MOLECULAR AND CELLULAR BIOLOGY, Feb. 1988, p. 551–556
0270-7306/88/020551-06$02.00/0

Vol. 8, No. 2

Yeast *STE7*, *STE11*, and *STE12* Genes Are Required for Expression of Cell-Type-Specific Genes

STANLEY FIELDS,[1] DEBORAH T. CHALEFF,[2]†* AND GEORGE F. SPRAGUE, JR.[3]

Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York 11794[1]; E. I. du Pont de Nemours & Co., Inc., Central Research and Development Department, Experimental Station, Wilmington, Delaware 19898[2]; and Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene, Oregon 97403[3]

Received 23 June 1987/Accepted 3 November 1987

Cell type specialization in yeast haploids involves the mutually exclusive expression of one of two sets of genes, the a-specific and α-specific genes. We demonstrated that the products of the *STE7*, *STE11*, and *STE12* genes were required for the expression of both gene sets. RNA levels transcribed from these gene sets were significantly decreased but not abolished in haploids containing a null mutation in the *STE7*, *STE11*, or *STE12* gene. Transcript levels from the a- and α-specific gene sets were not further reduced in strains harboring mutations in all three *STE* genes, suggesting that *STE7*, *STE11*, and *STE12* are required for the same aspect of transcription. We further showed that the requirement for these products was not the same for each member of a particular gene set. However, for any given a- or α-specific gene, the effect on RNA levels of any of the three *ste* mutations was similar.

Haploids of the yeast *Saccharomyces cerevisiae* exist as one of two cell types, mating types **a** and α. Cells of opposite cell type are able to mate with each other to form the third cell type, the **a**/α diploid. Unlike haploids, diploids are unable to mate but are capable of undergoing meiosis and sporulation. Each haploid cell type expresses two sets of genes. The α-specific gene set is expressed only in α cells, whereas the **a**-specific gene set is expressed only in **a** cells. The α- and **a**-specific genes encode proteins that allow cells of opposite mating type to conjugate. These products include unique pheromones secreted by cells of each mating type, specific cell surface receptors to these pheromones, as well as additional functions required for pheromone biosynthesis or degradation (2–4, 6, 12, 16, 22, 23, 30, 35, 42). A third set of genes, denoted haploid specific, are expressed in haploids of either cell type, but expression is absent or diminished in diploid cells. Members of each set of genes have been isolated as cloned DNA and used to demonstrate that control of this expression occurs at the mRNA level (3, 4, 11, 13, 18–20, 24, 29, 30, 34, 38, 42, 43).

Current understanding of the control of this cell type specialization has focused on the role of the mating type locus (*MAT*), the genetic locus that differs in **a** and α cells. **a** cells carry the *MAT***a** allele, α cells the *MAT*α allele, and **a**/α cells both alleles. Genetic analysis of cells with mutations at this locus resulted in the following model for cell type specialization (39; reviewed in references 15 and 37). The *MAT*α locus encodes two activities: *MAT*α*1*, an activator of the α-specific genes, and *MAT*α*2*, a negative regulator of the **a**-specific genes. Thus, α cells express α-specific but not **a**-specific genes. The *MAT***a** allele has no known role in an **a** cell. Rather, in these cells, **a**-specific genes are expressed due to the absence of the *MAT*α*2* repressor, and α-specific genes are not expressed due to the absence of the *MAT*α*1* product. In diploid cells, the *MAT*α*2* and *MAT***a***1* products act in concert to repress expression both of the haploid-specific genes and of *MAT*α*1*. Thus, in **a**/α cells, the **a**-, α-, and haploid-specific genes are not expressed (see Fig. 5).

This model for mating type regulation proposes that only the α-specific genes have a *MAT*-encoded activator; expression of **a**-specific genes depends simply on the absence of the *MAT*α*2* repressor. However, the existence of additional activators for both the **a**-specific genes and the α-specific genes is not precluded by this model. Indeed, the possible existence of additional activators was suggested by the finding that *MAT*α*1*, when transcribed from a constitutive promoter, could promote α-specific gene expression in **a** cells but not in **a**/α cells (1). One explanation for this result is that α-specific gene expression requires an activator(s) present in haploids but absent in diploids.

Because mating competence depends on the appropriate expression of cell-type-specific genes, mutations in genes encoding activators for expression of the **a**- or α-specific gene sets would be expected to lead to a nonmating (sterile) phenotype. Several genes (*STE4*, *STE5*, *STE7*, *STE11*, and *STE12*) that are required for mating by haploid cells of both mating types have been identified (14, 24–26). Conditional mutations in these genes confer not only sterility at the non-permissive temperature, but failure to produce and respond to pheromone as well, suggesting a general defect in cell type expression (14, 24, 25). In addition, *ste12* point mutations lead to a reduction in **a**- and α-specific transcript levels (10). We further examine **a**-, α-, and haploid-specific gene expression by analyzing null mutations of the *STE7*, *STE11*, and *STE12* genes. We demonstrate that RNA levels of the **a**- and α-specific genes are reduced in the *ste* mutants and that the reduction of any given **a**- or α-specific transcript is identical in these mutants. However, the requirement for the *STE* genes differs between individual members of both sets of genes.

MATERIALS AND METHODS

Strains. The strains used in this study are listed in Table 1. All of the strains used are isogenic to haploid strain EG123 (34). Haploids DC129 and DC130 contain the *ste7* deletion, *ste7*-Δ*1*, whereas DC127 carries *ste7*-Δ*2* (5). These strains were constructed by substitutive transformation (31) as

* Corresponding author.
† Present address: American Cyanamid Co., P.O. Box 400, Princeton, NJ 08540.

TABLE 1. Strains used in this study

Strain	Relevant genotype[a]	Source or reference
EG123	*MAT***a** *STE*$^+$	34
DC38[b]	*MAT***a** *STE*$^+$	5
DC39[b]	*MAT*α *ste11*-Δ1	5
DC40[b]	*MAT***a** *ste11*-Δ1	5
DC41[b]	*MAT*α *STE*$^+$	5
246.1.1	*MAT*α *STE*$^+$	34
DC127	*MAT***a** *ste7*-Δ2	This study
DC129	*MAT***a** *ste7*-Δ1	This study
DC130	*MAT*α *ste7*-Δ1	This study
198	*mat*α1- *STE*$^+$	P. Siliciano
199	*MAT***a** *STE*$^+$ *MAT*α STE$^+$	P. Siliciano
SF167-5a	*MAT***a** *ste12*::*LEU2*$^+$	11
SF167-1c	*MAT*α *ste12*::*LEU2*$^+$	11
YY408	*MAT*α *ste7*-Δ1 *ste11*-Δ1	This study
YY709	*MAT***a** *ste-7*-Δ1 *ste11*-Δ1	This study
YY710	*MAT*α *ste7*-Δ1 *ste11*-Δ1 *ste12*::*LEU2*$^+$	This study
YY711	*MAT*α *ste7*-Δ1 *ste11*-Δ1 *ste12*::*LEU2*$^+$	This study

[a] All strains are isogenic to EG123 (34), carrying the additional mutations *his4-519 leu2 trp1 ura3 can1-101*.

[b] DC38, DC39, DC40, and DC41 are sister spores from a single tetrad derived from diploid DC26 (5).

described in Chaleff and Tatchell (5). Their molecular structures were confirmed by blot hybridization (36) (data not shown).

The *ste7 ste11* double mutants YY408 and YY709 and *ste7 ste11 ste12* triple mutants YY710 and YY711 were constructed as follows. DC129 was crossed to DC39 after introducing pSTE7.2 (Table 2) into DC129 and pSTE11.1 into DC39, alleviating the mating defect of each strain. Mitotic diploid segregants that had lost both plasmids were identified and subjected to tetrad analysis. A *MAT*α *ste7 ste11* double mutant, YY408, was identified from a tetrad segregating 2 Ste$^+$:2 Ste$^-$ spores; it proved mating competent only when transformed with both pSTE11.1 and pSTE7.2. The isogenic derivative, YY709, was constructed by cotransforming YY408 carrying pSTE7.2 and pSTE11.1 with YEp24 (to allow identification of transformants) and a linear *Hin*dIII fragment containing the *MAT***a** allele. The resultant YY709 transformant (Ste$^+$) secretes **a**-factor rather than α-factor, indicating that the *MAT*α allele has been replaced by *MAT***a**. YY408 and YY709 cells were cured of all plasmids to recover the Ste$^-$ phenotype and transformed with a linear *Sac*I-*Sph*I fragment containing the *ste12*::*LEU2*$^+$ mutation (11). That the resultant Leu$^+$ transformants, YY710 (derived from YY408) and YY711 (derived from YY709), contained the *ste12*::*LEU2*$^+$ mutation was verified by Southern blot hybridization analysis (36) (data not shown).

Plasmids. The plasmids used either as radiolabeled probes or in the double and triple mutant strain constructions are listed in Table 2.

RNA preparation and analysis. Total and polyadenylated [poly(A)$^+$] RNA was prepared as described previously (5, 10, 38). RNA was examined by Northern (RNA) blot hybridization analysis (41) by denaturation in either glyoxal (41) or 6% formaldehyde and 50% formamide (7). Autoradiograms were scanned by densitometry as described previously (10). All plasmid probes were radiolabeled with ^{32}P by nick translation (32).

RESULTS

Conditional (temperature-sensitive) mutations in the *STE7*, *STE11*, and *STE12* genes were isolated by Hartwell (14) on the basis of resistance to the growth-inhibitory effects on **a** cells of α-factor, the pheromone secreted by α cells. Subsequent genetic analysis of these mutants revealed the requirement of these genes for mating in cells of either haploid mating type. In addition, the *ste7*, *ste11*, and *ste12* mutants exhibited a pleiotropic phenotype that suggests defective expression of haploid functions involved in mating at the restrictive temperature. For example, *ste* mutants are partially defective in the synthesis or secretion of pheromone, in agglutination, in response to the pheromone secreted by cells of the opposite mating type (14), and in the accumulation of the α-factor receptor (17). In addition, *ste12* point mutations, which did not completely abolish mating and were presumably leaky, led to a 3- to 50-fold decrease in the level of α- and **a**-specific transcripts (10).

We used cloned DNA from these three genes to construct a set of strains of each mating type that contained deletion mutations for each gene (5, 11). Steady-state levels of **a**-, α-, and haploid-specific RNAs were assayed in wild-type and *ste* mutants, in **a**/α diploids, and in *mat*α*1* mutant strains by Northern blot hybridization (41). In all experiments, each filter was probed for transcription from a gene (*URA3*) whose expression is indifferent to cell type to ensure that comparable amounts of RNA were applied to each lane. These control transcripts are shown only for representative blots.

a-Specific genes are positively regulated by *STE7*, *STE11*, and *STE12*. Steady-state levels of RNA transcribed from four known **a**-specific genes (*STE6*, *STE2*, *MFa1*, and *MFa2*) were determined. *MFa1* and *MFa2* encode **a**-factor, the pheromone secreted by **a** cells (3). The *STE6* gene is required for the biosynthesis of **a**-factor (6, 42). The *STE2* gene encodes part or all of the receptor for α-factor, the pheromone secreted by α cells (16). The amount of *STE6* (top panel, Fig. 1) and *STE2* (middle panel, Fig. 1) RNA was reduced approximately fivefold in the *ste7*, *ste11*, and *ste12* mutants. Significantly lower levels of RNA from the **a**-factor genes were observed in the *ste* strains: a 15-fold reduction in *MFa2* RNA (bottom panel, Fig. 1) was observed, whereas no *MFa1* RNA (data not shown) was detected in the three *ste* mutants. As expected, no *STE6*, *STE2*, *MFa1*, or *MFa2* RNA was observed in the wild-type *MAT*α haploid or *MAT***a**/*MAT*α diploid cells.

TABLE 2. Plasmids used in this study

Plasmid and use	Relevant genes	Source or reference
Strain constructions		
pSTE7.2	*STE7*, *TRP1*	5
pSTE11.1	*STE11*, *LEU2*	5
pSUL16	*ste12*::*LEU2*	11
YEp24	*URA3*	
Probes		
pC6L-2	*STE6*, *URA3*	43
pC6-17	*STE6*, *URA3*	K. Wilson
pAB14	*STE2*	A. Burkholder
pSM29	*MFa2*	S. Michaelis
pBH3	*MFα1*	22
pBR-MFα2	*MFα2*	23
pSL7	*STE3*, *URA3*	38
YIp5-BH2	*HO*, *URA3*	18
YIp5	*URA3*	

α-Specific genes are positively regulated by *STE7*, *STE11*, and *STE12*. Expression of three α-specific genes, *STE3*, *MFα1*, and *MFα2*, was assayed. The *STE3* gene is believed to encode the **a**-factor receptor (2, 12, 30), and *MFα1* and *MFα2* encode α-factor (22, 23, 35). Expression of *STE3* was depressed approximately fivefold in the *ste7*, *ste11*, and *ste12* mutants relative to the *MATα STE*$^+$ control strain (Fig. 2, top panel). A severe reduction in the levels of transcript from the α-factor genes was observed in the set of *ste* mutants. *MFα1* RNA was reduced 40-fold relative to the *MATα STE*$^+$ control, whereas no *MFα2* RNA was detected in the mutants, corresponding to a reduction of at least 100-fold (Fig. 2, middle and bottom panels, respectively). As expected, no *STE3*, *MFα1*, or *MFα2* RNA was detected in *MAT***a**, *matα1*, or *MAT***a**/*MATα* cells.

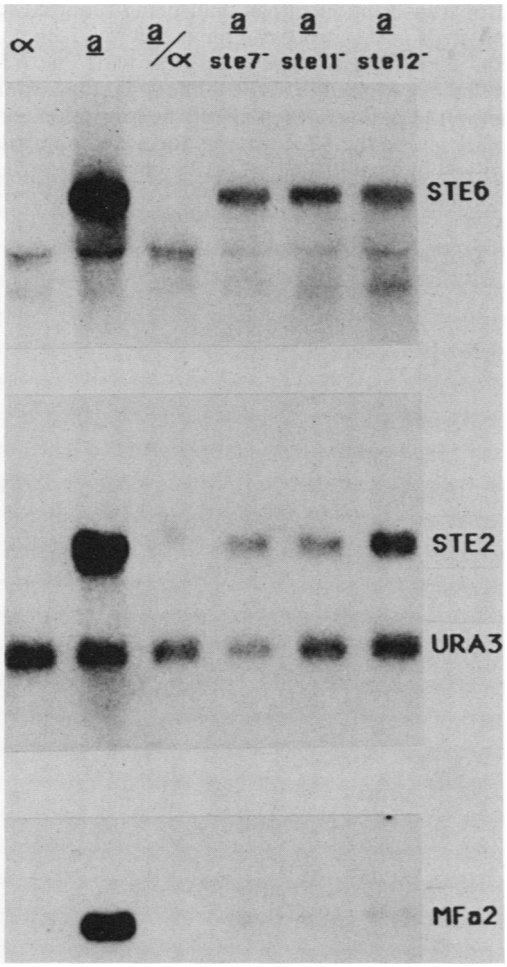

FIG. 1. Regulation of **a**-specific genes by *STE7*, *STE11*, and *STE12*. (Top and middle) Poly(A)$^+$ RNA was isolated from strains DC41 (lane 1), DC38 (lane 2), 199 (lane 3), DC127 (lane 4), DC40 (lane 5), and SF167-5a (lane 6), denatured by treatment with glyoxal, fractionated by agarose gel electrophoresis, and blotted to nitrocellulose. (Bottom) Total RNA was isolated from strains 246.1.1 (lane 1), EG123 (lane 2), 199 (lane 3), DC129 (lane 4), DC40 (lane 5), and SF167-5a (lane 6), denatured in formaldehyde and formamide, fractionated by agarose gel electrophoresis, and blotted to nitrocellulose. Plasmid probes pC6L-2 (*STE6*), pAB14 (*STE2*), pSM29 (*MFa2*), and YIp5 (*URA3*) were labeled by nick translation. The relevant genotype is indicated above each lane. The positions of the *STE6*, *STE2*, *URA3*, and *MFa2* transcripts are indicated on the right. Although all filters were normalized for the amount of RNA loaded in each lane by hybridization with a constitutively transcribed gene, *URA3*, this is displayed only in the *STE2* blot.

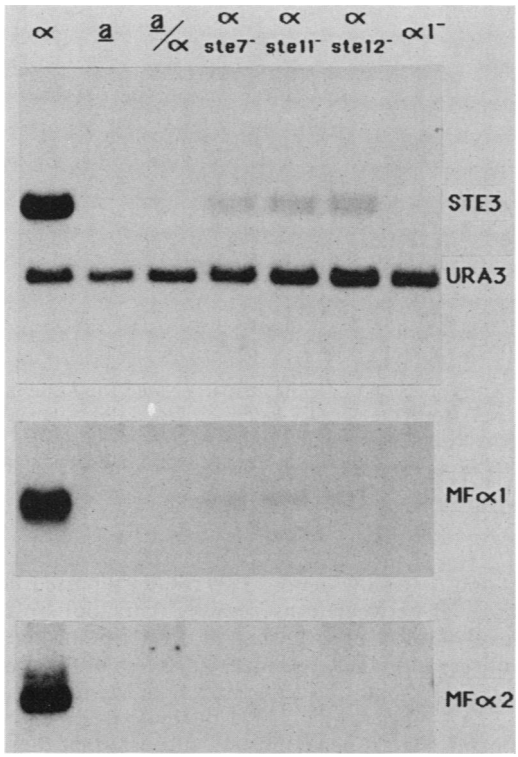

FIG. 2. Regulation of α-specific genes by *STE7*, *STE11*, and *STE12*. Total RNA was isolated from strains 246.1.1 (lane 1), EG123 (lane 2), 199 (lane 3), DC130 (lane 4), DC39 (lane 5), SF167-1c (lane 6), and 198 (lane 7), denatured in formaldehyde and formamide, and analyzed as described in the legend to Fig. 1. Plasmids pSL7 (*STE3*), pBH3 (*MFα1*), pBR-MFα2 (*MFα2*), and YIp5 (*URA3*) were used as probes.

Expression of haploid-specific genes in *ste7*, *ste11*, and *ste12* mutants. Several haploid-specific genes, i.e., genes expressed in haploids of both mating types but not expressed in **a**/α diploids, have been identified. The expression of two such genes, *HO* (18) and *STE5* (25), was measured in the *ste7*, *ste11*, and *ste12* null mutants. The steady-state level of RNA transcribed from the *HO* gene, which encodes an endonuclease involved in mating type interconversion (21), was not affected by mutations in the *STE7*, *STE11*, or *STE12* gene (Fig. 3). The expression of *STE5*, another gene required for mating in either haploid cell type (14), was similarly unaffected (data not shown).

Expression of cell-type-specific genes in triple *ste* mutants. The results of the above experiments demonstrated that while the *STE7*, *STE11*, and *STE12* products were required for expression of **a** and α cell-type-specific functions, the requirement for these gene products was not always absolute. Moreover, the level of reduction of a particular cell-type-specific RNA was similar in all the *ste* mutants. To determine whether these effects were additive, we compared expression of *STE3* and *STE6* in *ste7 ste11 ste12* triple mutants with that in the individual single mutants. The levels of *STE3* (Fig. 4, top panel) and *STE6* (Fig. 4, bottom panel) RNA were identical in the single and triple mutants.

DISCUSSION

The results of these experiments establish the requirement for the *STE7*, *STE11*, and *STE12* genes in RNA production

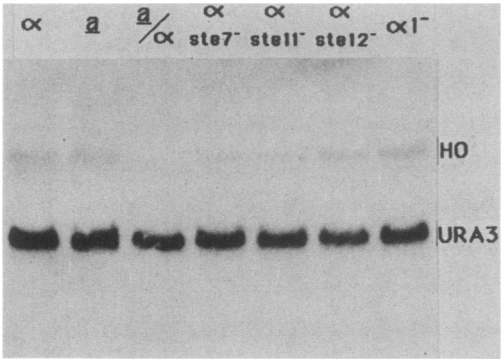

FIG. 3. Expression of *HO* in *ste7*, *ste11*, and *ste12* null mutants. Total RNA from the strains listed in Fig. 2 was denatured in formaldehyde and formamide and analyzed as described in the legend to Fig. 1. The filter was hybridized with radiolabeled plasmid YIp5-BH2 (*HO URA3*).

from **a**- and α-specific genes. The results reported here do not distinguish between transcriptional activation, regulation at the level of mRNA processing, or transcript turnover. The recent observation that sequences upstream of the transcription start site of both the *STE3* and *MFa2* genes are able to confer *STE7*, *STE11*, and *STE12* dependence to expression from a heterologous gene strongly suggests that these gene products regulate transcription initiation (E. Jarvis, G. F. Sprague, Jr., and S. Fields, unpublished results). We have shown that the *STE* genes are not necessary for expression of the entire haploid-specific gene set: *HO* and *STE5* transcription is independent of *STE7*, *STE11*, and *STE12*. By contrast, expression of other haploid-specific genes, namely *FUS1* (28), Ty1, and genes whose expression is under Ty1 control (ROAM mutations), is decreased in strains carrying point mutations in the *STE7*, *STE11*, and *STE12* genes (8, 9).

The requirement for the *STE* gene products for **a**- and α-specific gene expression is not absolute. The effect on transcript levels observed in strains carrying mutations in any of the *STE* genes ranged from a decrease of approximately 5- to 10-fold (*STE3*, *STE2*, and *STE6*) to an almost complete absence (*MFα2* and *MFa1*), corresponding to a reduction of at least 100-fold. However, the effect on the expression of any given member of each cell-type-specific gene set was similar, if not identical, in each of the three *ste* mutants. One explanation for this differential effect is that the *STE7*, *STE11*, and *STE12* products optimize the affinity of the transcription machinery for the promoter elements at each **a**- and α-specific gene. In the absence of the *STE* products, the transcription machinery has different inherent affinities for each promoter. That is, in some cases the *STE* products increase the affinity by 50-fold, whereas in other cases the affinity is increased by only a factor of 5. A second possible explanation is that the **a**- and α-specific gene promoters each have two components, one of which is sensitive to regulation by the *STE* genes, the other of which is unaffected by the *STE* products. In this view, the basal components from different genes would have different capacities to promote transcription. The lack of an absolute requirement for the *STE* products is in contrast to the complete dependence on the *MATα1* product for α-specific gene expression and suggests that the *MATα1* product functions differently from the *STE* gene products.

The existence of regulatory functions that are common to both the **a**- and α-specific genes provides an additional regulatory layer in cell type specialization (Fig. 5). The ability to express either haploid cell type is conferred by positive regulators that include *STE7*, *STE11*, and *STE12*. Expression of these functions in the absence of *MAT*-encoded products leads to **a**-specific gene transcription. Superimposed on this level of control in an α cell are the two regulatory components encoded by the *MATα* allele. The *MATα1* protein acts in concert with the *STE* products to allow expression of the α-specific genes, whereas the *MATα2* protein represses **a**-specific gene expression even in the presence of the *STE7*, *STE11*, and *STE12* positive regulatory factors.

The finding that the levels of *STE3* and *STE6* transcripts were identical in the single and triple mutants argues that the *STE7*, *STE11*, and *STE12* products function in the same aspect of transcription rather than being required for independent steps in this process. One possibility is that the *STE* products constitute a linear pathway, with one *STE* gene product being required to activate the next; the terminal *STE* product in the pathway then affects transcription. A second possibility is that the *STE* proteins form a complex that may be involved in establishing an active chromatin structure or may act in the transcription process itself. Either possibility

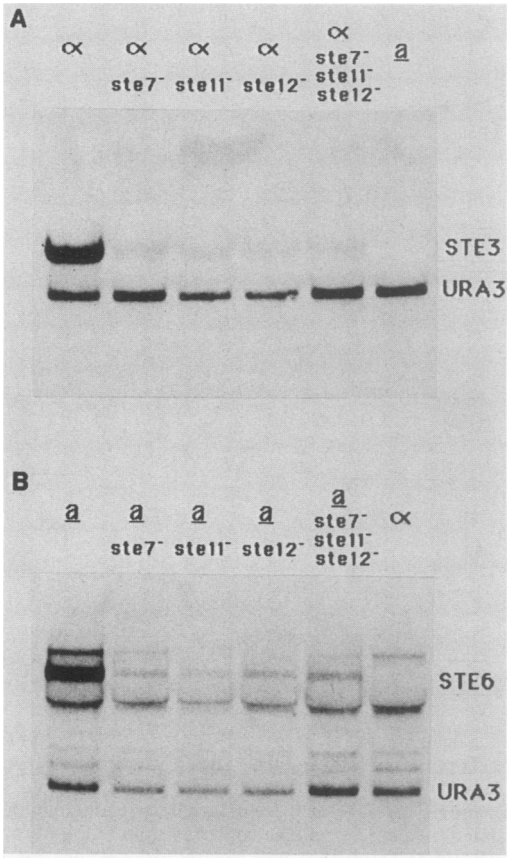

FIG. 4. Expression of *STE3* and *STE6* in *ste7*, *ste11*, and *ste12* single and triple mutants. (A) Poly(A)$^+$ RNA from strains DC41 (lane 1), DC130 (lane 2), DC39 (lane 3), SF167-1c (lane 4), YY710 (lane 5), and DC38 (lane 6) was denatured in formaldehyde and formamide and analyzed as described in the legend to Fig. 1, with plasmid pSL7 (*STE3 URA3*) as the probe. (B) Poly(A)$^+$ RNA from strains DC38 (lane 1), DC129 (lane 2), DC40 (lane 3), SF167-5a (lane 4), YY711 (lane 5) and DC41 (lane 6) was denatured in formaldehyde and formamide and analyzed. The filter was probed with pC6-17 (*STE6 URA3*).

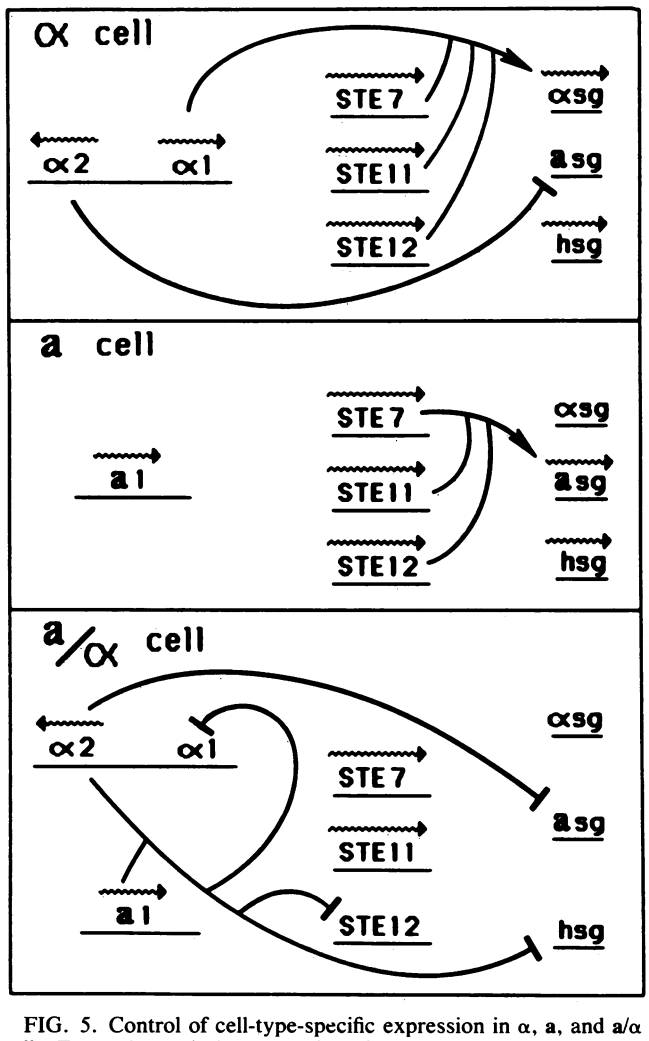

FIG. 5. Control of cell-type-specific expression in α, a, and a/α cells. For each panel, the expression of the mating type locus (*MAT*) is located on the left, the *STE7*, *STE11*, and *STE12* genes in the middle, and the a-, α-, and haploid-specific genes on the right. Gene expression is indicated by the wavy lines. Solid lines terminating with arrowheads indicate stimulation of expression; solid lines terminating with bars indicate inhibition of expression. Abbreviations: αsg, α-specific genes; asg, a-specific genes; hsg, haploid-specific genes.

must eventually accommodate the recent finding that *STE7* encodes a protein kinase (40; D. T. Chaleff, unpublished results) and that the predicted *STE11* protein is also highly homologous to protein kinases (L. Connell, R. Focht, D. T. Chaleff, and B. Errede, unpublished results). In the pathway model, the *STE7* and *STE11* proteins may function indirectly in the transcriptional activation of the two cell-type-specific gene sets by phosphorylating other products (e.g., the *STE12* protein). In the second model, the complex of *STE* products may activate transcription of cell-type-specific genes by phosphorylating itself, a transcription factor, or a subunit of RNA polymerase.

Jenness et al. (17), analyzing temperature-sensitive mutations of *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*, showed that their *ste7* and *ste11* (but not *ste12*) mutant strains accumulated significant numbers of α-factor-binding sites at the restrictive temperature. They thus argue that the severe inability of these strains to respond to α-factor was due to defects in postreceptor functions. Our results with null mutations of *STE7*, *STE11*, and *STE12* revealed a fivefold reduction of expression of the gene encoding the α-factor receptor. Two explanations can be considered to rationalize the inability of *ste7*, *ste11*, and *ste12* mutants to respond to pheromone despite having only a modest reduction in the expression of the receptor gene. First, *STE7*, *STE11*, and *STE12* may be necessary for transcription of other genes that encode products required for response to pheromone. Transcription of some of these genes may show a strict requirement for the *STE* products, as we have observed for some of the a- and α-specific genes. Alternatively, the *STE* products may act in pheromone response at other levels besides transcription.

ACKNOWLEDGMENTS

We thank R. Chaleff, F. Chumley, and B. Valent for critical reading of the manuscript, A. Burkholder, S. Michaelis, K. Wilson, and P. Siliciano for strains and plasmids, and Betty Wolfe for preparation of the manuscript. S.F. is grateful to I. Herskowitz for providing advice, as well as facilities and support (Public Health Service research grant AI-18738) during part of this research.

This work was supported by du Pont, grants from the American Cancer Society (MV-308) and the National Science Foundation (DCB-8601949) to S.F., and by a research grant from the Public Health Service (GM-30027) and a Faculty Research Award (FRA-282) from the American Cancer Society to G.F.S.

LITERATURE CITED

1. **Ammerer, G., G. F. Sprague, and A. Bender.** 1985. Control of yeast α-specific genes: evidence for two blocks to expression in *MATa*/*MATα* diploids. Proc. Natl. Acad. Sci. USA **82**:5855–5859.
2. **Bender, A., and G. F. Sprague.** 1986. Yeast peptide pheromones, a-factor and α-factor, activate a common response mechanism in their target cells. Cell **47**:929–937.
3. **Brake, A. J., C. Brenner, R. Najarian, P. Laybourn, and J. Merryweather.** 1985. Structure of genes encoding precursors of the yeast peptide mating pheromone a-factor, p. 103–108. *In* M. J. Gething (ed.), Protein transport and secretion. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Brake, A. J., D. F. Julius, and J. Thorner.** 1983. A functional prepro-α-factor gene in *Saccharomyces* yeasts can contain three, four, or five repeats of the mature pheromone sequence. Mol. Cell. Biol. **3**:1440–1450.
5. **Chaleff, D. T., and K. Tatchell.** 1985. Molecular cloning and characterization of the *STE7* and *STE11* genes of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5**:1878–1886.
6. **Chan, R. K., L. M. Melnick, L. C. Blair, and J. Thorner.** 1983. Extracellular suppression allows mating by pheromone-deficient sterile mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **155**:903–906.
7. **Derman, E., K. Krauter, L. Walling, C. Weinberger, M. Ray, and J. E. Darnell, Jr.** 1981. Transcriptional control in production of liver-specific mRNAs. Cell **23**:731–739.
8. **Dubois, E., E. Jacobs, and J. C. Jauniaux.** 1982. Expression of the ROAM mutations in *Saccharomyces cerevisiae*: involvement of *trans*-acting regulatory elements and relation with Ty1 transcription. EMBO J. **1**:1133–1140.
9. **Errede, B., T. S. Cardillo, F. Sherman, E. Dubois, J. Deschamps, and J. M. Wiame.** 1980. Mating signals control expression of mutants resulting from insertion of a transposable repetitive element adjacent to diverse yeast genes. Cell **22**:427–436.
10. **Fields, S., and I. Herskowitz.** 1985. The yeast *STE12* product is required for expression of two sets of cell-type-specific genes. Cell **42**:923–930.
11. **Fields, S., and I. Herskowitz.** 1987. Regulation by the yeast mating type locus of *STE12*, a gene required for cell-type-specific expression. Mol. Cell. Biol. **7**:3818–3821.
12. **Hagen, D. C., G. McCaffrey, and G. F. Sprague.** 1986. Evidence the yeast *STE3* gene encodes a receptor for the peptide phero-

mone a-factor: gene sequence and implications for the structure of the presumed receptor. Proc. Natl. Acad. Sci. USA **83**:1418–1422.

13. **Hartig, A., J. Holly, G. Saari, and V. L. MacKay.** 1986. Multiple regulation of *STE2*, a mating-type-specific gene of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:2106–2114.
14. **Hartwell, L. H.** 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. J. Cell. Biol. **85**:811–822.
15. **Herskowitz, I., and Y. Oshima.** 1981. Control of cell type in *Saccharomyces cerevisiae*: mating type and mating type interconversion, p. 181–209. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Jenness, D. D., A. C. Burkholder, and L. H. Hartwell.** 1983. Binding of α-factor pheromone to yeast **a** cells: chemical and genetic evidence for an α-factor receptor. Cell **35**:521–529.
17. **Jenness, D. D., B. S. Goldman, and L. H. Hartwell.** 1987. *Saccharomyces cerevisiae* mutants unresponsive to α-factor pheromone: α-factor binding and extragenic suppression. Mol. Cell. Biol. **7**:1311–1319.
18. **Jensen, R., G. F. Sprague, and I. Herskowitz.** 1983. Regulation of yeast mating type interconversion: feedback control of *HO* gene expression by the mating type locus. Proc. Natl. Acad. Sci. USA **80**:3035–3039.
19. **Johnson, A. D., and I. Herskowitz.** 1985. A repressor (*MATα2* product) and its operator control expression of a set of cell type specific genes in yeast. Cell **42**:237–247.
20. **Klar, A., J. N. Strathern, J. R. Broach, and J. B. Hicks.** 1981. Regulation of transcription in expressed and unexpressed mating type cassettes of yeast. Nature (London) **289**:239–244.
21. **Kostriken, R., and F. Heffron.** 1984. The product of the *HO* gene is a nuclease: purification and characterization of the enzyme. Cold Spring Harbor Symp. Quant. Biol. **49**:89–96.
22. **Kurjan, J.** 1985. α-Factor structural gene mutations in *Saccharomyces cerevisiae*: effects on α-factor production and mating. Mol. Cell. Biol. **5**:787–796.
23. **Kurjan, J., and I. Herskowitz.** 1982. Structure of a yeast pheromone gene (*MFα*): a putative α-factor precursor contains four tandem copies of mature α-factor. Cell **30**:933–943.
24. **MacKay, V.** 1983. Cloning of yeast *STE* genes in 2μm vectors. Methods Enzymol. **101**:325–343.
25. **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.
26. **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.
27. **Manney, T. R., and V. Woods.** 1976. Mutants of *Saccharomyces cerevisiae* resistant to the α mating type factor. Genetics **82**: 639–644.
28. **McCaffrey, G., F. J. Clay, K. Kelsay, and G. F. Sprague.** 1987. Identification and regulation of a gene required for cell fusion during mating of the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:2680–2690.
29. **Mitchell, A. P., and I. Herskowitz.** 1986. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature (London) **319**:738–742.
30. **Nakayama, N., A. Miyajima, and K. Arai.** 1985. Nucleotide sequence of *STE2* and *STE3*, cell-type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J. **4**:2643–2648.
31. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78**:6354–6358.
32. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling DNA to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
33. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
34. **Siliciano, P., and K. Tatchell.** 1984. Transcription and regulatory signals at the mating type locus in yeast. Cell **37**:969–978.
35. **Singh, A., E. Y. Chen, J. M. Lugoboy, C. N. Chang, R. A. Hitzeman, and P. H. Seeburg.** 1983. *Saccharomyces cerevisiae* contains two discrete genes coding for the α-factor pheromone. Nucleic Acids Res. **11**:4049–4063.
36. **Southern, E. M.** 1975. Detecting specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503–517.
37. **Sprague, G. F., L. C. Blair, and J. Thorner.** 1983. Cell interactions and regulation of cell type in the yeast *Saccharomyces cerevisiae*. Annu. Rev. Microbiol. **37**:623–660.
38. **Sprague, G. F., R. Jensen, and I. Herskowitz.** 1983. Control of yeast cell type by the mating type locus: positive regulation of the α-specific gene *STE3* by the *MATα1* gene product. Cell **32**:409–415.
39. **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.
40. **Teague, M. A., D. T. Chaleff, and B. Errede.** 1986. Nucleotide sequence of the yeast regulatory gene *STE7* predicts a protein homologous to protein kinases. Proc. Natl. Acad. Sci. USA **83**:7371–7375.
41. **Thomas, P.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
42. **Wilson, K., and I. Herskowitz.** 1984. Negative regulation of *STE6* gene expression by the *α2* product of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:2420–2427.
43. **Wilson, K., and I. Herskowitz.** 1986. Sequences upstream of the *STE6* gene required for its expression and regulation by the mating type locus in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **83**:2536–2540.