MUTANTS OF *SACCHAROMYCES CEREVISIAE* UNRESPONSIVE TO CELL DIVISION CONTROL BY POLYPEPTIDE MATING HORMONE

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ABSTRACT

Temperature-sensitive mutations that produce insensitivity to division arrest by α-factor, a mating pheromone, were isolated in an *MATa* strain of *Saccharomyces cerevisiae* and shown by complementation studies to define eight genes. All of these mutations (designated *ste*) produce sterility at the restrictive temperature in *MATa* cells, and mutations in seven of the genes produce sterility in *MATα* cells. In no case was the sterility associated with these mutations correctible by including wild-type cells of the same mating type in the mating test nor did any of the mutants inhibit mating of the wild-type cells; the defect appears to be intrinsic to the cell for mutations in each of the genes.

Apparently, none of the mutants is defective exclusively in division arrest by α-factor, as the sterility of none is suppressed by a temperature-sensitive *cdc* 28 mutation (the latter imposes division arrest at the correct cell cycle stage for mating).

The mutants were examined for features that are inducible in *MATa* cells by α-factor (agglutinin synthesis as well as division arrest) and for the characteristics that constitutively distinguish *MATa* from *MATα* cells (a-factor production, α-factor destruction). *ste2* Mutants are defective specifically in the two inducible properties, whereas *ste4, 5, 7, 8, 9, 11,* and *12* mutants are defective, to varying degrees, in constitutive as well as inducible aspects. Mutations in *ste8* and *9* assume a polar budding pattern unlike either *MATa* or *MATα* cells but characteristic of *MATa/α* cells.

This study defines seven genes that function in two cell types (*MATa* and α) to control the differentiation of cell type and one gene, *ste2*, that functions exclusively in *MATa* cells to mediate responsiveness to polypeptide hormone.

In most eukaryotic cells for which information exists, division is controlled in G1, before the initiation of DNA synthesis, whether by hormones, nutrients, or during development. An understanding of how division is controlled will require knowledge of the gene products that function in division control and an analysis of their roles. Mutants of *Saccharomyces cerevisiae* have been used to define two genetic programs in the cell cycle and to locate a step in G1, "start", common to both pathways where division is controlled (11). Completion of start results in the duplication of

the spindle-pole body, a microtubule organizing region imbedded in the nuclear membrane (2). At least four gene products perform essential functions at start (Reed, S., in press).

Cell division in *S. cerevisiae* is controlled at start both by polypeptide hormones and by nutrients. Polypeptide hormones are produced by each of the two mating types, a-factor by *MAT*a cells (1) and α-factor by *MAT*α cells (29); these hormones arrest division in the cell of opposite mating type at start (3, 31) and, together, they synchronize the cell cycles of the two partners in conjugation (10).

The purpose of the work reported here was to define the genes that mediate the arrest of division in response to polypeptide hormones. Mutants of an *MAT*a strain were selected for their resistance to division arrest by α-factor. It was known from previous work that most of the mutants selected in this way would be sterile (21). An analysis of the number of genes that are essential for mating hormone responsiveness and fertility was precluded in previous studies (17, 18, 21) because of the sterility associated with such mutants. Hence, the present work was restricted to the study of temperature-sensitive mutants to facilitate complementation tests.

*MAT*a cells display several features associated with mating that distinguish them from *MAT*α cells (5, 20). *MAT*a cells produce a-factor (31), destroy α-factor (4, 13, 19, 30), and are inducible by α-factor for *a* agglutinin (7) and for cell division arrest at start (3). *MAT*α cells, in contrast, produce α-factor (16, 29) and are inducible for cell division arrest at start by a-factor (31); commonly, *MAT*α cells produce α agglutinin constitutively but some are inducible by a-factor (27). To gain some insight into the specific roles played by the genes identified in this study, hormone production, hormone destruction, and agglutination were monitored in the mutants.

Although the two cell types have many phenotypic differences, they contain the same genetic information, differing only in the information that is being expressed at the *MAT* locus (12, 14). Indeed, *MAT*a cells can be converted to *MAT*α and vice versa, possibly through a rearrangement of DNA involving the *MAT* locus. It was of interest therefore to determine whether the mutants isolated in *MAT*a cells have any effect when in an *MAT*α cell. MacKay and Manney (18) found that mutations in *ste4* and *5* produced sterility in both mating types, whereas mutations in *ste2* were specific for *MAT*a cells and those in *ste3* were specific for *MAT*α.

MATERIALS AND METHODS

Strains

The parent strain of the *ste* mutants, 381G *MAT*a *SUP4-3 cry1 his4-580 trp1 ade2-1 tyr1 lys2* (381G), carries a temperature-sensitive amber nonsense suppressor (*SUP4-3*), two amber markers (*his4-580* and *trp1*), three ochre markers (*ade2-1, tyr1,* and *lys2*), recessive resistance marker *cry1* closely linked to the mating-type locus *MAT*a, and probably the cytoplasmic element [psi], although the latter was not confirmed by direct test. This strain was derived from strain 381-11-1 by selection of a spontaneous clone that grew on YEPD plates at pH 3.5, so that mutant selection could be performed at low pH where the degradation of α-factor is diminished. Strain 381-11-1 was derived from crosses involving strains FM11 (24) provided by Dr. Gerald Fink (Cornell University, Ithaca, N. Y.) and *cry5*, a mutant resistant to cryptopleurine at the *cry1* locus (28) derived from strain A364A (9) by Dr. Calvin McLaughlin (University of California, Irvine, Calif.). To derive *MAT*α *ste* strains for complementation, the *MAT*a *ste* strains were crossed with 382-31 *MAT*α *his4-580 met2 ura1*, the resulting diploid was sporulated, and tetrads were dissected. Other strains used here include EMS63 *MAT*α *his2* (provided by Gerald Fink), 5003-38B (provided by Seymour Fogel, University of California, Berkeley, Calif.), and X2180-1A and X2180-1B (Yeast Genetics Stock Center, Donner Laboratory, University of California, Berkeley, Calif.).

Genetic Analysis

Procedures for mating, diploid isolation, and tetrad analysis are standard (22).

Chemicals and Mating Factors

Cryptopleurine was obtained from Chemasea Mfg. Pty. Ltd., Sydney, Australia, and cycloheximide from Sigma Chemical Co., St. Louis, Mo. Partially purified α-factor was prepared by the method of Duntze et al., (6) as modified by Chan (4); this preparation was used in assays of destructive activity. Both a- and α-mating-factor preparations for the induction of agglutinin were prepared as culture supernates from dense cultures of strains X2180-1A and X2180-1B, respectively, grown in YM-1 medium (9).

Mutant Isolation

An overnight stock of the parent strain (381G) was mutagenized with ethylmethane sulfonate (23) to ~50% survival, diluted into numerous tubes containing YM-1 medium, and grown for a 20-fold increase in cell density overnight at 34°C. Samples were plated on YM-1 plates (adjusted to pH 3.5 after autoclaving) at sufficient density to give between 10 and 100 mutants per plate and incubated at 34°C. A crude preparation of α-factor containing about 10^3 U was spread over the agar surface. Mutants were cloned on nonselective plates and tested for mating with *MAT*a and *MAT*α testers. Most clones were found to be nonmaters at both temperatures; ~10% were maters at 22° and nonmaters at 34°C. Mutants with different numbers came from different tubes and are presumed of independent origin.

Quantitative Mating Experiments

Cells were grown overnight at 22° or 34°C in YM-1 medium and collected at a density of <2 × 10⁷ cells/ml. 2 × 10⁶ Cells of each mutant were mixed with 2 × 10⁶ cells of strain EMS63 *MAT*α, collected on a filter (type HA; Millipore Corp., Bedford, Mass.), and washed with 10 ml of YM-1, and the filter was transferred to a plate containing YM-1 medium with 2% Noble agar (Difco Laboratories, Detroit, Mich.), which produces higher yields of diploids than medium with Bacto agar. After 6 h at 22° or 34°C, the cells were resuspended in 5 ml of YNB medium (15) without ammonium sulfate or glucose, sonicated for 10 s to disperse clumps, diluted, and plated onto selective nutritional plates.

Agglutination

Cells were grown overnight in YM-1 medium at 20°–22°C. Agglutinin was induced at 22° and 34°C as follows. One part of the culture was adjusted to 4 × 10⁶ cells/ml, received 100 U/ml of α-factor, and was incubated for 1 h at 22°C. The other part was adjusted to 2.6 × 10⁶ cells/ml and was incubated 2 h at 34°C; at the end of the 34°C preincubation, this culture received 100 U/ml of α-factor and was incubated for 1 h more at 34°C. Both cultures received 100 µg/ml cycloheximide after the induction period. Induction of agglutinin is arrested by cycloheximide.

The presence of agglutinin was then quantitated by mixing 1 ml of induced *MAT*a with 1 ml of strain EMS63 *MAT*α (this strain is constitutive for the *MAT*α agglutinin and was pregrown in YM-1 medium at 22°C). The mixture was centrifuged at 700 g in 13 × 100-mm test tubes for 5 min; the tube was then covered and inverted six times to resuspend the pellet and allowed to settle at 1 g for 20 min. Large clumps of cells resulting from agglutination will have settled in this period, whereas nonagglutinated mixtures will not. The OD₆₆₀ of the supernate was then read and converted to the equivalent cell number by an empirical curve. The mixture was prepared in triplicate and the three values were averaged. The cell number for a culture treated identically but containing two ml of the *MAT*a strain and another culture containing 2 ml of the *MAT*α strain were treated identically. The agglutination index was calculated as follows: agglutination index, A.I. = ([(A + B)/2] − C)/(A + B)/2 where A is the cell number observed in the *MAT*a culture (nonagglutinated cells); B, the cell number in the *MAT*α culture; and C, the cell number in the mixture. Thus, if C = 0 (complete agglutination), the A.I. = 1.0, and if C = (A + B)/2 (no agglutination), A.I. = 0.

a-Factor Assay

a-Factor was assayed by its ability to induce agglutinin in an *MAT*α strain. Most *MAT*α strains are constitutive for agglutinin but strain 5003-38B is highly inducible. Strain 5003-38B was grown overnight in YM-1 medium containing glycerol as carbon source (to reduce the basal level of agglutinin) at 22°C, adjusted to a density of 5 × 10⁶ cells/ml, and sonicated lightly to break up clumps. The a-factor preparation to be assayed was then diluted to give a final volume of 1 ml in YM-1 containing glucose as carbon source and mixed with 4 ml of the 5003-38B culture. The cultures were incubated at 20°–22°C for 2 h in tubes rotating on a rollodrum (New Brunswick Scientific Co., Inc., Edison, N. J.). The induction was terminated by addition of 100 µg/ml cycloheximide, and agglutinin was assayed immediately, although the cells can be stored cold for a few hours. An *MAT*a strain (381G), fully induced for agglutinin, was prepared by adjusting a culture

growing in YM-1 medium to 5 × 10⁶ cells/ml at 22°C, adding 0.10 vol of medium containing ~10⁹ U/ml α-factor, incubating for 1 h, adding another 0.10 vol of α-factor containing medium, and incubating for 1 h more. Agglutinin induction was assayed by mixing 1 ml of the induced 5003-38B strain with 1 ml of the induced *MAT*a strain (381G). The agglutination index was determined as described for the agglutination assay except that the cells were allowed to settle for 15 min at 1 g before the OD 660 was read. A unit of a-factor is the amount that will induce an A.I. of 0.5 in strain 5003-38B. A series of threefold dilutions of the a-factor preparation to be assayed were made and the A.I. was plotted against the log of the dilution. A series of three points spanning the 0.5 A.I. were obtained and the activity of the preparation was determined by drawing a line through these three points to estimate the amount of the preparation that would produce an A.I. of 0.5.

α-Factor Assay

α-Factor was assayed in the same manner as a-factor. The inducible *MAT*a strain (381G) was grown overnight in YM-1 medium (containing glucose as carbon source) at 22°C, adjusted to a density of 5 × 10⁶ cells/ml. The α-factor preparation to be assayed was diluted into 1 ml of YM-1 and added to 4 ml of the 381G culture. The cultures were incubated at 22°C on a rollodrum for 1 h. Induction was terminated by the addition of 100 µg/ml cycloheximide. An *MAT*α strain, fully induced for agglutinin, was prepared by adjusting a culture of EMS63 growing in YM-1 to a density of 4 × 10⁶ cells/ml, adding 0.10 vol of medium containing ~10⁹ U/ml of a-factor, incubating for 1 h at 22°C, adding another 0.10 vol of medium containing a-factor, and incubating for 1 h more. Cycloheximide was added to produce a final concentration of 100 µg/ml, and the induced *MAT*α culture was kept on ice and used for the rest of the day.

The induction of agglutinin in the 381G *MAT*a strain was assayed by mixing 1 ml of the 381G culture with 1 ml of the induced EMS63 *MAT*α culture and determining the agglutination index as described for the agglutination assay. A unit of α-factor is defined as the amount that will induce an A.I. of 0.4 in strain 381G. A series of threefold dilutions of the α-factor preparation to be assayed were made and the A.I. was plotted against the log of the dilution. A series of three points spanning the 0.4 A.I. were obtained and the activity of the preparation was determined by drawing a line through these three points to estimate the amount of the preparation that would produce an A.I. of 0.4.

α-Factor Destruction

Cells were grown overnight in YM-1 at 34°C, adjusted to a density of 1 × 10⁷ cells/ml, and diluted to 10 ml with YM-1 that was preheated to 34°C and contained sufficient α-factor to give a final concentration of ~10³ U/ml. A culture without cells was incubated as control. At .5, 1, 2, and 4 h, 2.5 ml of medium were removed and centrifuged to remove cells, and the supernates were frozen in dry ice-acetone and stored at −20°C. Samples were then thawed and assayed as described below for the quantity of α-factor remaining. Because the assay was only reproducible to within a factor of ~2 when these experiments were performed, an initial assay was performed to find the time at which most but not all of the activity had disappeared. The activity remaining at this time point was then assayed quantitatively and the destructive activity of the mutant as compared to that of the parent strain was calculated, considering both the final activity and the

amount of growth that had occurred in each culture during the period of incubation. To correct for the growth in the different cultures, the decrease in α-factor activity that occurred during the time interval, t, was divided by $(e^{at} - 1)/a$, where $a = \ln 2/t_2$ and t_2 = the population doubling time at 34°C (83 min). The values obtained are the units of α-factor destroyed per milliliter per minute by 10^7 cells.

Time-Lapse Photomicroscopy for Budding Pattern

Cells were grown overnight in YM-1 medium at 34°C to a density of ~10^7/ml. They were agitated on a vortex mixer for 20 s and a drop was placed on a slab of agar maintained at 34°C and consisting of YM-1 medium containing 2% Noble agar (Difco) ~1-mm thick resting on a microscope slide. Cells were allowed to settle for 20 s, the slide was placed vertically to allow the liquid to run off the cells, and once the surface was dry, a mesh of nylon screen was placed over the cells to facilitate field location. A sleeve, surrounding the piece of agar, was sealed to the slide with grease, and a cover slip was placed over the sleeve between pictures to prevent drying. Photographs were taken through a 16 × objective by use of dark field optics with Kodak TriX Pan film ASA 400 every 30 min for 3 h. Unbudded cells or cells with a single bud were followed on the series of photographs until the cell produced a second bud. The initial position of the second bud was recorded as equatorial if it was located in the same hemisphere as the first bud and polar if located in the opposite hemisphere.

RESULTS

Mutant Isolation

Mutants were obtained by mutagenizing strain 381G MATa and selecting for growth on plates containing α-factor at 34°C. Clones were then tested for mating with an MATα strain at 22° and at 34°C, and 239 clones that displayed temperature sensitivity for mating were selected for further characterization.

Complementation Tests

Complementation tests were performed by the scheme outlined in Fig. 1. Representative mutants were mated to strain 382-31 MATα at 22°C and the resulting diploids were sporulated and the asci dissected. For seven of the eight complementation groups eventually identified, MATα strains that were temperature sensitive for mating were obtained among the spores. For one complementation group (ste2), none of the MATα spores produced clones sterile at 34°C, whereas about one-half of the MATa spores produced clones sterile at 34°C; this result indicated that a mutation unlinked to the MAT locus was present that conferred sterility specifically in MATa cells. MATα strains carrying the ste2 mutation were identified

1. Parent strain: 381G MATa cry1 SUP4
2. Mutagenize and plate on α-factor to select: 381G-50B ste2 MATa cry1 SUP4
3. Mate with 382-31 MATα CRY⁺, sporulate and dissect to obtain: ste2 MATα CRY⁺
4. Cross all ste MATa by ste MATα to obtain:

MATa cry1	ste x
MATα CRY⁺	ste y

5. Plate on cryptopleurine to select:

MATa cry1	ste x
MATa cry1	ste y

6. Test for temperature-sensitivity of mating to assign complementation groups.

FIGURE 1 Flow chart for complementation studies.

by further outcrosses and these strains were used in complementation studies. An MATα strain carrying an ste mutation and appropriate nutritional markers was crossed to all temperature-sensitive MATa ste mutants and the resulting MATa/α diploids were isolated by prototrophic selection. These diploids were replica plated onto YEPD plates spread with 0.1 ml of 250 μg/ml cryptopleurine (Chemasea) in methanol. The majority of the colonies that grew up on these plates mated at 22°C and were presumed to be MATa/a diploids, heterozygous for the two ste mutations. If they did not mate at 34°C, the mutants were assigned to the same complementation group. A possible source of error would arise if the ste locus is linked to MAT, but none of the mutants studied here is so linked.

In this way, mutants were assigned to eight complementation groups. The distribution of alleles per group is shown in Table I. Complementation studies with ste mutants from the MacKay and Manney collection (17, 18) showed that three of these groups (ste2, 4, and 5) corresponded to loci previously described.

Segregation

Crosses with mutants in groups ste4, 5, 7, 8, 9, 11, and 12 segregated primarily two sterile to two fertile spores when tested at 34°C. Hence, all are the result of single gene defects. None were linked to the MAT locus or closely centromere linked (data not shown).

Mating

The mutants were tested at 22° (permissive) and 34°C (restrictive) after pregrowth at the same temperature for their mating efficiencies (Table II). Most mutants mate between 0.2 and 1.0 as effi-

TABLE I

Complementation Groups and MAT Specificity of
ste Mutants

Gene*	Strains‡	Allele*	Total§	MAT‖	Suppres- sible¶
ste2	50B	3	5	a	0/5
	61C	4			
	84D	5			
	90E	6			
ste4	63B	3	32	a, α	3/5
	68B*	4			
	82B	5			
	118B	6			
	234A	7			
ste5	42E	3	25	a, α	3/9
	64C	4			
	66A	5			
	101A	6			
	206C	7			
ste7	43A	1	12	a, α	0/6
	79A	2			
	93F	3			
	214A	4			
ste8	52B	1	5	a, α	0/3
	59A	2			
	76D	3			
	85D	4			
	91A	5			
ste9	62C	1	5	a, α	2/4
	95D*	2			
	99G	3			
	204SB*	4			
	236F	5			
ste11	41A	1	83	a, α	0/6
	44B	2			
	53A	3			
	57A	4			
ste12	59C	1	21	a, α	0/5
	85F	2			
	94B	3			
	97C	4			
	117A	5			

* The complementation groups have been assigned the designation ste (for sterility, in accord with the nomenclature of MacKay and Manney [17, 18]). Complementation studies with strains provided by MacKay and Manney show that three of the eight groups correspond to ste2, 4 and 5; the first two alleles of ste2, 4, and 5 are those isolated by MacKay and Manney.
‡ Strains harboring these mutations in the 381G back-

ciently as the parent strain at 22°C and between 10^{-4} and 10^{-6} as well at 34°C. Thus for all eight genes the frequency of conjugation is drastically reduced by the mutation conferring resistance to division arrest by mating factor.

Suppressibility

The mutants were derived in a background containing a temperature-sensitive amber nonsense suppressor, SUP4-3. Hence the temperature-sensitive fertility of the mutants can be the result either of a thermolabile gene product or of a nonsense mutation whose suppression is thermolabile. Segregation studies were performed by crossing the mutants to strain 382-31, which does not carry the temperature-sensitive amber suppressor but does carry the same suppressible nutritional markers present in the parent strain of the mutants trp1 and his4. Hence segregation of the temperature-sensitive suppressor could be followed unambiguously by the trp and his phenotypes of the segregants and the existence of nonsense ste mutants was evidenced by the presence of nonconditional sterility in segregants containing the ste mutation but lacking the SUP4-3 mutation and temperature-sensitive fertility in segregants containing both the ste and SUP4-3 loci. Eight nonsense ste mutants were discovered from among 43 mutants analyzed (Table I).

Intrinsic or Extrinsic Defect

Because extracellular components (mating hormones and agglutinins) are involved in mating, it is possible that some of the mutants are defective solely in some of these or other extracellular agents. If so, their mating defect might be corrected by the presence of nonmutant cells of the same mating type. Therefore quantitative mating experiments were carried out between temperature-sensitive mutants representative of each complementation group and an MATα tester strain in the presence of nonmutant cells of MATa. Nutritional

ground are designated 381G-50B, etc. * Indicates a nonsense mutation. The first strain listed for each gene is on deposit at the Yeast Genetics Stock Center.
§ The total number of mutations found in each complementation group.
‖ When present in a strain of the indicated MAT phenotype, the mutation eliminates fertility.
¶ The number of alleles suppressible by SUP4-3 divided by the total number of alleles analyzed by tetrad analysis.

TABLE II

Mating Frequencies of ste Mutants at 22° and 34°C

Gene	Strain	Mating efficiency* (mutant/381G)	
		22°C	34°C
ste2	50B	1.0	2×10^{-5}
	90E	1.2	1×10^{-4}
ste4	63B	0.2	7×10^{-4}
	68B	0.2	$<1 \times 10^{-4}$
	82E		
ste5	42E	1.4	6×10^{-3}
	64C	0.02	1×10^{-5}
	101A	0.01	$<3 \times 10^{-6}$
ste7	79A	0.3	1×10^{-6}
	214A	0.5	1×10^{-5}
ste8	59A	0.6	3×10^{-4}
	91A	0.5	$<1 \times 10^{-6}$
ste9	62C	0.4	2×10^{-4}
	95D	0.3	4×10^{-6}
	236F	0.3	2×10^{-3}
ste11	41A	0.6	1×10^{-4}
	44B	1.0	4×10^{-4}
	53A	0.7	2×10^{-5}
ste12	59C	0.5	$<1 \times 10^{-6}$
	85F	0.4	$<1 \times 10^{-6}$

	Diploids formed‡	
Strain	22°C	34°C
381G	$2.88 \pm 0.82 \times 10^6$	$2.62 \pm 0.87 \times 10^7$

* Strains were pregrown overnight at the temperature used for the mating. The number is the number of diploids formed in matings between the mutant strain and an $MAT\alpha$ tester, divided by the same number for matings between the parent strain 381G and the same $MAT\alpha$ tester.

‡ The average number of diploids formed and the standard deviation in matings between the parent strain 381G and the tester $MAT\alpha$ strain in seven experiments. One unusually high experiment was eliminated from the calculations.

markers were arranged so that only matings between the MATa temperature-sensitive mutant and tester $MAT\alpha$ were scored. The results in Table III (column headed *helper a*) demonstrate that the mating defect of these mutants is not corrected by the presence of nonmutant cells. The control for

all experiments is along the diagonal of Table III, which records diploids formed in mating mixtures containing a single MATa strain and the tester $MAT\alpha$.

A mutant from each *ste* gene was also mixed with cells of the parent strain (381G) and mated to a test $MAT\alpha$ strain to see if the mutant cells produced an extracellular inhibitor of mating. In no case did the mutant cells inhibit mating between the parent strain and the test $MAT\alpha$ strain (Table III, column headed STE^+).

Finally, a mutant from each *ste* gene was mixed with mutants of all other *ste* genes in all pairing combinations to see if any pairs of mutants could cross-feed one another for their mating defect. In no case was mating significantly stimulated with heterologous pairs of *ste* mutants over that seen with each *ste* mutant by itself (Table III). The genetic defect in each of these mutants appears to be in some cellular process intrinsic to the cell.

Morphological Alteration

After division arrest of MATa cells by α-factor, the cells continue protein and RNA synthesis; they become larger and elongated (the resulting cells are called shmoos). Mutants representative of each complementation group were pregrown at 34°C and sufficient α-factor was added to induce shmoo formation in the parent strain, 381G. None of the mutant cultures revealed such shmoos.

Agglutination

Most MATa strains and some $MAT\alpha$ strains are inducible by mating factor for a surface agglutinin; the agglutinin is specific for the cell of opposite mating type. The parent strain of the mutants, 381G, is highly inducible, exhibiting an agglutination index <0.10 without prior exposure to α-factor and >0.70 after induction with α-factor (Table IV). Most mutant strains exhibit a fairly high agglutination index when pregrown at 22°C and induced with α-factor at 22°C, the permissive temperature for mating (Table IV). However, a few strains exhibit low agglutination indices even under the permissive conditions. With few exceptions the mutants are no longer inducible for agglutinin after pregrowth for 2 h at 34°C (Table IV). One allele of *ste2* and one allele of *ste4* are exceptions in that they retain some inducibility for agglutinin after pregrowth at 34°C. I conclude that the products of all of the *ste* genes are necessary for agglutinin induction.

TABLE III

Tests for Defects in Extrinsic Mating Factors Among ste Mutants

Strain	Helper a 265-7-4	STE+ 381G	ste2 90E	ste4 68B	ste5 101A	ste7 79A	ste8 59A	ste9 95D	ste11 53A	ste12 85F
Helper a	<50									
STE+	2.1×10^7	1.7×10^7								
ste2	2.1×10^2	1.7×10^7	2.5×10^2							
ste4	<50	2.9×10^7	4.4×10^2	<50						
ste5	<50	2.0×10^7	3.6×10^2	<50	<50					
ste7	<50	2.1×10^7	1.8×10^2	<50	<50	<50				
ste8	<50	1.7×10^7	2.6×10^2	<50	<50	<50	<50			
ste9	<50	3.6×10^7	2.5×10^2	<50	<50	<50	<50	<50		
ste11	<50	2.3×10^7	2.5×10^2	<50	<50	<50	<50	<50	<50	
ste12	<50	1.4×10^7	2.2×10^2	<50	<50	<50	<50	<50	<50	<50

Cells were pregrown at 34°C, the restrictive temperature, and each mating mixture received 1×10^6 cells of each of two MATa strains (one from the left column and one from the top row) as well as 2×10^6 of the tester MATα, 265-9-4. The helper MATa (column helper a) strain was 265-7-4. Incubations were at 34°C and other procedures as in Quantitative Mating Experiments under Materials and Methods. Numbers record the total number of diploids formed exclusive of matings between 265-7-4 and 265-9-4.

Mating Factor Production

The a-factor is produced constitutively by MATa strains and can be quantitatively assayed by its ability to induce agglutinin in an inducible MATα strain. The parent strain, 381G, accumulates a constant amount of a-factor per cell in the culture medium throughout its exponential growth cycle (unpublished observations). Mutants pregrown at 34°C were assayed for the amount of a-factor accumulated in the medium (Table V). Lesions in ste8 and 9 reduce a-factor production to levels that are undetectable by my assay (1 or 2% of the parent strain). Lesions in ste4, 5, 7, 11, and 12 reduce a-factor production to levels between 10 and 60% of the parent strain but do not eliminate it. Lesions in ste2 appear to elevate a-factor production by 250%.

A mixing experiment was performed to determine whether or not the absence of a-factor activity in supernates from mutants in ste8 and ste9 resulted from an inhibitor of a-factor or from the absence of a-factor. A standard curve to determine a-factor activity in the parent strain (381G) supernate was run with and without 0.1 ml of culture supernate from strain 59A (ste8) or strain 62C (ste9). The curves were the same indicating that no inhibitor was detectable in the ste8 or 9 extracts (data not shown).

Mating Factor Destruction

MATa strains destroy the biological activity of α-factor apparently by endopeptidic cleavage (30).

The capacity of MATa strains bearing various ste mutations to destroy the biologic activity of α-factor was monitored (Table VI). The mutant strains were pregrown at 34°C (the restrictive temperature), α-factor was added to the growth medium, and the cultures were maintained at 34°C. Samples were withdrawn and centrifuged to remove the cells, and the remaining α-factor was assayed by its ability to induce agglutinin on a naive, tester MATa strain.

Mutations in ste8 and 9 eliminate detectable mating factor destruction, in ste11 and 12 reduce destructive activity to ~5% of that in the parent strain, in ste4, 5, and 7 to ~15-20% of the parent strain, and in ste2 have no effect upon destructive activity.

Budding

MATa and MATα cell budding is equatorial; the mother and daughter bud near the site of previous bud or birth scar (8). Diploid MATa/α cell budding is polar; the bud of the mother cell usually appears at the opposite end of the elliptical cell from its last bud scar; the daughter is less predictable. MATa strains bearing various ste mutations were grown at 34°C and examined for their budding pattern by time-lapse photography. The fraction of mother cells displaying polar budding or equatorial budding was determined (Table VII). Mutations in two ste genes, 8 and 9, alter the budding pattern of the MATa strain from the characteristically equatorial pattern of the 381G

parent strain to a predominantly polar pattern characteristic of *MATa/α* strains.

Suppression by cdc 28

The sterility of a mutant that is defective specifically in the arrest of cell division by mating factor might be suppressed by a second mutation that imposes division arrest at start, the stage from which conjugation is permitted (25). Double mutants carrying an allele of one *ste* gene and a *cdc* 28 mutation were constructed to test this possibility. Crosses were performed between one allele of each *ste* gene and a strain (638-1) carrying the *cdc* 28-4 allele, and the doubly heterozygous diploids were sporulated and dissected (Table VIII). For *ste4, 5, 7, 8, 9, 11*, and *12*, both the fertility and growth at 34°C segregated $2^-:2^+$. The ratios of PD:NPD:TT indicated that the *ste* and *cdc* lesions were not closely linked. Because NPD tetrads yield two double mutants (ste^-, cdc^-) and TT asci each yield one double mutant, it is clear from these data that the sterile phenotype is not being strongly suppressed by the *cdc* 28 lesion. The cross with *ste2* did not segregate $2^+:2^-$ for fertility, because *ste2* is expressed only in *MATa* cells; nevertheless, six segregants with both ste^- and cdc^- phenotypes were obtained, again indicating lack of suppression.

These qualitative data might be misleading, because mating frequencies might vary by orders of magnitude and still be scored the same by the replica plating techniques used in this analysis. Therefore, strains that were double mutants, ste^- and cdc^-, were compared in quantitative mating experiments to strains that were ste^- cdc^+ as a more sensitive test for suppression of ste^- by *cdc* 28^- (Table IX). None of the ste^- mutations is significantly suppressed by the *cdc* 28^- mutation (*ste9* was not tested). I conclude that none of the ste^- mutants is defective exclusively in division arrest by α-factor.

Furthermore, because growth at 36°C segregates $2^+:2^-$ in each of the crosses, none of the *ste* mutations suppresses the growth defect of *cdc* 28.

DISCUSSION

The mutants described in this paper were selected for their resistance to division arrest by α-factor because I was particularly interested in defining the genes that control cell division. It was clear from previous work (21) that mutations in several genes could produce this phenotype and that some

TABLE IV

Agglutination of ste Mutants

| Gene | Strain | Index | |
		22°C	34°C
ste2	50B	.77	.15
	61C	.74	.16
	84D	.71	.19
	90E	.76	.49
*ste4**	63B	.64	.06
	68B	.46	.22
	82B	.69	.01
	118B	.65	−.01
	234A	.63	.01
*ste5**	42E	.81	.09
	64C	.43	.06
	66A	.55	.05
	206C	.54	.04
*ste7**	43A	.40	.00
	79A	.64	.03
	214A	.43	.01
ste8	52B	.69	.05
	59A	.74	.03
	76D	.59	.07
	85D	.17	.00
	91A	.78	.02
ste9	62C	.37	.06
	95D	.06	
	99G	.05	
	204SB	.28	
	204SC	.47	.10
ste11	41A	.75	.00
	44B	.77	.06
	53A	.65	.03
	57A	.50	.04
*ste12**	59C	.70	.04
	94B	.54	.02
	117A	.70	−.01
	381G	.77 ± .064‡	.64 ± 0.49§

* Some alleles of these genes gave low values for agglutination at 22°C and were not tested at 34°C.
‡ Average and SD of seven determinations.
§ Average and SD of three determinations.

of them would be pleiotropic. Eight genes whose function are necessary in *MATa* strains for division arrest by α-factor were defined in the present study by complementation studies among >200

TABLE V

a-Factor production in ste mutants at 34°C

Gene	Strain	a-Factor	Mutant/381G*
		$U/10^7$ cells	
ste2	50B	544	
	61C	1,388	
	84D	1,041	
	90E	1,075	
	avg.	1,012	2.53
ste4	63B	141	
	82B	68	
	118B	147	
	234A	145	
	avg.	125	0.31
ste5	42E	380	
	64C	222	
	66A	226	
	206C	106	
	avg.	233	0.58
ste7	43A	122	
	79A	100	
	214A	38	
	avg.	87	0.22
ste8	52B	<5	
	59A	<5	
	76D	<5	
	91A	≤5	
	avg.	<5	<0.01
ste9	62C	<5	
	95D	<5	
	204SB	13	
	236F	≤5	
	avg.	<7	<0.02
ste11	41A	64	
	44B	77	
	53A	95	
	57A	77	
	avg.	90	0.23
ste12	59C	22	
	94B	57	
	117A	30	
	avg.	36	0.09
	381G	399 ± 148‡	

* The average units per milliliter for all the alleles tested of one gene divided by the average value for the parent strain 381G.

‡ The average and SD for eight determinations of two different cultures.

TABLE VI

The Destruction of α-Factor by ste Mutants at 34°C

Gene	Strain	Destructive activity	Mutant/381G
ste2	50B	38.2	1.0
	90E	41.0	1.1
ste4	63B	8.0	0.21
	82B	7.8	0.21
ste5	42E	7.8	0.21
	64C	7.8	0.21
ste7	93F	5.6	0.15
	214A	5.1	0.14
ste8	52B	<1.0	<0.03
	59A	<1.0	<0.03
	85D	<1.0	<0.03
	91A	<1.0	<0.03
ste9	62C	<1.0	<0.03
	99G	<1.0	<0.03
	236F	1.9	0.05
	236F	1.2	0.03
ste11	44B	1.7	0.045
	57A	1.6	0.043
ste12	85F	1.5	0.040
	97C	1.7	0.045
	381G	37.6	1.0

temperature-sensitive mutants. Mutations in all eight genes were highly pleiotropic (Table X). All genes are essential for fertility in MATa strains, a fact that is consistent with previous findings of Manney and Woods (21) showing that all of 100 nonconditional mutants isolated for resistance to α-factor were also sterile. Neither study precludes the possibility that mutants will be found that retain some fertility and yet are resistant to division arrest, because negative observations are always subject to revision upon more intensive search and because in the present work attention was limited to mutants that were conditionally fertile. Although we confirmed the finding of Manney that almost all mutants isolated for resistance to division arrest are also sterile, we have found a few mutants that grew on mating factor–containing plates and yet retained some fertility; these have not as yet been studied in detail. It is noteworthy that in the previous work of MacKay and Manney (17, 18), as well as the present study,

none of the *ste* mutations mapped to the *MATa* locus.

All of the mutations produce defects intrinsic to the cell as evidenced by their inability to be cor-

TABLE VII

Budding Patterns of ste Mutants at 34°C

Gene	Strain	Equato- rial	Polar	% Polar
ste2	50B	173	8	4.4
	90E	160	6	3.6
ste4	63B	130	10	7.1
	82B	158	10	5.9
ste5	42E	121	16	11.7
	64C	71	13	15.5
ste7	43A	172	11	6.0
	214A	93	12	11.4
ste8	59A	51	71	58.2
	91A	63	76	54.6
ste9	62C	80	134	62.6
	236F	100	154	60.6
ste11	41A	138	11	7.4
	44B	117	9	7.1
ste12	59C	116	15	11.4
	117A	100	1	1.0
	381G *MATa*	91	3	3.2
	381G *MATα*	111	4	3.5
	381G *MATa/α*	25	69	73.4

rected in mating mixtures by the presence of a nonmutant *MATa* strain. This suggests that none of the mutants is defective solely in the production of an extracellular component. Furthermore, none of the mutants inhibits mating of a nonmutant *MATa* in the same mixture, suggesting that the sterility is not a result solely of the overproduction of an extracellular component. However, a negative result in these experiments must be interpreted with caution. It is possible that although the extracellular agents are present in the medium, their biological activity is effective only over short distances. For example, it may be that mutants defective specifically in the production of mating factor will not be phenotypically corrected merely by the addition of mating factor to the medium, and, until such a mutant is known and its response in such a test determined, rigorous interpretation of negative results is impossible. One reason to suspect that the topographical distribution of mating factors, destructive enzymes, receptors, or agglutinins might be important is that matings always occur between two cells rather than among three or more (10, 26), and no explanation yet exists for this restriction.

Seven of the eight genes are necessary for fertility in both mating types. This finding is quite interesting in view of the dramatic differences between the *MATa* and *MATα* cell types. It is almost certain that the chemical structures of the agglutinins, mating factors, mating factor receptors, and the enzymes that destroy the mating factors are different in the two mating types, and yet it appears that many of the gene products that control the expression of these differences are the same. Perhaps the *ste* gene products are intrinsi-

TABLE VIII

Suppression of ste by cdc 28: Tetrad Data

Gene	Strain	Cross	ste 2⁺:2⁻	cdc 2⁺:2⁻	Segregation		
					PD :	NPD :	TT
ste4	63B	1,046	9/9	9/9	0	1	7
ste5	64C	1,048	8/8	8/8	2	0	6
ste7	79A	1,047	12/13	13/13	2	1	10
ste8	76D	1,050	8/8	8/8	2	0	6
ste9	204SB	1,051	4/4	4/4	0	1	3
ste11	53A	1,044	11/11	10/11	2	1	7
ste12	59C	1,045	14/15	15/15	3	1	10
					No. of cdc⁻, ste⁻, db mut		
ste2	61C	1,049	3/14	14/14	6		

TABLE IX

Suppression of ste⁻ by cdc 28: Quantitative Mating

Cross	Spore	ste	cdc 28	Mating (diploids formed) 22°C	34°C
1,049	1–2	2	–	2.3×10^6	2.6×10^3
	4–4	2	–	1.5×10^6	1.1×10^3
	2–3	2	+	5.0×10^6	3.9×10^2
	3–4	2	+	5.0×10^6	1.4×10^3
1,046	1–2	4	–	7.0×10^5	<50
	2–1	4	–	3.0×10^6	<50
	1–3	4	+	1.5×10^3	<50
	2–2	4	+	9.0×10^5	<50
1,048	4–4	5	–	$<5 \times 10^2$	<50
	6–4	5	–	2.5×10^4	<50
	1–4	5	+	1.1×10^4	<50
	2–3	5	+	—	<50
1,047	5–3	7	–	1.8×10^6	<50
	7–4	7	–	8.5×10^5	<50
	2–3	7	+	4.1×10^6	90
	3–1	7	+	3.9×10^5	<50
1,050	1–4	8	–	1.3×10^6	<50
	10–4	8	–	1.5×10^6	<50
	3–2	8	+	7.0×10^5	3.9×10^2
1,044	1–2	11	–	3.4×10^6	7.0×10^3
	2–2	11	–	2.5×10^6	<50
	2–1	11	+	4.4×10^6	<50
	3–2	11	+	5.5×10^6	9.5×10^2
1,045	3–2	12	–	5.5×10^6	95
	10–2	12	+	1.1×10^7	<50
	13–4	12	+	4.6×10^6	<50
1,044	1–4	+	–	9.5×10^6	1.8×10^7
	3–1	+	–	3.2×10^6	3.8×10^6
	1–2	+	+	1.7×10^7	3.8×10^7
	3–4	+	+	7.5×10^6	1.6×10^7

The strains were grown overnight at 20°–22°C, adjusted to 2×10^6 cells/ml, and divided into two portions. One was incubated at 34°C for 2 h and the other at 22°C for 2.5 h, after which 1 ml of each was mixed with 3×10^6 cells of EMS63 *MATα* and placed onto Millipore filters. The rest of the protocol was as in Quantitative Mating Experiments under Materials and Methods.

cally nonspecific and their specific consequences in each *MAT* cell type are imposed by the function of the *MAT* locus. It will be of interest to see if the phenotypes of the *ste* mutations in a *MATα* strain are the same as they are in the *MATa* strain.

One context in which to consider the pleiotropy of these mutants (Table X) is in terms of inducible and constitutive functions. Constitutive functions include a-factor production, α-factor destruction, the budding pattern, and the presence of a-factor receptor; the presence or absence of receptor was not assayed directly, because procedures for its assay have not yet been worked out. The inducible functions are division arrest and agglutinin synthesis. Mutants deficient in constitutive as well as inducible functions might be specifically defective in the expression of constitutive functions and the lack of inducible function could be a secondary consequence of a deficiency of mating factor receptor. This class includes seven of the eight genes described, *ste4*, *5*, *7*, *8*, *9*, *11*, and *12*. However, there is a strong distinction between *ste4*, *5*, *7*, *11*, and *12* on the one hand, which display reduced but detectable a-factor production and α-factor destruction and retain an equatorial budding pattern, and *ste8* and *9* on the other, which display no constitutive functions and bud in a polar pattern. Mutants in *ste8* and *9* have phenotypes indistinguishable from *MATa/α* diploids; Jasper Rine (University of Oregon, Eugene, Oregon) has reported to me (personal communication) that *ste8* and *9* are allelic to *sir3* and *4* respectively, genes whose products are thought to repress the silent *MAT* alleles at HMa and HMα.

Mutations in only one gene (*ste2*) display no defect in constitutive functions. This gene is specific for *MATa* and is defective for both inducible functions, division arrest and agglutination. All of these properties are consistent with the possibility that *ste2* codes for the α-factor receptor, a possibility suggested previously by MacKay and Manney (17, 18). The reason for the hyperproduction of a-factor by *ste2* mutants is unclear.

No mutants specifically defective in division arrest were found. It was considered possible that the pleiotropic phenotypes of some of the mutants were secondary manifestations of a primary defect in division arrest; hence I tested for phenotypic correction of the fertility defect by making strains doubly mutant for an *ste* mutation and for a mutation, *cdc* 28, which imposes cell division arrest at the same step in the cell cycle as mating factor. No such double mutants were phenotypically corrected for fertility when division arrest was imposed by temperature shift.

TABLE X

Summary of Phenotypes of ste Mutants

ste	Inducible functions		Constitutive functions		Budding pattern	MAT specificity
	Division arrest	Agglutination	a-Factor production	α-Factor destruction		
+	+	+	1.0	1.0	Equatorial	
8, 9	−	−	0	0	Polar	a & α
4	−	−	0.3	0.2	Equatorial	a & α
5	−	−	0.6	0.2	Equatorial	a & α
7	−	−	0.2	0.15	Equatorial	a & α
11	−	−	0.2	0.04	Equatorial	a & α
12	−	−	0.1	0.04	Equatorial	a & α
2	−	−	2.5	1.0	Equatorial	a

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