GENE CONVERSION OF THE MATING-TYPE LOCUS IN *SACCHAROMYCES CEREVISIAE*[1]

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ABSTRACT

Tetrad analysis of *MAT***a**/*MAT*α diploids of *Saccharomyces cerevisiae* generally yields 2 *MAT***a**:2*MAT*α meiotic products. About 1 to 1.8% of the tetrads yield aberrant segregations for this marker. Described here are experiments that determine whether the aberrant meiotic segregations at the mating-type locus are ascribable to gene conversions or to *MAT* switches, that is, to mating-type interconversions. Diploid strains incapable of switching *MAT***a** to *MAT*α, or the converse, nevertheless display changes of *MAT***a** to *MAT*α, or the reverse. These events must be attributed to gene conversion. Further, we suggest that *MAT***a** and *MAT*α alleles may represent nonhomologous sequences of DNA since they fail to display postmeiotic segregations.

GENE conversions resulting in aberrant tetrad segregations, principally of the *3A*:*1a* and *1A*:*3a* types rather than *2A*:*2a*, constitute apparent exceptions to Mendel's first law. These events provide a primary and critical data base for analyzing the molecular mechanisms of genetic recombination. Currently, genetic evidence favors the view that conversion events are generated by a DNA excision and resynthesis process, which in effect changes the heteroduplex regions formed by the association of polynucleotide chains from homologous chromatids into homoduplexes (FOGEL and MORTIMER 1970).

In the budding yeast, *Saccharomyces cerevisiae*, the frequency of gene conversion varies from one locus to another. The mating-type locus (*MAT*) displays "apparent" gene conversions with a frequency of about 1 to 1.8%, whereas *SUP6*, an ochre suppressor, is converted in 15 to 20% of tetrads (ROMAN 1963; TAKAHASHI 1964; FOGEL, HURST and MORTIMER 1971; HURST, FOGEL and MORTIMER 1972).

Mating type in this yeast is controlled by two alleles of *MAT*, *i.e.*, *MAT***a** and *MAT*α, located about 25 centimorgans from the centromere on the right arm of chromosome *III* (MORTIMER and HAWTHORNE 1969). In heterothallic (*ho*) strains, the *MAT* alleles are relatively stable during vegetative cell division, but rare switches from *MAT***a** to *MAT*α and the reverse occur at a frequency of about 10^{-6} (HAWTHORNE 1963b; RABIN 1970). In contrast, homothallic (*HO*) strains

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effect switches in either direction at the mating type locus as often as every cell generation (WINGE and ROBERTS 1949; HAWTHORNE 1963a; OSHIMA and TAKANO 1971; HICKS and HERSKOWITZ 1976). The switched loci are heritable and the switching is controlled by three loci: *HO*, *HMα* (alternate allele *hmα*) and *HMa* (alternate allele *hma*). Strains of the genotype *HO HMα hma* switch *MATα* to *MAT***a**, but not back, and correspondingly *HO hmα HMa* strains switch *MAT***a** to *MATα*, but not the reverse. Hence, *HMa* and *HMα* control the direction of switching. The *HO HMα HMa* strains change *MATα* and *MAT***a** reversibly (HAWTHORNE 1963a; OSHIMA and TAKANO 1971; NAUMOV and TOLSTORUKOV 1973; HARASHIMA, NOGI and OSHIMA 1974; KLAR and FOGEL 1977). Standard heterothallic laboratory strains carry the genotype *ho HMα HMa*. Hence, they switch both *MAT* alleles reversibly although at very low rates. The *HMa* and *HMα* loci are loosely linked to *MAT*, and the map position of *HO* is unknown at present (HARASHIMA and OSHIMA 1977; KLAR and FOGEL 1977).

Whether the occasional aberrant meiotic segregations at *MAT* are attributable to *ho*-mediated switches or are *bona fide* gene conversions is not known. However, a choice between these alternatives can be approached by constructing strains that are unable to switch *MAT***a** to *MATα* and then analyzing unselected tetrads to determine whether *MAT***a** to *MATα* changes occur. If such events are observed, they are most probably ascribable to gene conversions. This study reports data indicating that authentic gene conversions occur at the mating-type locus.

MATERIALS AND METHODS

Strains: All strains used in the present study are described in Table 1.

Techniques: All media for growth and sporulation and techniques for micromanipulation and tetrad analysis have been described (MORTIMER and HAWTHORNE 1969). Hybrids were constructed by cell-to-spore matings. These matings were initiated by placing cells and spores in direct contact with each other on dissection agar. Subsequently, the zygotes were isolated by micromanipulation. The hybrid nature of the zygotic clones was verified by appropriate complementation responses for the markers carried by the parent strains.

TABLE 1

List of strains used

Strain #	Genotype*
J3	*MATα, HO, hmα, HMa, cry1, arg4–1, leu2, gal1, MAL*
J4	*MAT***a***/MATα, HO/HO, hmα/hmα, HMa/HMa, thr4/thr4, his4/his4, leu2/leu2, lys2/lys2, MAL/MAL*
J20	*MAT***a***, HO, HMα, hma, cry1, his4, his2, leu2, lys2, metx, gal1, Mal*
J25	*MAT***a***/MATα, HO/HO, HMα/HMα, hma/hma, thr4/thr4, trp1/trp1, his4/his4, his2/his2, leu2/leu2, gal1/gal1*

* The terminology and genetic symbols are those proposed by the Nomenclature Committee for Yeast Genetics (PLISCHKE *et al.* 1976). However, the old terminology for homothallism genes is retained (HARASHIMA, NOGI and OSHIMA 1974).

RESULTS

The MAT**a** *allele converts to the* MATα *allele*: A hybrid between J20 and J25 (*MAT***a**/*MAT*α *HO/HO HM*α/*HM*α *hma/hma* was constructed by cell-to-spore mating as described in MATERIALS AND METHODS. The hybrid was sporulated and unselected tetrads were analyzed. Nearly all of the tetrads (369/377) produced 2 nonmating: 2 *MAT***a** segregants, as expected. The nonmating segregants are capable of sporulation, hence are judged to be *MAT***a**/*MAT*α diploids. In this communication, such segregants will be referred to as *MAT***a**/*MAT*α. This result is obtained because each ascus contains 2 *MAT*α:2 *MAT***a** spores, and as the *MAT*α spores grow and undergo mitotic divisions, *HO*-mediated switches from *MAT*α to *MAT***a** occur, most often as early as the four cell stage. The immediately adjacent sister cells carrying the opposite mating type fuse to establish "diploidized" *MAT***a**/*MAT*α clones (HAWTHORNE 1963a; TAKANO and OSHIMA 1970; HICKS and HERSKOWITZ 1976). The*MAT***a**/*MAT*α diploids do not exhibit further switching. The two remaining *MAT***a** spores grow to haploid clones since they do not seem to exhibit a mating-type switch in the absence of *HM*a.

As indicated in Table 2, eight tetrads yielded a segregation pattern other than 2 diploid:2 haploid *MAT***a**. Two tetrads produced 3 *MAT***a**/*MAT*α:1 *MAT***a** and six tetrads produced 1 *MAT***a**/*MAT*α:3 *MAT***a** segregants. The segregation pattern, 3 *MAT***a**/*MAT*α:1 *MAT***a** is presumed to originate from an ascus containing 3 *MAT*α:1 *MAT***a** spores since only *MAT*α progenies can switch to establish diploid clones. Because the J20 × J25 hybrid is homozygous for *hma* and therefore cannot switch *MAT***a** to *MAT*α, the additional *MAT*α spore is interpreted to result from a conventional gene conversion of *MAT***a** to *MAT*α. The three diploidized segregants from one of these tetrads were sporulated and subjected to tetrad analysis. They all yielded 2 *MAT***a**/*MAT*α: 2 *MAT***a** segregants among ten tetrads analyzed, suggesting that the additional *MAT***a**/*MAT*α segregant in the aberrant tetrads did not arise as a consequence of a mutation of *hma* to *HM*a. The six tetrads containing 1 *MAT***a**/*MAT*α:3 *MAT***a** segregants might result either from gene conversions or switches of *MAT*α to *MAT***a**. The extra *MAT***a** segregant clones do not exhibit detectable sporulation; we conclude that these clones are comprised of only *MAT***a** cells.

The MATα *allele converts to the* MAT**a** *allele*: A hybrid between J3 × J4 (*MAT***a**/*MAT*α *HO/HO hm*α/*hm*α *HM*a/*HM*a) was sporulated and unselected

TABLE 2

Aberrant segregations among 377 unselected tetrads from the hybrid J20 × J25
(MAT**a**/MATα HO/HO HMα/HMα hma/hma)

# Asci	Type	*cry1* and *thr4* combination	Conversion
2	3 *MAT***a**/*MAT*α: 1 *MAT***a**	parental	*MAT***a** → *MAT*α
3	1 *MAT***a**/*MAT*α: 3 *MAT***a**	parental	*MAT*α → *MAT***a**
3	1 *MAT***a**/*MAT*α: 3 *MAT***a**	recombined	*MAT*α → *MAT***a**

tetrads were analyzed. Most of the tetrads (407/412) yielded the expected 2 *MAT***a**/*MAT*α:2 *MAT*α segregants. The result is obtained because both *MAT***a** segregants diploidize by the occurrence of frequent early switches within their progenies and subsequent matings between the resultant cells of opposite mating type. The *MAT*α spores fail to switch their offspring since the meiotic products from J3 × J4 lack *HM*α. Hence, the *MAT*α spores grow to produce stable haploid *MAT*α clones. As shown in Table 3, five tetrads displayed an aberrant segregation pattern. A single tetrad yielded 3 *MAT***a**/*MAT*α:1 *MAT*α segregation. Because in this strain only *MAT***a** spores can produce diploid *MAT***a**/*MAT*α clones by switching and mating, the above tetrad is presumed to originate from an ascus containing 3 *MAT***a**:1 *MAT*α spores. Apparently one of the *MAT*α alleles had changed to *MAT***a**. Since switching of *MAT*α to *MAT***a** cannot occur in this hybrid (which lacks *HM*α), the additional *MAT***a** spore is interpreted to result from a conventional gene conversion of *MAT*α to *MAT***a**. The three "diploidized" segregants from this tetrad were sporulated and subjected to tetrad analysis. They all yielded 2 *MAT***a**/*MAT*α:2 *MAT*α segregants, suggesting that the additional *MAT***a**/*MAT*α segregant in the original aberrant tetrad did not arise as a consequence of a mutation (or change) of *hm*α to *HM*α. The four remaining tetrads yielded aberrant segregations of the type 1 *MAT***a**/*MAT*α:3 *MAT*α (Table 3). We are uncertain whether these additional *MAT*α segregants arose by gene conversion or switches. Since the extra *MAT*α segregant clones do not exhibit detectable sporulation, we conclude that these clones are comprised of only *MAT*α cells.

DISCUSSION

These experiments were aimed at establishing whether gene conversion of *MAT* occurs in yeast. TAKAHASHI (1964) reported UV-induced enhancement of meiotic gene conversion frequency at *MAT*. However, the aberrant segregations that he reported for *MAT* cannot be apportioned between gene conversions and switches. The results presented here allow us to conclude that *MAT* alleles undergo *bona fide* gene conversion in about 1.65% of unselected tetrads. The results presented here provide no information as to whether aberrant segregations at *MAT* can also occur as a consequence of switches during meiosis and sporulation in strains where the direction of switching is not controlled. Also, it should be noted that the results reported here pertain to strains homozygous for *HO*; whether similar results are obtained for *ho* strains remains to be tested.

TABLE 3

Aberrant segregations among 412 unselected tetrads from the hybrid J3 × J4
(MAT**a**/MATα HO/HO hmα/hmα HMa/HMa)

# Asci	Type	*cry1* and *thr4* combination	Conversion
1	3 *MAT***a**/*MAT*α: 1 *MAT*α	parental	*MAT*α → *MAT***a**
1	1 *MAT***a**/*MAT*α: 3 *MAT*α	parental	*MAT***a** → *MAT*α
3	1 *MAT***a**/*MAT*α: 3 *MAT*α	recombined	*MAT***a** → *MAT*α

TAKANO, KUSUMI and OSHIMA (1973) reported a naturally occurring variant of *MAT*α, *MAT*α^{inc} (*inc*, for inconvertible), which cannot be switched by *HO* at high frequency. Only about 1/1000 cells switch to produce *MAT***a**. When the *MAT***a** derivative is switched, only normal *MAT*α is produced and *MAT*α^{inc} is not recovered. Hence, the *MAT*α^{inc} allele is "healed" by switching. TAKANO, KUSUMI and OSHIMA (1973) observed that *MAT*α^{inc}/*MAT***a***HO*/*HO HM*α/*HM*α *HMa*/*HM*α strain yielded 2 *MAT***a**/*MAT*α:2 *MAT*α segregation pattern in 325 among 330 tetrads studied. Since the *MAT*α segregants do not diploidize, they presumably carry the *MAT*α^{inc} allele. They found five "aberrant" tetrads with 1 *MAT***a**/*MAT*α:3 *MAT*α segregants. We interpret this result by supposing that the additional *MAT*α segregants must carry the *MAT*α^{inc} allele; otherwise it would switch and produce diploidized (*MAT***a**/*MAT*α) clones. We believe that these aberrant tetrads provide evidence concerning the fidelity of gene conversion at *MAT*, *i.e.*, the converted allele derives from information residing at a corresponding site in the interacting homologue (FOGEL and MORTIMER 1970).

Genetic evidence favors the view that meiotic conversion events are produced by correcting the heteroduplex regions formed by polynucleotide chains from homologous chromatids. It is noteworthy that no postmeiotic segregations (PMS) at *MAT* were observed among about 23,000 tetrads analyzed (FOGEL, MORTIMER, LUSNAK and TOVARES, unpublished observations); such events would be detected as diploid nonmater or dual mater ascosporal clones. A PMS event, ordinarily detected as a sectored ascosporal clones (ESPOSITO 1971) is taken to represent a heteroduplex DNA segment that failed to undergo excision and resynthesis and was resolved instead by the ensuing replication cycle. Virtually all the markers studied in yeast, with the exception of *MAT*, undergo PMS. Recent observations by FOGEL, MORTIMER and LUSNAK (unpublished data) show that *his4* deletion/+ heterozygotes fail to yield PMS events in 2100 unselected tetrads that contain 89 conversions. Two *his4* deletions were analyzed, and both convert with a frequency of 3.5 to 5%. Other alleles of this locus readily yield PMS events. These data on the *his4* deletions suggest an explanation for the lack of PMS events at *MAT*. One can envision *MAT***a** and *MAT*α as nonhomologous DNA sequences. Accordingly, any heteroduplex formed between nonsister DNA strands encompassing this region should generate a distortion or mispairing that is sufficient to guarantee the occurrence of the excision-repair process. At best, the absence of PMS at *MAT* is only suggestive of the DNA nonhomology between the *MAT***a** and the *MAT*α loci; the final answer must await physical studies of the *MAT* region. However, consistent with this notion is the observation that reciprocal recombination between *MAT***a** and *MAT*α loci has not been reported, while mutant alleles of *MAT*α have been observed to recombine (STRATHERN 1977).

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