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 *MATα* 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 *MATα* haploids with respect to mating efficiency, sporulation in diploids formed with *MAT***a** strains, secretion of α-factor, and failure to secrete the *MAT***a**-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 *MATα2* 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 *MAT***a** 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 *MATα*. They characterized four nonmating mutants: VC2, VN33, VC73, and VP1 that are closely linked to *MATα* (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 *MATα* is complex, coding for a *MATα1* function, which is defective in VC2 (*matα1-2*) and VN33 (*matα1-5*), and a *MATα2* function, which is defective in VC73 (*matα2-1*) and VP1 (*matα2-4*). The *MATα1* function, inferred from the phenotypes of VC2 and VC33, acts as a positive regulator of α-specific functions, such as secretion of the mating pheromone, α-factor, synthesis of the α-specific surface agglutinin, and the ability to respond to the **a**-specific pheromone, **a**-factor. The *MATα2* function inferred from the behavior of *matα2-1* mutants acts as a negative regulator of **a**-specific functions, such as secretion of **a**-factor, synthesis of the Barrier activity (which inactivates α-factor [6]), synthesis of the **a**-specific surface agglutinin, and as a positive regulator of sporulation. *matα2-4* is different from *matα2-1* only in that it does not impair normal sporulation. According to this model, the phenotype of a *MATα* cell results from expressing the α-specific functions and not expressing the **a**-specific ones. The phenotype of a *MAT***a** cell results from expressing the **a**-specific functions, because there is no *MATα2* function, and not expressing the α-specific functions, because there is no *MATα1* function. This handily accounts for the failure to find any nonmating mutants that map at the *MAT***a** locus (3, 4, 7, 8).

The failure of *matα2* mutants to secrete α-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 α-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 α-factor defect in *matα2* mutants but not of the mating defect. This also shows that the mating defect is not merely the result of the α-factor deficiency. The nonmating phenotype of VC73 and VP1 is apparently a consequence of simultaneous expression of the other **a**-specific and the α-specific functions. A double mutant (DC65) isolated from a cross between VC73 and VN33, and believed to carry mutations in both *MATα* functional units (*matα1-5* and *matα2-1*), has the *MAT***a** mating phenotype, as predicted by the model (5).

This model also accounts for the sporulation phenotype by postulating an interaction between *MAT***a** and *MATα2* that

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acts as a positive regulator of sporulation-specific functions and as a negative regulator of α-specific functions (5). This feature accounts for the mutants of *MAT***a** 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 *matα2* 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). *MATα tup1* strains secrete **a**-factor, express the Barrier function, do not secrete α-factor, have impaired ability to mate with *MAT***a** cells, and prevent sporulation when homozygous. Unlike *matα2* mutants, however, they mate at a low frequency with *MATα* cells, have an abnormal morphology resembling the "shmoo" 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 *matα2* 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 (YEPD), 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, YEPD was buffered to pH 4.8 with citrate-phosphate as described by Fink and Styles (18); for strains containing either *ade1* or *ade2*, YEPD 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	*MAT***a** *gal2*	R. K. Mortimer
X2180-1B	*MATα gal2*	R. K. Mortimer
XT1219-1A	*MAT***a** *trp1 his2 ade1 gal1*	T. Manney
XT1219-18A	*MAT***a** *trp1 his2 ade1 gal1*	T. Manney
XP300-26C	*MATα thr4 his6 lys1 gal2*	T. Manney
XP300-26D	*MATα ade2-1 trp5 his6 lys1 gal2*	T. Manney
XP300-28A	*MAT***a** *ade2-1 trp5 his6 lys1 gal2*	T. Manney
XP300-29B	*MAT***a** *his6 lys1 trp5 ade2 gal2*	T. Manney
CR7	*cry1-7 MATα thr4 his6 lys1 gal2*	This work
PD7	*MATα thr4 trp5 his6 lys1 gal2*	This work
PE34	*cry1-7 matα2-34 thr4 his6 lys1 gal2*	This work
XP422-3C	*cry1-7 matα2-34 thr4 trp5 his6 lys1 gal2*	This work
XP422-14B	*cry1-7 matα2-34 thr4 ade2 his6 lys1 trp5 gal2*	This work
XT1172-S245c	*MATα his6 ade6 trp5-1 met1 can1 gal2*	T. Manney
CR22	*cry1-22 MATα his6 ade6 trp5-1 met1 can1 gal2*	This work
VN33	*matα1-5 his6 ade6 leu1 trp5-1 met1 can1 gal2*	V. MacKay
CR29	*cry1-29 matα1-5 his6 ade6 leu1 trp5-1 met1 can1 gal2*	This work
VC73	*matα2-1 his6 ade6 leu1 trp5-1 met1 can1 gal2*	V. MacKay
CR30	*cry1-30 matα2-1 his6 ade6 leu1 trp5-1 met1 can1 gal2*	This work
VP1	*matα2-4 his6 ade6 leu1 trp5-1 met1 can1 gal2*	V. MacKay
CR34	*cry1-34 matα2-4 his6 ade6 leu1 trp5-1 met1 can1 gal2*	This work
DC65	*matα1-5 matα2-1 leu2 ade6 leu1 lys2*	J. Strathern
ts187	*MAT***a** *prt1 ade2 ura1 tyr1 his7 lys2 gal1*	L. Hartwell
RC629	*MAT***a** *sst1-2 ade2 ura1 his6 met1 can1 cyh2 gal2*	R. Chan
RC757	*MATα sst2-1 his6 met1 can1 cyh2 rme gal2*	R. Chan
G130D2-18B	*MAT***a** *bar1-1 ade2 met1 cyh2 leu1 rme can1 ura3 gal2*	G. Sprague
G190-4C	*MAT***a** *bar1-1 cyh2 leu1 met1 can1 rme*	G. Sprague
XMB4-12b	*MAT***a** *sst1-1 arg9 ilv3 ura1 gal2(?)*	L. Blair
XP546-25A	*cry1 matα2-34 thr4 trp5 sst1-2 his6 ura1 gal2*	This work
XP633-1C	*MATα thr4 tup1-7 lys1-1 can1 gal2*	This work
XY507-7A	*MAT***a** *umr7-1 his5-2 lys1-1 ura4-1 gal1 gal2*	J. Lemontt
XY507-5A	*MATα umr7-1 his5-2 lys1-1 ura4-1*	J. Lemontt
Monosomic diploid		
XP562	*cry1 MAT***a** *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 *ura* (uracil). Additional symbols include the following: **a** or α (mating type), *can* (resistance to canavanine), *cyh* (resistance to cycloheximide), *cry* (resistance to cryptopleurine), *gal* (inability to ferment galactose), *prt* (protein synthesis), *rme* (regulator of meiosis), *sst* (supersensitive to α-factor), *tup* (ability to take up dTMP), and *umr* (resistance to UV mutagenesis at *CAN1*).

fractions, 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 ethylmethanesulfonate (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 *MATα* cells were mixed with cells of a *MAT***a** 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 *MATα* cells and 5×10^6 *MAT***a** cells per plate. These plates were incubated at 36°C for 2 d to allow the *MATα* cells to form colonies. 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 **a**-factor, α-factor, and Barrier activity made use of the inhibition of supersensitive strains by **a**-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. 2×10^6 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.

a-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 1.6×10^6 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 *MAT***a** 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. 1×10^6 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 asci 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 DuLong 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 *cry1* as cryptopleurine-resistant segregants. Homozy-

TABLE II
List of Crosses

Strain number	Cross
XP173	XP300-29B × XP300-26C
XP422	XP300-29B × PE34
XP545	XP422-3C × G130D2-18B
XP546	XP422-3C × RC629
XP633	PD7 × G190-4C
XP562	XP300-26D × XP300-48A
XP580	XP422-14B × CR22
XP581	XP422-14B × CR29
XP582	XP422-14B × CR30
XP583	XP422-14B × CR34
XP655	XY507-7A × PD7
XP656	XY507-5A × PD7
XP657	XY507-5A × XP300-26C
XP720	XP300-29B × PD7
XP658	PD7 × XP300-26D

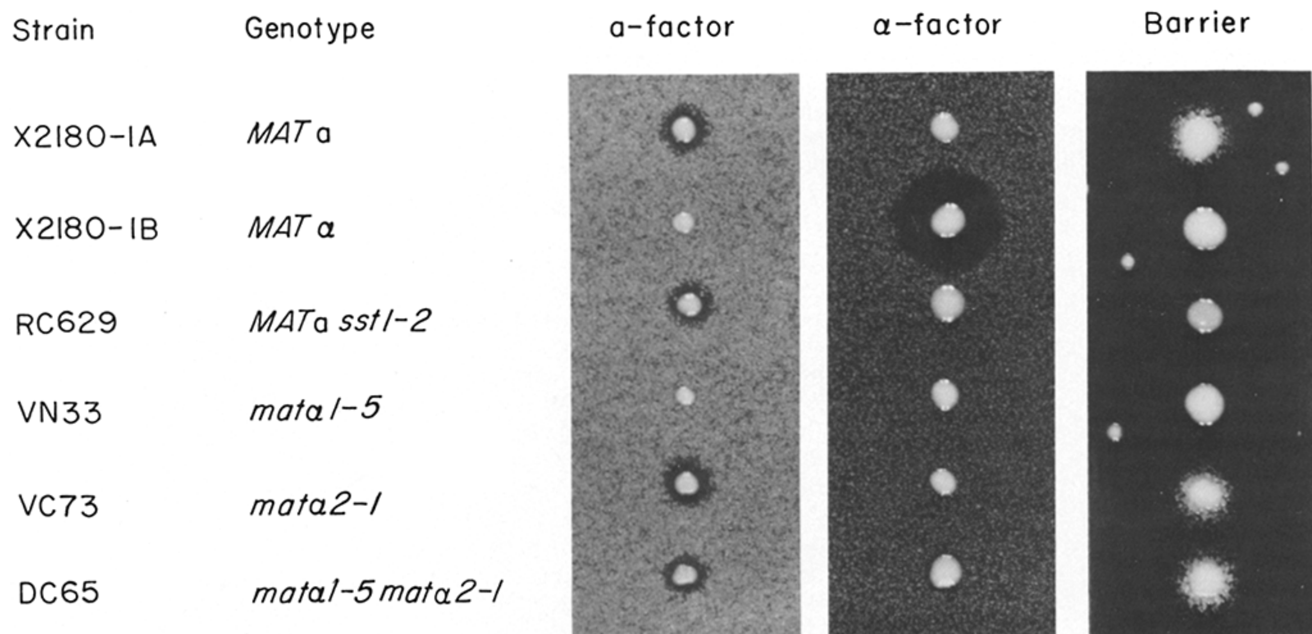

FIGURE 1 Qualitative tests for secretion of **a**-factor, α-factor, and Barrier activity for strains with known phenotypes.

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, α-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" α-factor-deficient mutants, taking advantage of their ability to mate at normal frequencies.

Use of the halo test for identifying α-factor, using *MAT***a** strains that are abnormally sensitive to inhibition, has led to our appreciation that these mutants are in fact temperature sensitive for α-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, α-factor, and Barrier function are illustrated in Fig. 2.

Fig. 2*A* shows **a**-factor production, detected by inhibition of the *MATα 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. 2*B* shows the production of α-factor, detected by inhibition of the *MAT***a** *sst1-1* strain, XMB4-12b, for the same strains. At 23°C, all of the strains except the X2180-1a (*MAT***a**) secrete large amounts of α-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. 2*C* the reversal of the α-factor-inhibition of the *MAT***a** *sst1-2* strain, RC629, is used to show the expression of the Barrier function in the same strains. At 23°C, only the *MAT***a** 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 α-factor secretion seen in Fig. 2*B*. 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 *MATα* 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 *ste* mutants and nonmating *mat* mutants, which are typically of the order of one per 10^6.

The effect of temperature on the sporulation efficiency of diploid strains produced by mating these mutants to *MAT***a** strains is shown in Table IV. The normal *MAT***a**/*MATα* 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 IIIR

The original mutant collection was screened for linkage to *MAT* by mitotic recombination. Each strain, which carried a *cry1* allele (cryptopleurine resistance) closely linked to *MAT*, was crossed to a *CRY1* (sensitive) *MAT***a** strain. Cryptopleurine-resistant mitotic segregants that mated as *MATα* were isolated and scored for α-factor production by the confrontation test. Diploids of this type constructed from PD7 and PE34 yielded segregants that were α-factor deficient, showing that if 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 *MAT***a** 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 asci that contained only α-factor-deficient *MATα* 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 asci (1 parental ditype [PD]:3 nonparental ditype [NPD]:20 tetratype [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 *MAT***a** background, the tetrads could not be scored completely relative to other markers. However, the 48 *MATα* spores that were scored showed a recombination frequency of 23% (11/48) between the α-factor deficiency and *thr4*.

PE34 Is Defective in the MATα2 Function

The relationship of the defect in PE34 to the *MAT* functions was examined by complementation tests with *matα1-5*, *matα2-1*, and *matα2-4*. 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 *matα1-5*, but not *matα2-1* or *matα2-4*. Accordingly, we have assigned this mutation the gene symbol *matα2-34*. The α-factor production and mating abilities of these hybrids are summarized in Table V.

The phenotypes of XP580 (*MATα/matα2-34*) and XP581 (*matα1-5/matα2-34*) were indistinguishable from normal *MATα* strains with respect to mating, **a**-factor, α-factor, and Barrier function at all temperatures tested, showing that *matα2-34* is recessive, and complementary to *matα1-5*. The phenotypes of XP582 (*matα2-34/matα2-1*) and XP583 (*matα2-34/matα2-4*) 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 α-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 α-factor. At 37°C, XP582 mated only weakly with the *MAT***a** 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

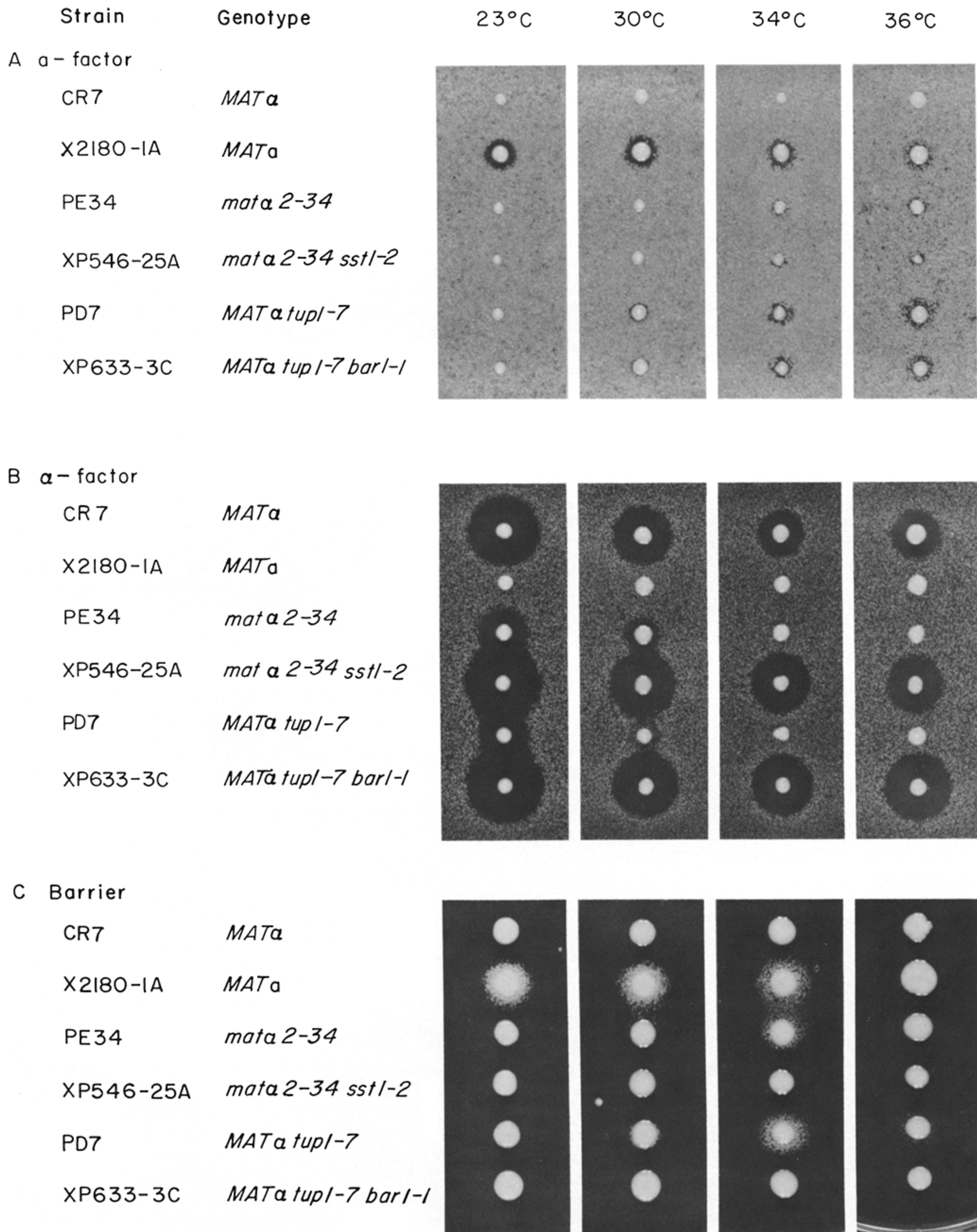

FIGURE 2 Qualitative test for secretion of **a**-factor (*A*), α-factor (*B*), and Barrier activity (*C*) for normal and mutant strains. Test procedures are described in Materials and Methods.

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 *MAT***a** 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

TABLE III

Mating Frequencies with MATa

Strain	Genotype	Mating efficiency*			
		23°C	30°C	35°C	37°C
PE34	*matα2-34*	58	21	1.1	1.1
XP546-25A	*matα2-34 sst1-2*	nt	150	nt	2.2
PD7	*MATα tup1-7*	45	12	1.9	0.9
XP633-1C	*MATα tup1-7 bar1-1*	nt	24	nt	1.1
CR7	*MATα*	(0.38)‡	(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/MATα*	35	75	48	29
XP422	*MATa/matα2-34*	35	50	12	2
XP720	*MATa/MATα tup1-7*	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 matα2 Alleles

Strain	MAT genotype	Mating ability*		α-Factor‡	
		30°C	37°C	23°C	34°C
XP580	*matα2-34/MATα*	+	+	+	+
XP581	*matα2-34/matα1-5*	+	+	+	+
XP582	*matα2-34/matα2-1*	+	±	+	−
XP583	*matα2-34/matα2-4*	+	−	+	−

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

varied from 83 to 94%. Spores were scored for mating type, sporulation, and α-factor secretion at 23°C, in addition to nutritional requirements. In every case the majority of asci conformed to expected segregation patterns and the monosomic and disomic spore phenotypes for mating and α-factor production, including temperature sensitivity, reiterated those observed in the original haploids and the diploids in Table V. Exceptional segregation patterns could be accounted for on the basis of nondisjunction, gene conversion, or false tetrads (data not shown).

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 **a**-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 morphology characteristics are not shared by the *matα2* mutants. To test for allelism between the mutation in PD7 and *tup1-16*, we constructed a series of hybrid diploids homozygous for *MATα* by taking advantage of the relatively high frequency of mating between *MATα tup1* strains and *MATα* 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 *matα2* alleles described above, the heteroallelic phenotype is intermediate between the parents, and clearly noncomplementing.

bar1 Suppresses the α-Factor Defect in matα2-34 and tup1-7

Sprague and Herskowitz (9) demonstrated that the α-factor defect in *matα2* mutants results from the expression of the Barrier function, and a similar explanation for this defect in *tup1* mutants was suggested by Lamontt et al. (12). We therefore anticipated that *bar1* mutations would suppress the temperature-sensitive α-factor defects in *matα2-34* 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 *matα2-34* strain with a *MAT***a** *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 *MAT***a** but not in the *MATα* spores. Its assignment is unambiguous in the PD and NPD asci if 2:2 segregation is assumed. Accordingly, the formation of α-factor at 34°C by the *matα2-34* spores in these asci demonstrates the suppression of the temperature-sensitive defect by *bar1*. This interpretation is further supported by the T asci. In every case where an ascus contains a Bar$^+$ *MAT***a** spore, there is a corresponding *matα2-34* spore that secretes α-factor at the restrictive temperature.

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

Table VI

Tests for Allelism of tup1-7 and tup1-16

Strain	Genotype	a-Factor	α-Factor	Barrier	Clumpy growth	Cell morphology
CR7	*MATα*	−	+	−	−	N*
PD7	*MATα tup1-7*	ts‡	ts	ts	ts	S
XY507-5A	*MATα tup1-16*	+	−	+	+	S
	MATα tup1-7					
XP656	*MATα tup1-16*	ts	ts	ts	ts	S
	MATα tup1-16					
XP657	*MATα TUP1*	−	+	−	−	N
	MATα tup1-7					
XP658	*MATα 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 matα2-34 by bar1 Tetrad Analysis of XP545 and XP546

Type	Genotypes	Phenotype		Number of asci		
		α-Factor	Bar	XP545	XP546	Total
PD	*MATa bar1*	−	−	7	4	11
	MATa bar1	−	−	—	—	—
	matα2-34 +	ts	−	—	—	—
	matα2-34 +	ts	−	—	—	—
NPD	*MATa +*	−	+	9	6	15
	MATa +	−	+	—	—	—
	matα2-34 bar1	+	−	—	—	—
	matα2-34 bar1	+	−	—	—	—
T	*MATa +*	−	+	20	11	31
	MATa bar1	−	−	—	—	—
	matα2-34 +	ts	−	—	—	—
	matα2-34 bar1	+	−	—	—	—

allele in the *Mat*a background. However, asci of class 1, of which two were found, permit unambiguous assignment of the *tup1-7* allele to the *MATα* spores on the basis of their temperature-sensitive **a**-factor expression. The occurrence of one Bar⁺ *MAT***a** spore implies that one of the *MATα tup1-7* spores carries *bar1-1*. Thus, we infer that spores producing both **a**-factor and α-factor at 34°C have the genotype *MATα tup1-7 bar1-1*. 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 *bar1* has no effect on the reduced mating efficiencies caused by *matα2-34* and *tup1-7* at their restrictive temperatures. This is fully in agreement with the findings and interpretations reported by Sprague and Herskowitz (9) for *matα2-1* and *matα2-4*.

Table VIII

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

Phenotypes			Inferred genotypes	Number of asci
a-factor	α-factor	Bar		
+	−	+	*MAT***a** + +	2
+	−	−	*MAT***a** + *bar1*	
ts	ts	−	*MATα tup1*	
ts	+	−	*MATα tup1 bar1*	
+	−	+	*MAT***a** *¹ +	1
+	−	+	*MAT***a** *	
−	+	−	*MATα* + *bar1*	
ts	+	−	*MATα tup1 bar1*	
+	−	+	*MAT***a** * +	1
+	−	−	*MAT***a** * *bar1*	
ts	+	−	*MATα tup1 bar1*	
−	+	−	*MATα* +	
+	−	+	*MAT***a** * +	5
+	−	−	*MAT***a** * *bar1*	
−	+	−	*MATα* + *bar1*	
ts	ts	−	*MATα tup1*	
+	−	+	*MAT***a** * +	2
+	−	−	*MAT***a** * *bar1*	
ts	+	−	*MATα tup1 bar1*	
−	+	−	*MATα* +	
+	−	+	*MAT***a** *tup1* +	
+	−	−	MATa tup1 bar1	1
−	+	−	*MATα* + *	
−	+	−	*MATα* + *	

Traits marked * could not be ascertained from the data.

DISCUSSION

These two mutants, PD7 (*tup1-7*) and PE34 (*matα2-34*) 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 *MATα2*, these are **a**-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 MATa *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 α1-α2 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 *matα2-matα1* 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 *matα2-34/matα2-1* and *matα2-34/matα2-4* 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 *matα2-34* 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 *matα2-34* 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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REFERENCES

1. Crandall, M., R. Egel, and V. L. MacKay. 1977. Physiology of mating in three yeasts. *Adv. Microb. Physiol.* 15:307–398.
2. Manney, T. R., and J. H. Meade. 1977. Cell-cell interactions during mating in *Saccharomyces cerevisiae*. Receptors and Recognition. (Series A) J. L. Reissig, editor. Chapman & Hall, London. 3:283–321.
3. MacKay, V., and T. R. Manney. 1974. Mutations affecting sexual conjugation and related processes in *Saccharomyces cerevisiae* I. Isolation and phenotypic characterization of nonmating mutants. *Genetics.* 76:255–271.
4. MacKay, V., and T. R.Manney. 1974. Mutations affecting sexual conjugation and related processes in *Saccharomyces cerevisiae*. II. Genetic analysis of nonmating mutants. *Genetics.* 76:273–288.
5. Strathern, J., J. Hicks, and I. Herskowitz. 1981. Control of cell type in yeast by the mating type locus: the α1-α2 hypothesis. *J. Mol. Biol.* 147:357–372.
6. Hicks, J. B., and I. Herskowitz. 1976. Evidence for a new diffusible element of mating pheromones in yeast. *Nature (Lond.).* 260:246–248.
7. Manney, T. R., and V. Woods. 1976. Mutants of *Saccharomyces cerevisiae* resistant to the α mating-type factor. *Genetics.* 82:639–644.
8. Hartwell, L. H. 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. *J. Cell Biol.* 85:811–822.
9. Sprague, G. F., Jr., and I. Herskowitz. 1981. Control of yeast cell type by the mating type locus. I. Identification and control of expression of the **a**-specific gene, *BAR1*. *J. Mol. Biol.* 153:305–321.
10. Kassir, Y., and G. Simchen. 1976. Regulation of mating and meiosis in yeast by the mating-type region. *Genetics.* 82:187–206.
11. Klar, A. J. S., J. N. Strathern, J. R. Broach, and J. B. Hicks. 1981. Regulation of

transcription in expressed and unexpressed mating type cassettes of yeast. *Nature (Lond.)*. 289:239–244.
12. Lemontt, J. F., D. R. Fugit, and V. L. MacKay. 1980. Pleiotropic mutations at the *TUP1* locus that affect the expression of mating-type-dependent functions in *Saccharomyces cerevisiae*. *Genetics*. 94:899–920.
13. Wickner, R. B. 1974. Mutants of *Saccharomyces cerevisiae* that incorporate deoxythymidine-5′-monophosphate into deoxyribonucleic acid *in vivo*. *J. Bacteriol.* 117:252–260.
14. Rothstein, R. J., and F. Sherman. 1980. Genes affecting the expression of cytochrome c in yeast: genetic mapping and genetic interactions. *Genetics*. 94:871–889.
15. Schamhart, D. H. J., A. M. A. ten Gerge, and K. W. van de Poll. 1975. Isolation of a catabolite repression mutant of yeast as a revertant of a strain that is maltose negative in the respiratory-deficient state. *J. Bacteriol.* 121:747–752.
16. Mortimer, R. K., and D. Schild. 1980. Genetic map of *Saccharomyces cerevisiae*. *Microbiol. Rev.* 44:519–571.
17. Meade, J. H., M. I. Riley, and T. R.Manney. 1977. Expression of cryptopleurine resistance in *Saccharomyces cerevisiae*. *J. Bacteriol.* 129:1428–1434.
18. Fink, G. R., and C. A. Styles. 1972. Curing of a killer factor in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA*. 69:2846–2849.
19. Duntze, W., D. Stotzler, E. Bucking-Throm, and S. Kalbitzer. 1973. Purification and partial characterization of α-factor, a mating-type specific inhibitor of cell reproduction from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 35:357–365.
20. Lipke, P. N. 1976. Biochemical studies on morphogenesis induced by a sex pheromone in *Saccharomyces cerevisiae*. Ph.D. Dissertation. University of California, Berkeley.
21. Tanaka, T., H. Kita, T. Murakami, and K. Narita. 1977. Purification and amino acid sequence of mating factor from *Saccharomyces cerevisiae*. *J. Biochem.* 82:1681–1687.
22. Chan, R. K., and C. A. Otte. 1982. Physiological characterization of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by a-factor and α-factor pheromones. *Mol. Cell. Biol.* 2:11–20.
23. Chan, R. K., and C. A. Otte. 1982. Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to GI arrest by a-factor and α-factor phermones. *Mol. Cell. Biol.* 2:21–29.
24. Duntze, W., V. MacKay, and T. R.Manney. 1970. *Saccharomyces cerevisiae:* a diffusible sex factor. *Science (Wash. DC)*. 168:1472–1473.
25. Mortimer, R. K., and D. C. Hawthorne. 1969. Yeast genetics. *In* The Yeasts. A. H. Rose and J. S. Harrison, editors. Academic Press, Inc., New York. 1:385–460.
26. Riley, M. I., and T. R. Manney. 1978. Tetraploid strains of *Saccharomyces cerevisiae* that are trisomic for chromosome III. *Genetics*. 89:667–684.
27. Stotzler, D., and W. Duntze. 1976. Isolation and characterization of four related peptides exhibiting α-factor activity from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 65:257–262.
28. Betz, R., V. L. MacKay, and W. Duntze. 1977. a-factor from *Saccharomyces* cerevisiae: partial characterization of a mating hormone produced by cells of mating type a. *J. Bacteriol.* 132:462–472.