Two Temperature-sensitive Mutants of *Saccharomyces cerevisiae* with Altered Expression of Mating-Type Functions

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ABSTRACT Two mutants of *Saccharomyces cerevisiae* have been isolated from normal haploid *MATa* strains and characterized as having temperature-sensitive, pleiotropic phenotypes for functions associated with mating. At the permissive temperature, 23°C, they were found to behave as normal *MATa* haploids with respect to mating efficiency, sporulation in diploids formed with *MATa* strains, secretion of a-factor, and failure to secrete the *MATa*-specific products, a-factor and Barrier. At higher temperatures they were found to decline in mating and sporulation efficiency and to express the a-specific functions. Genetic analysis established that one of these mutants, PE34, carries a temperature-sensitive allele of the *MATa*2 gene and that the other, PD7, carries a temperature-sensitive allele of the *TUP1* gene.

In the yeast *Saccharomyces cerevisiae*, mating type and the ability to sporulate are controlled by two alternative alleles of the mating-type locus *MAT*. Cells that express either the *MATa* or the *MATα* allele, regardless of ploidy or dosage, can mate with cells that express the alternative allele. Cells that are heterozygous at this locus can sporulate. Normally, mating and sporulation are mutually exclusive (1, 2). Although mating and sporulation are determined by this single locus, these phenotypes involve the expression of many specific and nonspecific functions coded by genes that are not linked to the mating-type locus. Consequently, the *MAT* locus appears to regulate the expression of the structural genes that are necessary for mating and sporulation (3, 4).

A specific and appealing model for this regulation has been proposed by Strathern et al. (5) to account for the behavior of mutants of *MATa*. They characterized four nonmating mutants: VC2, VN33, VC73, and VP1 that are closely linked to *MATa* (4). They found that the mutations in VC73 and VP1 do not complement each other, but do complement the ones in VC2 and VN33, which also fail to complement one another. Accordingly, they proposed that *MATa* is complex, coding for a *MATa1* function, which is defective in VC2 (*mata1-2*) and VN33 (*mata1-5*), and a *MATa2* function, which is defective in VC73 (*mata2-1*) and VP1 (*mata2-4*). The *MATa1* function, inferred from the phenotypes of VC2 and VC33, acts as a positive regulator of a-specific functions, such as secretion of a-factor, synthesis of the Barrier activity (which inactivates a-factor [6]), synthesis of the a-specific surface agglutinin, and as a positive regulator of sporulation. *mata2-4* is different from *mata2-1* only in that it does not impair normal sporulation. According to this model, the phenotype of a *MATa* cell results from expressing the a-specific functions and not expressing the a-specific ones. The phenotype of a *MATa* cell results from expressing the a-specific functions, because there is no *MATa2* function, and not expressing the a-specific functions, because there is no *MATa1* function. This handily accounts for the failure to find any nonmating mutants that map at the *MATa* locus (3, 4, 7, 8).

The failure of *mata2* mutants to secrete a-factor is an indirect result of the expression of an a-specific function. Sprague and Herskowitz (9) have reported compelling evidence that these strains produce a-factor, but simultaneously inactivate it by expressing the a-specific Barrier function (6). They isolated mutants defective in the Barrier function (*bar*) that act as suppressors of the a-factor defect in *mata2* mutants but not of the mating defect. This also shows that the mating defect is not merely the result of the a-factor deficiency. The nonmating phenotype of VC73 and VP1 is apparently a consequence of simultaneous expression of the other a-specific and the a-specific functions. A double mutant (DC65) isolated from a cross between VC73 and VN33, and believed to carry mutations in both *MATa* functional units (*mata1-5 and mata2-1*), has the *MATa* mating phenotype, as predicted by the model (5).

This model also accounts for the sporulation phenotype by postulating an interaction between *MATa* and *MATa2* that
acts as a positive regulator of sporulation-specific functions and as a negative regulator of a-specific functions (5). This feature accounts for the mutants of MATa that are defective only in the sporulation function (10). The predictions of this model have received strong support at the molecular level by the studies of transcription reported by Klar et al. (11).

Lamontt et al. (12) have described a class of mutants, isolated independently by several investigators (13–15), that share some of the characteristics of mata2 mutants but do not map at MAT. These mutations map at the tup1 locus, 36.1 cm distal to MAT on chromosome III, and have additional abnormalities, not obviously related to mating type (16). MATa tup1 strains secrete a-factor, express the Barrier function, do not secrete alpha-factor, have impaired ability to mate with MATa cells, and prevent sporulation when homozygous. Unlike mata2 mutants, however, they mate at a low frequency with MATa cells, have an abnormal morphology resembling the “shmoof” shape of cells responding to mating pheromone, are able to take up deoxythymidine monophosphate (DTMP), are defective in UV-induced resistance to canavanine, produce high levels of iso-2-cytochrome c, and exhibit clumpy growth. This diverse array of abnormalities, some mating-type related, some not, may reflect a major regulatory function served by the normal TUP1 product (12).

In this paper we describe the properties of two mutants with temperature-sensitive phenotypes; one, PE34, has an altered mata2 function and the other, PD7, has an altered tup1 function. The results indicate that these strains are temperature-sensitive for their abilities to negatively regulate the a-specific mating-type-controlled functions.

MATERIALS AND METHODS

Yeast Strains: Tables I and II list the yeast strains used in these studies, their genotypes, and their sources.

Media: For routine culturing, strains were grown on a yeast-extract-peptone-dextrose medium (YEPE), and nutritional requirements and drug resistances were scored on defined synthetic media prepared by supplementing Difco Yeast Nitrogen Base (Difco Laboratories, Detroit, MI), as described previously (3, 17). Where indicated, YEPE was buffered to pH 4.8 with citrate-phosphate as described by Fink and Styles (18); for strains containing either ade1 or ade2, YEPE was supplemented with adenine (80 mg/liter). Sporulation medium contained 1% potassium acetate and 0.25% Difco yeast extract.

α-Factor: α-Factor was isolated from strain X2180-1B by the general method described by Duntze et al. (19), using pyridine-acetate buffer for elution from the Amberlite CG-50 column, as described by Lipke (20). Peak active

### TABLE I

#### List of Strains

| Haploids | Strain number | Genotype* | Source |
|----------|---------------|-----------|--------|
| X2180-1A | MATa gal2     |           | R. K. Mortimer |
| X2180-1B | MATa gal2     |           | R. K. Mortimer |
| XT1219-1A | MATa trp1 his2 ade1 gal1 |     | T. Manney |
| XT1219-18A | MATa trp1 his2 ade1 gal1 |     | T. Manney |
| XP300-26C | MATa thr4 his6 lys1 gal2 |     | T. Manney |
| XP300-26D | MATa ade2-1 trp5 his6 lys1 gal2 |     | T. Manney |
| XP300-28A | MATa ade2-1 trp5 his6 lys1 gal2 |     | T. Manney |
| XP300-29B | MATa his6 lys1 trp5 ade2 gal2 |     | T. Manney |
| CR7 | cry1-7 MATa thr4 his6 lys1 gal2 |     | This work |
| PD7 | MATa thr4 trp5 his6 lys1 gal2 |     | This work |
| PE34 | cry1-7 mata2-34 thr4 his6 lys1 gal2 |     | This work |
| XP422-3C | cry1-7 mata2-34 thr4 trp5 his6 lys1 gal2 |     | This work |
| XP422-14B | cry1-7 mata2-34 thr4 ade2 his6 lys1 trp5 gal2 |     | This work |
| XT1772-524c | MATa his6 ade6 trp5-1 met1 can1 gal2 |     | T. Manney |
| CR22 | cry1-22 MATa his6 ade6 trp5-1 met1 can1 gal2 |     | This work |
| VN33 | mata1-5 his6 ade6 leu1 trp5-1 met1 can1 gal2 |     | V. MacKay |
| CR29 | cry1-29 mata1-5 his6 ade6 leu1 trp5-1 met1 can1 gal2 |     | This work |
| VC73 | mata2-1 his6 ade6 leu1 trp5-1 met1 can1 gal2 |     | V. MacKay |
| CR30 | cry1-30 mata2-1 his6 ade6 leu1 trp5-1 met1 can1 gal2 |     | This work |
| VP1 | mata2-4 his6 ade6 leu1 trp5-1 met1 can1 gal2 |     | V. MacKay |
| CR34 | cry1-34 mata2-4 his6 ade6 leu1 trp5-1 met1 can1 gal2 |     | This work |
| DC65 | mata1-5 mata2-1 leu2 ade6 leu1 lys2 |     | J. Strathern |
| ts187 | MATa prtl1 ade2 ural tyrl his7 lys2 gal1 |     | L. Hartwell |
| RC629 | MATa sst1-2 ade2 ural his6 met1 can1 chy2 gal2 |     | R. Chan |
| RC757 | MATa sst2-1 his6 met1 can1 chy2 ural gal2 |     | R. Chan |
| G130D2-18B | MATa bar1-1 ade2 met1 chy2 leu1 rme can1 ural3 gal2 | | G. Sprague |
| G190-4C | MATa bar1-1 chy2 leu1 met1 can1 rme | | G. Sprague |
| XM84-12b | MATa sst1-1 arg9 ilv3 ural1 gal2(t) | | L. Blair |
| XP546-25A | cry1 mata2-34 thr4 trp5 sst1-2 his6 ural1 gal2 | | This work |
| XP633-1C | MATa thr4 tup1-7 lys1-1 can1 gal2 | | This work |
| XY507-7A | MATa umr7-1 his5-2 lys1-1 urad-1 gal1 gal2 | | J. Lemontt |
| XY507-5A | MATa umr7-1 his5-2 lys1-1 urad-4-1 | | J. Lemontt |

Monosomic diploid

XP562 | cry1 MATa ade2-1 trp5-18 his6 lys1 gal2 | null | ade2-1 trp5-18 his6 lys1 gal2 |

* Gene symbols indicate mutations leading to requirements for the following: ade (adenine), arg (arginine), his (histidine), ilv (isoleucine-valine), leu (leucine), lys (lysine), met (methionine), trp (tryptophan), and ural (uracil). Additional symbols include the following: a-o (mating type), can (resistance to canavanine), chy (resistance to cycloheximide), cry (resistance to cyclophosphate), gal (inability to ferment galactose), prf (protein synthesis), rme (regulator of meiosis), sst (supersensitive to α-factor), tup (ability to take up DTMP), and umr (resistance to UV mutagenesis at CAN).
fractioiiis, eluted from Sephadex LH20 in methanol/acetic acid/water (21), were stored in methanol at -20°C.

α-Factor activity was estimated by dilution end point by using the failure to form a halo around wells in an agar plate loaded with the dilution samples as the end point. The halos were detected as described below for the qualitative α-factor halo test.

Isolation of α-Factor-deficient Mutants: The mutant isolation method has been described previously (2). Either strain XP300-26C (for PD7) or CR7 (for PE34) was grown overnight in liquid YEPD, centrifuged, resuspended in 0.1 M phosphate buffer (K+), pH 7.0, and ethylmethanesulfonylate (25 μl/ml), and then incubated at 30°C for 1 h. Treated cells were diluted into 0.2 M sodium thiosulfate. The mutagen-treated MATa cells were mixed with cells of a MATa temperature-sensitive strain (ts187) and plated in 2.5 ml of 0.7% agar on a plate of YEPD, at pH 4.8, to give 100 MATa cells and 5 × 10⁴ MATα cells per plate. These plates were incubated at 36°C for 2 d to allow the MATa colonies to form: the ts187 cells did not grow significantly at this temperature. The plates were then incubated at 22°C, allowing the ts187 cells to form a confluent lawn of growth, except where inhibited by α-factor secreted by the MATα colonies. Normal colonies produced a small halo of inhibition, but α-factor-deficient mutants did not.

Scoring of Mating-associated Functions: Qualitative tests for secretion of α-factor, α-factor, and Barrier activity made use of the inhibition of supersensitive strains by α-factor and α-factor (22, 23). These tests yield positive results with appropriate wild-type strains at all temperatures from 23 to 36°C. α-Factor secretion was detected by a halo formed in a confluent lawn of a spontaneous petite isolate of XMB4-12b. 2 × 10⁶ cells from a stationary YEPD-grown culture were suspended in 2 ml of 0.75% agar at 50°C and immediately poured on a prewarmed (37°C) plate of pH 4.8-YEPD agar. Plates were allowed to stand at room temperature for 1–2 h before test cells were applied by spotting or replica plating. Halos reaching maximum visibility after 2–3 d of incubation, depending on the temperature. α-Factor secretion was also scored by the confrontation test (3, 24). Fig. 1 illustrates the test for a normal MATα strain and for several strains that do not secrete α-factor.

α-Factor secretion was detected by an analogous test using inhibition of RC757. The plates were prepared as described for the α-factor test, except that 1.6 × 10⁶ cells/plate were used. Halos were visible after 1–2 d, depending on the temperature. Examples for several strains are illustrated in Fig. 1.

Secretion of Barrier activity was detected by the formation of a fringe of small colonies on a lawn of α-factor-inhibited RC629 cells. The secreted Barrier apparently inhibits the α-factor, allowing the cells to recover. Lawns were prepared on unbuffered YEPD by the procedure described for the α-factor test, except that 8 U of α-factor were added to the soft agar before the cells were added. Fringes were clearly visible after 2–3 d, depending on the temperature. Examples for Bar+ and Bar- strains are shown in Fig. 1.

Mating was scored qualitatively by the complementation tests described by MacKay and Manney (3) using the strains XT1219-1A and XT1219-18A as MATa and MATα testers respectively.

Mating ability was estimated quantitatively by measuring diploid formation with the same test strains. Test cells and testers were grown overnight on YEPD and washed with water. 1 × 10⁶ cells of each mating type were mixed in 1 ml of fresh YEPD in a 1.5-ml microcentrifuge tube. The capped tubes were then suspended in a thermostated, circulating water bath to maintain the temperature within a 0.4°C range. After overnight incubation, the cells were washed with water, dispersed by sonification, and plated at appropriate dilutions on minimal medium to estimate the number of diploids, and on YEPD to estimate the total number of cells. Colonies were counted after 3 d.

Sporulation ability was scored on diploid cultures grown on YEPD agar for 1 d at 30°C and then transferred to sporulation medium and incubated for 5 d at the indicated temperature. The sporulation mixture was suspended in water and the frequencies of ascis and cells were counted through the microscope. At least 200 objects were scored at each point.

Clumpy growth was detected by visual observation. Cells were grown in 10 ml YEPD cultures overnight with shaking at 200 rpm on a rotary shaker in 50-ml DurLong flasks. Flasks were removed from the shaker and the cells allowed to settle. Clumped cultures were conspicuous and unambiguous.

Abnormal morphology, or "shmooing" was detected by microscopic observation of stationary, YEPD-grown cultures.

Genetic Methods: The standard procedures described by Mortimer and Hawthorne (25) were used. Rare diploids were isolated by prototroph selection. Mitotic recombinants homozygous for mating type were selected from diploids heterozygous for cryl as cryptopleurine-resistant segregants. Homozygotes
gosity at the mating-type locus was confirmed by mating as described above. Cryptopleurine-resistant mutants were isolated as described previously (17).

RESULTS

Isolation of the Mutants

The mutants PD7 and PE34 were found in a collection of 250 mutants isolated in two experiments as nonsterile, a-factor-deficient mutants. The PD mutants were isolated from XP300-26C and the PE mutants from CR7, a cryptopleurine-resistant mutant of XP300-26C. The PD mutants were subsequently made cryptopleurine resistant by a mutation at cry. A preliminary description of the mutant isolation and some of the PD isolates has been published previously (2). The isolation procedure used to identify these mutants (see Materials and Methods) was designed to facilitate the isolation of temperature-sensitive mutants. However, in our initial characterization by the confrontation test we failed to recognize the temperature-sensitive character of PD7 and PE34. Consequently, we first studied them as "leaky" a-factor-deficient mutants, taking advantage of their ability to mate at normal frequencies.

Use of the halo test for identifying a-factor, using MATa strains that are abnormally sensitive to inhibition, has led to our appreciation that these mutants are in fact temperature sensitive for a-factor secretion as well as for several a-specific functions.

Temperature-sensitive Phenotypes of PD7 and PE34

The pleiotropic, temperature-sensitive phenotypes of these mutants for the expression of a-factor, a-factor, and Barrier function are illustrated in Fig. 2.

Fig. 2A shows a-factor production, detected by inhibition of the MATa sst2-1 strain, RC757. Neither mutant produces detectable a-factor at 23°C, but above 30°C for PD7 and 34°C for PE34, the expression of this a-specific function is significant.

Fig. 2B shows the production of a-factor, detected by inhibition of the MATa sst1-1 strain, XMB4-12b, for the same strains. At 23°C, all of the strains except the X2180-1a (MATa) secrete large amounts of a-factor, although the mutants do not secrete full wildtype levels. At 30°C, the secretion is significantly reduced in both PD7 and PE34, and at the higher temperatures it is undetectable.

In Fig. 2C the reversal of the a-factor-inhibition of the MATa sst1-2 strain, RC629, is used to show the expression of the Barrier function in the same strains. At 23°C, only the MATa strain has detectable activity, but at the higher temperatures both the PD7 and PE34 strains are Bar-. The expression of the Barrier function parallels the deficiency in a-factor secretion seen in Fig. 2B. At 36°C, even the wildtype strain is Barrier negative.

The effect of temperature on the mating efficiencies of PD7 and PE34 relative to that of their normal MATa parent is summarized in Table III. The results clearly demonstrate that the mating efficiency in these mutants is temperature-sensitive. It should be noted, however, that the mating frequencies observed at 35 and 37°C reflect only minor effects in comparison with the mating frequencies of sre mutants and nonmating mat mutants, which are typically of the order of one per 10³.

The effect of temperature on the sporulation efficiency of diploid strains produced by mating these mutants to MATa strains is shown in Table IV. The normal MATa/MATa diploid, XP173, shows a strong optimum for sporulation at 30°C. At 35°C and higher no sporulation was observed. Both of the mutation-bearing diploids exhibit some temperature-dependent reduction of sporulation efficiency.

Localization on Chromosome III R

The original mutant collection was screened for linkage to MAT by mitotic recombination. Each strain, which carried a cryl allele (cryptopleurine resistance) closely linked to MAT, was crossed to a CRY1 (sensitive) MATa strain. Cryptopleurine-resistant mitotic segregants that mated as MATa were isolated and scored for a-factor production by the confrontation test. Diploids of this type constructed from PD7 and PE34 yielded segregants that were a-factor deficient, showing that these mutants are not dominant, they are either closely linked to the MAT locus, or distal to it on the right arm of chromosome III.

Linkage to MAT

Each strain was crossed to a normal MATa strain (XP300-29B), the diploid sporulated, and asci dissected and analyzed. An indication of strong linkage between the mutation in PE34 and MAT was given by a sample of 12 ascii that contained only a-factor-deficient MATa spores (data not shown). In contrast, the defect in PD7 was not closely linked to MAT, as demonstrated by a high frequency of recombinant-bearing ascii (1 parental dihybrid [PD]: 3 nonparental dihybrid [NPD]: 20 tetraparental [T]). This result, taken with the mitotic segregation placing the mutation on this chromosome arm, placed the PD7 mutation distal to MAT. Due to the fact that this defect has no phenotype that we can detect in a MATa background, the tetrads could not be scored completely relative to other markers. However, the 48 MATa spores that were scored showed a recombination frequency of 23% (11/48) between the a-factor deficiency and thr4.

PE34 Is Defective in the MATa2 Function

The relationship of the defect in PE34 to the MAT functions was examined by complementation tests with mata-5, mata2-1, and mata2-4. Diploids were constructed by prototrophic selection for rare matings. The properties of these diploids, which will be described, support the conclusion that the temperature-sensitive mutation in PE34 complements mata-5, but not mata2-1 or mata2-4. Accordingly, we have assigned this mutation the gene symbol mata2-34. The a-factor production and mating abilities of these hybrids are summarized in Table V.

The phenotypes of XP580 (MATa/mata2-34) and XP581 (mata1-5/mata2-34) were indistinguishable from normal MATa strains with respect to mating, a-factor, a-factor, and Barrier function at all temperatures tested, showing that mata2-34 is recessive, and complementary to mata-5. The phenotypes of XP582 (mata2-34/mata2-1) and XP583 (mata2-34/mata2-4) were qualitatively the same as those of PE34, but more extreme at each temperature. At 23°C they make no detectable a-factor or Barrier activity, but their a-factor halos are smaller than normal. At 30°C their phenotypes are distinctly mutant; there is definite a-factor and Barrier function and no visible a-factor. At 37°C, XP582 mated only weakly with the MATa tester and XP583 gave no indication of mating ability by the complementation test. This reflects substantially less mating than observed for PE34 at this temperature. The phenotypes
are intermediate between the parents, and they are clearly noncomplementing.

The genotypes of these nonsporulating diploids were confirmed by trisomic tetraploid analysis (26). Each of the strains in Table V was crossed with Xp562, a MATa diploid monosomic for chromosome III, and zygotes were isolated by micromanipulation. The resulting trisomic tetraploids were sporulated and 18 to 22 asci dissected and analyzed. Spore viability

| Strain    | Genotype                          | 23°C | 30°C | 34°C | 36°C |
|-----------|-----------------------------------|------|------|------|------|
| A α-factor|                                   |      |      |      |      |
| CR7       | MATα                              |      |      |      |      |
| X2180-IA  | MATα                              |      |      |      |      |
| PE34      | mata 2-34                         |      |      |      |      |
| XP546-25A | mata 2-34 sstl-2                  |      |      |      |      |
| PD7       | MATα tup1-7                       |      |      |      |      |
| XP633-3C  | MATα tup1-7 barl-1                |      |      |      |      |
| B α-factor|                                   |      |      |      |      |
| CR7       | MATα                              |      |      |      |      |
| X2180-IA  | MATα                              |      |      |      |      |
| PE34      | mata 2-34                         |      |      |      |      |
| XP546-25A | mata 2-34 sstl-2                  |      |      |      |      |
| PD7       | MATα tup1-7                       |      |      |      |      |
| XP633-3C  | MATα tup1-7 barl-1                |      |      |      |      |
| C Barrier |                                   |      |      |      |      |
| CR7       | MATα                              |      |      |      |      |
| X2180-IA  | MATα                              |      |      |      |      |
| PE34      | mata 2-34                         |      |      |      |      |
| XP546-25A | mata 2-34 sstl-2                  |      |      |      |      |
| PD7       | MATα tup1-7                       |      |      |      |      |
| XP633-3C  | MATα tup1-7 barl-1                |      |      |      |      |

Figure 2. Qualitative test for secretion of α-factor (A), α-factor (B), and Barrier activity (C) for normal and mutant strains. Test procedures are described in Materials and Methods.
varied from 83 to 94%. Spores were scored for mating type, spore morphology characteristics are not shared by the mata2 mutants. To test for allelism between the mutation in PD7 and tup1-16, we constructed a series of hybrid diploids homozygous for MATa by taking advantage of the relatively high frequency of mating between MATa tup1 strains and MATa strains (12). The desired diploids were easily recovered by prototroph selection at 30°C.

Table VI summarizes the properties of several haploid and diploid strains that demonstrate that tup1-16 does not complement the mutation in PD7 at its restrictive temperature. This shows that PD7 does indeed carry a temperature-sensitive mutation at the TUP1 locus. On the basis of these results, we have assigned the symbol tup1-7 to this mutation. The normal functions expressed by the heterozygous diploids XP657 (tup1-16/+) and XP658 (tup1-7/+), and by the heteroallelic diploid XP656 (tup1-16/tup1-7) at 23°C demonstrate that these mutations are recessive for all of the characteristics listed. The phenotype of the heteroallelic diploid is qualitatively the same as that of PD7, but more extreme at each temperature. As with the mata2 alleles described above, the heteroallelic phenotype is intermediate between the parents, and clearly noncomplementing.

**bar1 Suppresses the α-Factor Defect in mata2-34 and tup1-7**

Sprague and Herskowitz (9) demonstrated that the α-factor defect in mata2 mutants results from the expression of the Barrier function, and a similar explanation for this defect in tup1 mutants was suggested by Lamont et al. (12). We therefore anticipated that bar1 mutations would suppress the temperature-sensitive α-factor defects in mata2-34 and tup1-7 strains. The results summarized in Tables VII and VIII demonstrate that this is indeed the case.

Table VII shows the observed phenotypes and inferred genotypes of spores segregating from two crosses of a mata2-34 strain with a MATa bar1 strain. XP545 carries the bar1-1 mutation and XP546 carries sst1-2, an allele of bar1-1 (9). The BAR1 phenotype can be scored directly in the MATa but not in the MATa spores. Its assignment is unambiguous in the PD and NPD ascis if 1:2 segregation is assumed. Accordingly, the formation of α-factor at 34°C by the mata2-34 spores in these ascis demonstrates the suppression of the temperature-sensitive defect by bar1. This interpretation is further supported by the T ascis. In every case where an ascus contains a Bar+ spore, there is a corresponding mata2-34 spore that secretes α-factor at the restrictive temperature.

Table VIII shows the results of an analogous cross between a MATa tup1-7 strain and a MATa bar1-1 strain. The analysis of this case is complicated by our inability to score the tup1-7

### Table III

**Mating Frequencies with MATa**

| Strain  | Genotype          | Mating efficiency* |
|---------|-------------------|--------------------|
|         | 23°C  | 30°C  | 32°C  | 34°C  |
| PE34    | mata2-34         | 58    | 21    | 1.1   | 1.1   |
| XP546-25A | mata2-34 sst1-2 | nt    | 150   | nt    | 2.2   |
| PD7     | MATa tup1-7      | 45    | 12    | 1.9   | 0.9   |
| XP633-1C | MATa tup1-7 bar1-1 | nt   | 24    | nt    | 1.1   |
| CR7     | MATa             | (0.38)$\dagger$ | (0.26) | (0.12) | (0.027) |

* Mating efficiency of each strain is its mating frequency expressed as percentage of the mating frequency of CR7 at the same temperature.
† Mating frequency of CR7 (diploids/viable cell). nt, not tested.

### Table IV

**Sporulation Frequencies**

| Strain  | Genotype          | Percent sporulation* |
|---------|-------------------|----------------------|
|         | 23°C  | 30°C  | 32°C  | 34°C  |
| XP173   | MATa/MATa         | 35    | 75    | 48    | 29    |
| XP422   | MATa/mata2-34     | 35    | 50    | 12    | 2     |
| XP720   | MATa/MATa         | 44    | 31    | 22    | 11    |

* Percent sporulation is 100 times the number of asci divided by the number of asci plus cells.
† No sporulation was observed at higher temperatures.

### Table V

**Complementation between the Defect in PE34 and Previously Described mata2 Alleles**

| Strain  | MAT genotype      | Mating ability* | α-Factor† |
|---------|-------------------|-----------------|-----------|
|         | 30°C  | 37°C  | 23°C  | 34°C  |
| XP580   | mata2-34/MATa     | +    | +    | +    | +    |
| XP581   | mata2-34/mata1-5  | +    | +    | +    | +    |
| XP582   | mata2-34/mata2-1  | +    | ±    | +    | −    |
| XP583   | mata2-34/mata2-4  | +    | -    | +    | -    |

* Mating with XT1219-1A judged by prototroph formation on minimal agar medium.
† Judged by halo formation.

### Table VI

**Strain Genotype 23°C 30°C 32°C 34°C**

| Strain  | Genotype          | 23°C  | 30°C  | 32°C  | 34°C  |
|---------|-------------------|-------|-------|-------|-------|
| XP422   | MATa/mata2-34     | 35    | 50    | 12    | 2     |
| XP720   | MATa/MATa         | 44    | 31    | 22    | 11    |
| XP173   | MATa/MATa         | 35    | 75    | 48    | 29    |
| XP580   | mata2-34/MATa     | +    | +    | +    | +    |
| XP581   | mata2-34/mata1-5  | +    | +    | +    | +    |
| XP582   | mata2-34/mata2-1  | +    | ±    | +    | −    |
| XP583   | mata2-34/mata2-4  | +    | −    | +    | −    |

PD7 Is Defective in the TUP1 Function

The phenotype of PD7 and its position on the genetic map are similar to the highly pleiotropic tup1 mutants (12). At temperatures above 34°C, PD7 shares with the previously reported tup1 mutants the inability to secrete α-factor, secretion of α-factor. Barrier function, abnormal, shmoo-like morphology (which is apparent even at 30°C), and clumpy growth. Curiously, PD7 and tup1 also share the characteristic of having normal cell morphology at 37°C, an observation not previously reported for the tup1 mutants. These abnormal growth and
TABLE VI

Tests for Allelism of tup1-7 and tup1-16

| Strain   | Genotype  | α-Factor | α-Factor | Barrier | Clumpy growth | Cell morphology |
|----------|-----------|----------|----------|---------|---------------|----------------|
| CR7      | MATa      | -        | +        | -       | -             | N*             |
| PD7      | MATa tup1-7 | ts†      | ts       | ts      | ts            | S              |
| XY517-15 | MATa tup1-16 | -        | -        | +       | +             | S              |
| XP56     | MATa tup1-7 | ts       | ts       | ts      | ts            | S              |
| XP57     | MATa TUP1  | -        | +        | -       | -             | N              |
| XP58     | MATa TUP1  | -        | +        | -       | -             | N              |

* N, normal; S, shmoo-shaped; all strains have normal cell morphology at 37°C.
† Responses indicated as ts are those illustrated for PD7 in Fig. 2.

TABLE VII

Suppression of the α Factor Defect in mata2-34 by bar1 Tetrad Analysis of XP545 and XP546

| Type | Genotypes | α-Factor | Bar | XP545 | XP546 | Total |
|------|------------|----------|-----|-------|-------|-------|
| PD   | MATa bar1  | -        | -   | 7     | 4     | 11    |
| NPD  | MATa bar1  | -        | -   | -     | -     | -     |
|      | mata2-34   | ts       | -   | -     | -     | -     |
|      | mata2-34   | ts       | -   | -     | -     | -     |
|      | MATa +     | -        | +   | 9     | 6     | 15    |
|      | MATa +     | -        | +   | -     | -     | -     |
|      | mata2-34   | +        | -   | -     | -     | -     |
|      | mata2-34   | +        | -   | -     | -     | -     |
|      | mata2-34   | +        | -   | -     | -     | -     |
|      | mata2-34   | +        | -   | -     | -     | -     |
|      | mata2-34   | +        | -   | -     | -     | -     |
| T    | MATa +     | -        | +   | 20    | 11    | 31    |
|      | MATa bar1  | -        | -   | -     | -     | -     |
|      | mata2-34   | ts       | -   | -     | -     | -     |
|      | mata2-34   | +        | -   | -     | -     | -     |
|      | mata2-34   | +        | -   | -     | -     | -     |

traits marked * could not be ascertained from the data.

TABLE VIII

Suppression of the α Factor Defect in tup1-7 by bar1 Tetrad Analysis of XP633

| Phenotypes | a-factor | α-factor | Bar | Inferred genotypes | Number of asci |
|------------|----------|----------|-----|--------------------|----------------|
| +          | -        | +        | MATa+ | + +               | 2              |
| +          | -        | -        | MATa bar1 | + +               | 5              |
| ts         | ts       | -        | MATatup1 | + +               | 2              |
| +          | -        | -        | MATatup1 | + +               | 2              |
| +          | -        | -        | MATatup1 | + +               | 1              |
| ts         | +        | -        | MATatup1 | + +               | 1              |
| ts         | -        | +        | MATa+ | + +               | 2              |
| +          | -        | +        | MATa+ | + +               | 2              |
| +          | -        | -        | MATatup1 | + +               | 1              |
| ts         | +        | -        | MATatup1 | + +               | 1              |
| +          | +        | -        | MATa+ | + +               | 1              |
| +          | -        | +        | MATa+ | + +               | 1              |

allele in the Mata background. However, asci of class 1, of which two were found, permit unambiguous assignment of the tup1-7 allele to the MATa spores on the basis of their temperature-sensitive α-factor expression. The occurrence of one Bar+ Mata spore implies that one of the MATa tup1-7 spores carries barl-1. Thus, we infer that spores producing both α-factor and α-factor at 34°C have genotype MATa tup1-7 barl-1. By similar reasoning, the rest of the observed asci can be assigned self-consistent genotypes.

Suppression of the temperature-sensitive α-factor defect in these mutants by bar1 demonstrates that this defect is indirect; both produce normal levels of α-factor, but it is inactivated by the Barrier function. Furthermore, the data in Table III show that barl has no effect on the reduced mating efficiencies caused by mata2-34 and tup1-7 at their restrictive temperatures. This is fully in agreement with the findings and interpretations reported by Sprague and Herskowitz (9) for mata2-1 and mata2-4.

DISCUSSION

These two mutants, PD7 (tup1-7) and PE34 (mata2-34) display only marginally detectable abnormalities at 23°C, but at higher temperatures they express the phenotypic characteristics of their nonconditional alleles. It has been suggested that both of these genes code for regulatory functions (9, 12), and the results reported here support that suggestion. The temperature-sensitive function in each case is apparently a negative regulator of a number of structural genes. In the case of MATa2, these are α-specific functions, and in the case of TUP1 they include these...
and apparently some functions not obviously related to MAT.

The conventional terminology of "permissive" and "restrictive" temperatures warrants some comment in this context. For these mutants we would consider 23°C to be the "permissive" temperature, corresponding to normal function. The "restrictive" temperature, corresponding to loss of function, is not as well defined. We interpret the quantitative gradations of expression of some of the affected functions to reflect quantitative expression of the negative regulator. We surmise, then, that at 23°C this repressor is fully active, and that at higher temperatures it becomes progressively less active. Consequently, normal MATα functions, such as α-factor secretion and mating ability, are normal at the permissive temperature and defective at the restrictive temperatures, while the a-specific functions, which are abnormal in a MATα strain, are expressed at the restrictive but not at the permissive temperature.

The ability to assess the expression of a-factor, α-factor, and the Barrier function has been greatly facilitated by the use of strains with the supersensitive phenotype. An important characteristic of the tests illustrated in Fig. 1 is their relative insensitivity to temperature over the range of interest for these mutants. As the control strains in Fig. 2 show, they are not totally insensitive to temperature, but their temperature dependence is small compared with that of the temperature-sensitive functions. A notable exception is that the Barrier function is not expressed at 36°C even in the MATα Bar1 strain.

These tests have the additional virtues of being quite sensitive and at the same time having a wide latitude of sensitivity. Although the size of a halo depends on the amount of pheromone or Barrier factor secreted into the agar, caution must be exercised when interpreting differences in halo sizes because the relationship is not linear and depends strongly on the ability of the molecule to diffuse through agar. α-Factor is secreted by the cells as a small, somewhat hydrophobic peptide that is sufficiently soluble in water to diffuse freely in agar (2, 27, 28). In contrast, a-factor, which is also a small peptide, is much more hydrophobic and is associated with high-molecular weight material. The Barrier factor is apparently a protein that is substantially larger than the tridecapeptide α-factor (Nath and Manney, unpublished data). The latter two activities diffuse very slowly in agar. Consequently, a given relative difference in two α-factor concentrations will result in a much larger difference in halo sizes than would result from the same relative difference in concentrations of a-factor or Barrier product. Therefore, the small differences in the sizes of the a-factor and Barrier halos, compared with the α-factor halos may not be a true quantitative reflection of the temperature-dependence of these functions. Furthermore, the variation in the α-factor concentration is only an indirect consequence of the variation in the amount of Barrier activity that is inactivating it.

Our results confirm the well-established observation that a-factor and Barrier are a-specific functions; neither of these functions is detectable in normal MATα cells. The results also support the a1–a2 hypothesis of Strathern et al. (5), which attributes to MATα2 the function of a negative regulator of these functions. The temperature-dependence of these functions in PE34 therefore most reasonably reflects temperature-sensitivity of this product, either the amount made, its activity, or its stability. The mating defect is evidently a consequence of the expression of some yet unidentified a-specific function that is incompatible with some α-specific function. This is implied by the finding that a mata2-mata1 double mutant mates as an a (5).

Another observation that bears on the quantitative nature of the MATα2 function is that of the intermediate phenotypes of the mata2-34/mata2-1 and mata2-34/mata2-4 diploids (Table V). At the permissive temperature (23°C) these strains behaved as normal MATα strains, but at the restrictive temperatures their phenotypes are more "restricted" than haploid mata2-34 strains at the same temperature. This could be understood as a simple MATα2 product dosage effect, supporting the idea that the effective concentration of this regulator modulates the expression of the a-specific structural genes.

Parallels between PE34 and PD7

The similarities between PE34 and PD7 with respect to their expression of a-specific functions is striking. MATα tup1-7 strains display all the same quantitative behavior as mata2-34 strains, both in response to temperature and to dosage in tup1-7/tup1-16 diploids (Table VI). It is difficult to avoid the conclusion that TUP1 also acts in the negative regulation of a-specific functions in MATα strains. But clearly, TUP1 does more; it apparently acts as a negative regulator of a much wider range of functions (12).

These temperature-sensitive mutants in two regulatory genes may prove to be unique tools for studying mating-type regulation. They provide a means for gaining quantitative control of the expression of the genes that are regulated. As a practical example, they facilitate the direct scoring of a-specific functions, such as Barrier and a-factor production in MATα strains, a distinct advantage for genetic analysis and strain construction. At a more fundamental level, we hope they will help correlate regulation at the molecular level with its consequences at the phenotypic level.

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