quot;/CRY a1 a2</td>
<td>37/39</td>
<td>4†</td>
</tr>
</tbody>
</table>

* 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 DO65; XBC, XHB6-14d and 17-15; XBD, XHB6-14d and DO65. Gene symbols are: cry, cry1-3; α-inc, MATα-inc; α" MATa; α1, matα-1; α2, matα-2; α1, matα.
† Two responders were haploid buds from the zygote; the third burst.

In all cases the MATα-inc allele was still present and coupled to cry1 (data not shown). Fifty haploid CRY segregants with the α phenotype were confirmed to be MATα by their ability to promote sporulation after mating with α ho cells. These results demonstrate that mating type interconversion does occur in matα1/MATα-inc ho/HO and matα1 matα2/MATα-inc diploids since matα1 and matα1 matα2 are readily switched to MATα. The α mating type locus, however, does not switch in these cells, although both mating type loci are able to switch in matα1/MATα and matα1 matα2/MATα ho/HO cells. Thus, the switching defect of MATα-inc in the presence of matα1 or matα1 matα2 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 α and α involves a heritable change of the mating type locus. (ii) Because MATα and MATα 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 MATα and MATα.

From the observation that matα1 mutations are healed efficiently to MATα, it was proposed that yeast cells contain an unexpressed copy (or copies) of MATα and, by extension, of MATα which are the sources of the functional MAT information (4, 5). If this is true, then mutations within MATα and MATα in addition to matα1 should also be efficiently healed. We have found this to be the case for the single MATα mutation, matα1, that we have tested. Klar et al. (21) have recently made similar observations on an independently isolated mutation of MATα. We have also shown that the matα2-1 mutation and double mutations matα1-5 matα2-1 and matα1-5 matα2-2 are efficiently healed to MATα. Thus, all mutations within MATα and MATα analyzed so far are healed. Oshima and colleagues (9, 25), from analysis of genes required for mating type interconversion, proposed that HMα and HMα 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 MATα information itself and HM α is equivalent to MATα information itself. Additional evidence for this equivalence comes from analysis of mutations (such as sir2) 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 MATα or MATα 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 mata1 and mata2 mutations can be healed simultaneously is consistent with the view that MATα 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 (11-15); 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 MATα/MATA and MATα/ MATα diploids (11-13) and, as shown here, in mata1/mata2, Mata1/mata1 mata2, and mata1/MATα 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 MATα1 and MATα2 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 pMATα1 and pMATα2) may also induce a negative regulator of the mating type interconversion machinery. Another possibility is that pMATα1 and pMATα2 play a more direct inhibitory role. For example, pMATα1 might inhibit transposition of the MATα cassette from HM α, and pMATα2 might inhibit transposition of the MATα 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 MATα or MATα 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, pMATα1 function coded by a MATα and pMATα2 coded by a MATα 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 MATα-inc cells that have switched (at low frequency) to MATα subsequently interconvert between MATα and MATα at normal frequency (18). This behavior could be explained if MATα-inc 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 MATα. MATα-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 mata1/ MATα-inc and mata1/mata2/MATα-inc diploids and, as shown by Takano and Arima (26), in MATα/MATα-inc diploids formed by protoplast fusion. Possible explanations for the behavior of MATα-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 MATα-inc is altered in a structural region necessary for interconversion (see Fig. 1). In the latter case, the “healing” of MATα-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.

We thank Jasper Rine, Flora Banuett, and Peter Kushner for comments on the manuscript and O. L. Radakof for S. dasaturatus. This work has been supported by a Research Career Development Award (AI-00163) and Research Grant (AI-13462) from the National Institutes of Health to I.H. and National Institutes of Health Molecular Biology Training Grant (P. von Hippel, Director).