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Multiple Regulation of *STE2*, a Mating-Type-Specific Gene of *Saccharomyces cerevisiae*

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The *Saccharomyces cerevisiae STE2* gene, which is required for pheromone response and conjugation specifically in mating-type a cells, was cloned by complementation of the *ste2* mutation. Transcription of *STE2* is repressed by the *MATα2* gene product, so that the 1.4-kilobase *STE2* RNA is detected only in a or *matα2* strains, not in α or a/α cells. However, *STE2* RNA levels are also increased by the mating pheromone α-factor and decreased in strains bearing mutations in the nonspecific *STE4* gene. Regulation of *STE2* expression in a cells is therefore achieved by several mechanisms.

Mating-type expression in the yeast *Saccharomyces cerevisiae* is an important model for development and regulation in eucaryotic cells (reviewed in references 14, 38, and 49). Two alleles, *MAT***a** and *MAT*α, determine the mating type of cells as **a** or α, respectively. Mating between **a** and α haploid cells results in a third cell type, the **a**/α diploid, which is unable to mate but can be induced to undergo meiosis and sporulation. To establish the mating type of a haploid cell, certain **a**- or α-specific characteristics must be expressed. For example, α cells secrete the mating pheromone α-factor and can respond to the pheromone **a**-factor, whereas **a** cells produce **a**-factor, are sensitive to α-factor, and secrete Barrier activity, a protein that reverses the effects of α-factor on **a** cells (reviewed in references 14, 49, and 58).

MacKay and Manney (30) proposed that the *MAT* alleles code for regulatory proteins, which control the expression of genes (or their products) that are unlinked to the *MAT* locus and that are necessary for establishing cell type and for conjugation. These unlinked genes (denoted *STE* for the sterile mutant phenotype) were identified by the isolation and subsequent genetic analysis of various nonmating mutants (13, 29, 30, 50; L. C. Blair, Ph.D. thesis, University of Oregon, Eugene, 1979; J. R. Rine, Ph.D. thesis, University of Oregon, Eugene, 1979). Some of the *STE* gene products, i.e., *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*, were shown to be necessary for mating in both cell types. Others, however, are cell type specific: **a** cells require the *STE2*, *STE6*, and *STE14* gene products for mating, whereas mutations in the *STE3*, *STE13*, *KEX2*, and *TUP1* genes block mating only in α cells. The last two have pleiotropic phenotypes that extend beyond their mating defects (26, 27) and therefore are not considered conjugation-specific genes.

A hypothesis for the regulation of the mating-type-specific functions has been proposed (53) in which *MAT*α codes for two regulatory proteins, α*1* and α*2*, in α cells. The α*1* protein acts as a positive regulator for the expression of α-specific genes, and α*2* is a negative regulator that blocks the expression of **a**-specific genes. In **a** cells, *MAT***a** codes for only one known functional product, **a***1*, which has no influence on mating but is required for sporulation in **a**/α diploids. The α*1*-α*2* hypothesis predicts that the expression of at least some mating-type-specific genes should be controlled by the *MAT* locus. Although this regulation could be transcriptional or posttranscriptional, the structural genes for α-factor and **a**-factor have been cloned and demonstrated to be transcribed only in α and **a** cells, respectively (4, 25, 47). In contrast, the *STE13* gene product, which is required for mating only in α cells, is produced in all three cell types; *STE13* apparently encodes a diaminopeptidase that is involved in processing mature α-factor (22) and is not a mating-type-specific function.

In this paper we describe the cloning of a putative **a**-specific gene, *STE2*, which has been proposed to encode the α-factor receptor on the surface of **a** cells (13, 18, 30). We have demonstrated that *STE2* is transcribed only in **a** cells and that its transcription is repressed by the *MAT*α*2* product, activated by the *STE4* gene product, and stimulated by exogenous α-factor.

(Part of this work was reported at the 11th International Conference on Yeast Genetics and Molecular Biology, Montpelier, France, 1982.)

MATERIALS AND METHODS

Strains and genetic methods. The *S. cerevisiae* strains used are listed in Table 1. Genetic methods and media used were described previously (29, 36): YEPD is a rich growth medium; MV is a chemically defined, minimal glucose medium; SC is MV with nutritional supplements to satisfy the growth requirements of the auxotrophic strains listed in Table 1; SC-leu lacks only leucine; SC-leu was supplemented with 1 M sorbitol. Temperature-sensitive mating mutants were grown at either 25°C (the permissive temperature) or 35°C (the restrictive temperature); all other strains were incubated at 30°C unless noted.

For mating tests, strains were replica plated to YEPD (or to SC-leu for transformants) and cross-streaked with mating tester strains JJ-1A and JJ-1C. After the strains had grown for approximately 24 h, the plates were replica plated to MV which would support growth only of diploids formed by mating. After another 24 h of incubation, growth of diploids indicated the mating type, as well as the ability or inability to mate, of the strains tested. For the screening of transformants for those bearing the *STE2* gene, transformant colonies from regeneration agar were resuspended and plated on SC-leu at approximately 1,000 cells per plate. The resulting

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TABLE 1. Yeast strains used

Strain	Genotype	Source or reference
X2180-1A	a *gal2*	YGSC[a]
JJ-1A	a *arg1 thr1*	M. Jagadish
JJ-1C	α *arg1 thr1*	M. Jagadish
XG41-14B	α *ade2 adeX his4 leu2 ura3 gal2*	15
G2	a *leu2-3,112 his3-11,15 trp1-289 ura3-52*	G. McKnight
AB20	α *his6 leu1 met1 trp5 can1 gal2*	41
XP635-10C	a *leu2-3,112 gal2 bar1*	T. R. Manney
XV627	a *ade2 his2 lys1 trp5 can1 gal2* + + + / α + + + *trp5 can1 gal2 his6 leu1 met1*	This work
XT1177-S47c	a *ade2 his2 lys1 trp5 can1 gal2*	29
VZ4	a *ste4-1* derived from XT1177-S47c	30
VAC1	a *ste5-1* derived from XT1177-S47c	30
VN33	*matα1 ade6 his6 leu1 met1 trp5 can1 gal2*	30
XCL632-2-2	*matα2-34 leu2-3,112 cry1-7 lys1 his6 gal2 thr4* + + / *matα2-34 leu2-3,112 cry1-7 lys1 his6 gal2 thr4 his4-519 can1*	32
23α75	*matα2*(αx75) *leu2 trp1 his4-519 ura3 can1-101*	56
17-16	*mata1 ade2 ura3 trp1 can1 cyh2 leu1 lysX*	23
XL4-S47	a *ste4-4 SUP4-3 his2* or *his4 arg1* or both	This work
XL4-S118	a *ste4-4 SUP4-3 ade2 his2* or *his4 thr1* or both	This work
381G-79A	a *ste7-2 SUP4-3 cry1 his4-580 trp1 ade2 tyr1 lys2*	13
381G-41A	a *ste11-1*, same genotype as 381G-79A	13
381G-59C	a *ste12-1*, same genotype as 381G-79A	13
XH6-10B	a *ste2-2 adeX leu2-3,112 lys1-1 can1*	This work
XH9-5C4	a *ste2-1 ade2-1 his3 leu2-3,112 can1*	This work

[a] YGSC, Yeast Genetics Stock Center, Berkeley, Calif.

colonies were replica plated to prewarmed lawns of strain JJ-1C on SC-leu, and the mating plates were incubated for 24 h before being replica plated to MV (selective for diploids).

Plasmids. Yeast-*Escherichia coli* shuttle vector YEp13 is derived from pBR322 (3) and carries the *S. cerevisiae* chromosomal *LEU2* gene and the origin of replication from the *S. cerevisiae* 2μm plasmid (5). The shuttle vector pZUC12 was obtained from M. Hansen, Novo Industri A/S, Bagsvaerd, Denmark; it contains these same yeast sequences in *E. coli* plasmid pUC12 (provided by J. Messing, University of Minnesota). Plasmid pLH3 (provided by L. Bell, ZymoGenetics) was constructed by isolating a 3-kilobase (kb) *Bgl*II fragment that contains the *S. cerevisiae* *LEU2* gene from plasmid YEp13. The *Bgl*II ends were blunt ended with DNA polymerase I (Klenow fragment), ligated with *Hin*dIII linkers, digested with *Hin*dIII, and inserted into pUC12.

Transformation. *E. coli* RR1 (3) and JM83 (33) cells were transformed as described by Bolivar et al. (3). Yeast strains were transformed by a modification (28) of the method of Beggs (2). Integrants were obtained by transformation with linearized plasmid DNA (42) digested at a unique site within the cloned fragment or with a linear chromosomal fragment.

Preparation of DNA and RNA. Plasmid DNA from *E. coli* was isolated by the method of Ish-Horowicz and Burke (17). Plasmid DNA from yeast for transformation into *E. coli* was usually isolated as described by Nasmyth and Reed (40), although *E. coli* transformants were not obtained from all preparations that were demonstrated to contain plasmid. In these cases, the method of Agaas was used to prepare plasmid from yeast (M. Cortelyou and V. L. MacKay, unpublished data); a 10-ml SC-leu culture was chilled on ice for 30 min and centrifuged, and the cells were washed in cold distilled water and suspended in 0.2 ml of SCE (1 M sorbitol, 0.1 M sodium citrate [pH 5.8], 10 mM EDTA) containing 0.8 mg of Zymolyase 5000 (Kirin Brewery, Japan) and 1% (vol/vol) β-mercaptoethanol. Incubation for 30 min at 37°C was followed by addition of 0.15 ml of STE (10% sodium dodecyl sulfate, 0.1 M Tris hydrochloride [pH 9.0], 10 mM EDTA), rapid mixing, and immediate boiling for 3 min. The lysis mixture was cooled on ice, 0.15 ml of 5 M potassium acetate (pH 5.6) was added, and the mixture was left on ice for 30 to 60 min before centrifugation for 5 min. For precipitation of DNA, 0.5 ml of 5 M ammonium acetate and 1 ml of isopropanol were added to the supernatant, which was then kept at −20°C for 15 min or longer. After centrifugation for 3 min, the precipitate was washed with 70% ethanol, dried in vacuo, and dissolved in 50 to 100 μl of TE (10 mM Tris hydrochloride [pH 8], 1 mM EDTA). For further purification, the DNA solution was extracted three times with phenol-chloroform (1:1), precipitated with ethanol, and resuspended in 50 μl of TE. This yeast plasmid preparation resulted in efficient *E. coli* transformation in all cases.

Yeast RNA was isolated as described by Richter et al. (43) from yeast cells grown in YEPD to a density of 2×10^7 to 3×10^7 cells per ml; poly(A)-enriched RNA samples were prepared by chromatography on oligo(dT)-cellulose (1). Incubation of a cells with α-factor was achieved by the addition of either approximately 4 U of the pheromone per ml, prepared as described previously (54), or an equivalent volume of 95% ethanol; the treated cultures were then incubated for the time indicated or for approximately one generation time before isolation of RNA.

Electrophoresis, transfers, and hybridizations. Electrophoresis of DNA fragments through agarose gels, subsequent transfer of denatured DNA fragments to nitrocellulose filters, and hybridization to nick-translated probes were done as described previously (35). Electrophoresis of RNA samples, transfers to nitrocellulose, and hybridizations were done as described by Thomas (57), except that dextran sulfate was omitted from the hybridization solutions. Nick translations were done essentially as described by Rigby et al. (44). Radiolabeled single-stranded probes in bacteriophage M13 were prepared by the method of Hu and Messing (16).

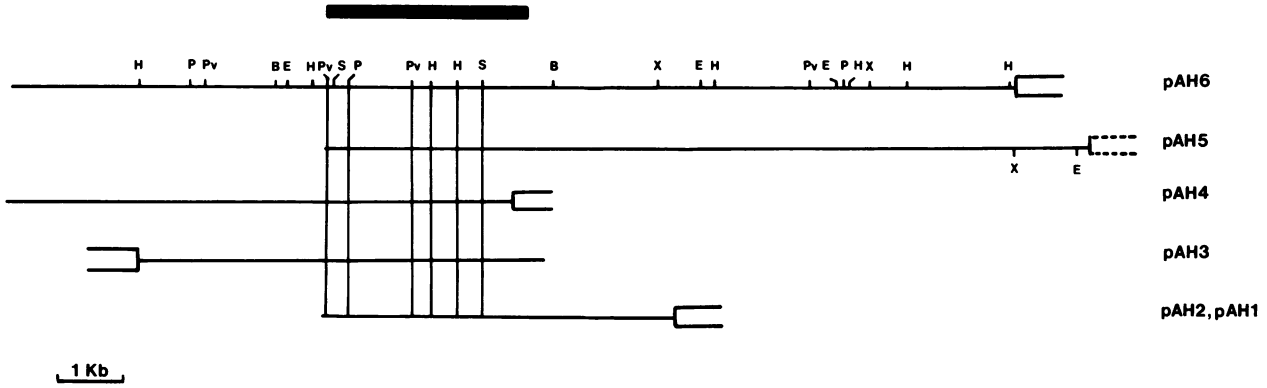


FIG. 1. Restriction map of the *STE2* plasmids pAH2 through pAH6. Only the inserts of the plasmids are shown. The orientation of each insert relative to the YEp13 vector is indicated by the open boxes on one side, which represent the *Hin*dIII-*Eco*RI-*Hin*dIII region of YEp13 (5). This portion of the vector has been deleted in pAH5. Subcloning the insert of pAH2 in pBR322 (yielding pAH1) was accomplished by digestion of pAH2 with *Eco*RI, dilution, and self-ligation of the pBR322-containing fragment. The region common to all plasmids is indicated by the closed bar. Symbols: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; X, *Xba*I.

Quantitation of autoradiograms. The autoradiograms were scanned lane by lane in a Beckman DU8 spectrophotometer, and relative peak areas were normalized by comparison with one lane of each autoradiogram set arbitrarily as one. Since the intensity of some of the autoradiogram bands is beyond the linear range, the values given should be considered only semiquantitative.

DNA sequencing. Restriction fragments were subcloned into derivatives of *E. coli* bacteriophage M13, and single-stranded template was prepared after transfection of *E. coli* JM101 or JM103 (33). Sequencing was done by the dideoxy method of Sanger et al. (46).

Enzymes. All restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories. DNase I and pancreatic RNase were from Worthington Diagnostics. DNA polymerase I was from Boehringer-Mannheim Biochemicals. Enzymes were generally used as recommended by the suppliers.

RESULTS

Isolation of DNA fragments that complement the *ste2* mutation. Strains XH6-10B and XH9-5C4 cannot mate because of the *ste2* mutation and were used as recipients for transformation with a clone bank of total yeast genomic fragments in vector YEp13 (41). Leu^+ transformants were screened for the ability to mate, as described in Materials and Methods. Approximately 15,000 original transformant colonies were screened, and six colonies were identified that had acquired the ability to mate. Altogether, these harbored five different plasmids that complemented the *ste2* mutation. The common region, presumably containing the *STE2* gene, is 2.6 kb long (Fig. 1); this localization was subsequently confirmed by the ability of a *Pst*I-*Bam*HI fragment from pAH1 subcloned into pZUC12 (plasmid pMT411) to complement the *ste2* mutation.

Complementation alone is not sufficient proof that the cloned gene is actually *STE2*, since overexpression of one gene might complement a mutation in another gene (28, 48). Therefore, plasmids pAH2 and pAH4 were integrated into the genome of XH6-10B (**a** *ste2*) by transformation with a linearized form (42) after digestion with *Bam*HI at the unique site in each plasmid (Fig. 1). Southern hybridization with *Eco*RI-digested genomic DNA from wild-type strains showed only one band, at approximately 10 kb, that hybridized to a fragment containing the 2.6-kb common region. Integration of pAH2 and pAH4 into the chromosome split the 10-kb genomic *Eco*RI fragment into two bands: 12.5 and 7 kb for integration of pAH2 and 9.5 and 4.1 kb for integration of pAH4 (data not shown). The appearance of the two new bands demonstrated that the integration occurred at the chromosomal location of the cloned gene. Tetrad analysis of these integrants proved that the cloned gene is *STE2*. The integrants were crossed with strain XG41-14B (α), and the resulting diploids were sporulated and subjected to tetrad analysis. In the 76 tetrads analyzed, no nonmating spores were found, indicating that the integration occurred within 0.65 centimorgan of the *ste2* mutation in XH6-10B. Although *LEU2* is normally linked to and between *HIS4* and *MAT*, in the integrants *LEU2* was unlinked to both *MAT* and *HIS4* (data not shown). Additional mapping (9) has assigned the *STE2* gene to chromosome VI (data not shown).

Identification of the *STE2* transcript. When the *Pst*I-*Bam*HI fragment from pAH1 was used to probe RNA samples from wild-type **a** strains, only a single transcript, of approximately 1.4 kb, was visible on the autoradiogram even after prolonged exposure (see, e.g., Fig. 2B, lane 1). However, in other experiments with the *Pst*I-*Hin*dIII or *Pvu*II-*Pvu*II fragment of pAH1 as probe, a minor transcript (approximately 1 kb) was occasionally seen (see Fig. 3 and 4). The 1.4-kb RNA was identified as the *STE2* transcript by gene disruption experiments (42, 45). A DNA fragment containing the yeast *LEU2* gene was inserted at several sites into the *Pst*I-*Bam*HI fragment of pAH1 subcloned into pUC12. *LEU2* was inserted at the *Sal*I site (pZV66) and in both orientations at the rightward *Hin*dIII site in the fragment (pZV64 and pZV65) (Fig. 2A). *LEU2* was also inserted between the two *Hin*dIII sites, replacing the approximately 0.4-kb *Hin*dIII-*Hin*dIII fragment of the *STE2* fragment (pZV63). The resulting plasmids were then digested with *Pst*I and *Bam*HI and used to transform yeast strain XP635-10C (**a** *bar1*) to leucine prototrophy; a majority of the transformants were expected to have the disrupted *STE2* fragment integrated in place of the homologous chromosomal *STE2* segment (42, 45). Because the *STE2* gene product is required for the **a** cell response to α-factor (13, 29, 30), disruption of the *STE2* gene and loss of the *STE2* product would confer insensitivity to α-factor. Sensitivity or

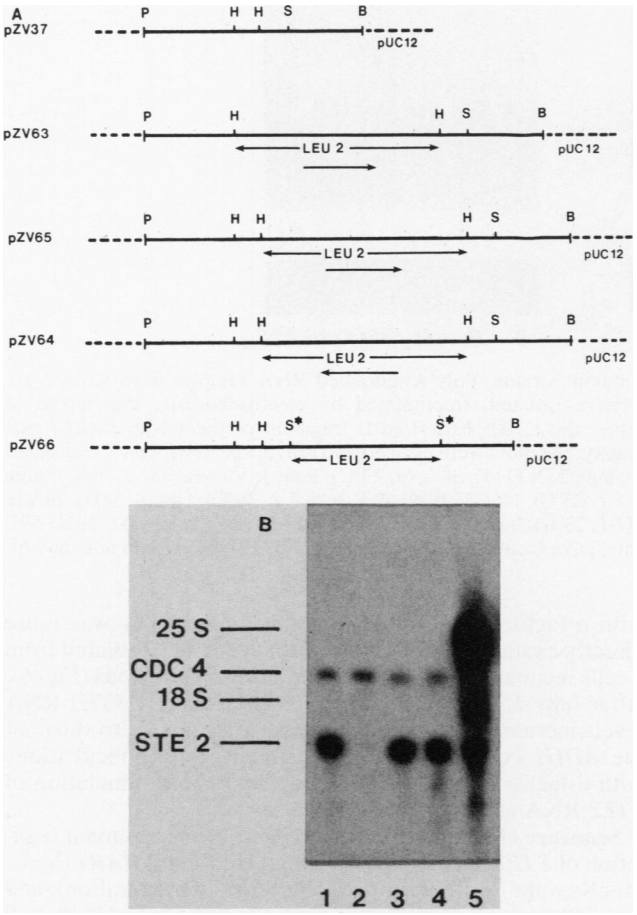


FIG. 2. Identification of the *STE2* transcript. (A) Plasmid pZV37 was derived by subcloning the *Pst*I-*Bam*HI fragment from pAH1 (Fig. 1) into pUC12. To construct plasmids pZV63 through pZV65, pZV37 was partially digested with *Hin*dIII and full-length linear fragments were isolated by agarose gel electrophoresis. These were ligated with a 3-kb *Hin*dIII fragment containing the yeast *LEU2* gene isolated from plasmid pLH3 and transformed into *E. coli* RR1 cells. The transformants were plated onto minimal medium containing ampicillin and lacking leucine. Transformants were screened for those containing the *LEU2* fragment within the *STE2* insert of pZV37. Plasmid pZV63 has the *LEU2* fragment inserted between the two *Hin*dIII sites, in place of the endogenous *Hin*dIII fragment; plasmids pZV64 and pZV65 have *LEU2* inserted at the rightward *Hin*dIII site in opposite orientations. Plasmid pZV66 was constructed by digesting pZV37 completely with *Sal*I and inserting the *Xho*I-*Sal*I *LEU2* fragment from YEp13. Since the orientation of the *LEU2* insert and therefore the location of the *Xho*I/*Sal*I junction were not determined, both junctions are denoted S*. All five plasmids shown were digested with *Pst*I and *Bam*HI, and the *STE2*-containing fragment was isolated after agarose gel electrophoresis. These fragments were used to transform yeast strain XP635-10C to leucine prototrophy. (B) Poly(A)-enriched RNA (10 μg) from each **a** strain was fractionated by electrophoresis, transferred to nitrocellulose, and probed with nick-translated pZV37 (see Materials and Methods). Lane 1, X2180-1A; lane 2, XP635-10C::pZV63; lane 3, XP635-10C::pZV65; lane 4, XP635-10C::pZV66; lane 5, XP635-10C bearing the autonomous plasmid pMT411 (*Pst*I-*Bam*HI fragment of pAH1 subcloned into vector pZUC12). RNA blots from XP635-10C::pZV37 are identical to lane 1 (data not shown).

resistance to α-factor was easily scored in the XP635-10C transformants, since this strain is supersensitive to α-factor (7). Eight independent Leu$^+$ transformants from each plasmid digest or from plasmid pMT411 were assayed for sensitivity (i.e., morphological response) to α-factor. None of those transformed with pMT411 or with the pZV64, pZV65, or pZV66 digests were resistant to α-factor, whereas six of eight transformed with the pZV63 digest had become resistant. These six are presumed to have integrated the pZV63 linear fragment in place of the chromosomal *STE2* gene, thereby abolishing *STE2* function because of the *LEU2* insertion in the gene.

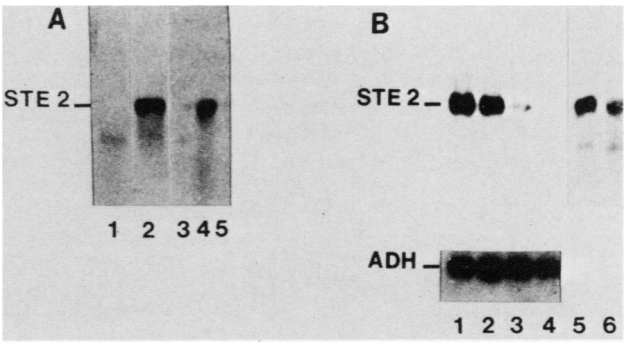


FIG. 3. Mating-type regulation of *STE2* transcription. Poly(A)-enriched RNA samples from various strains grown at the indicated temperature were isolated, fractionated by electrophoresis, transferred to nitrocellulose, and probed with nick-translated fragments isolated from pAH1 (either the 1.3-kb *Pst*I-*Hin*dIII fragment or the 1.1-kb *Pvu*II-*Pvu*II fragment) (see Materials and Methods). After hybridization and autoradiography, the blots were cleaned and rehybridized with nick-translated pY9T6 which contains the *ADH1* gene (kindly donated by E. T. Young, University of Washington, Seattle). The *ADH* control is shown for some of the lanes. (A) Lane 1, XT1177-S47c (**a**, 30°C); lane 2, AB20 (α, 30°C); lane 3, XV627 (**a**/α, 30°C); lane 4, 17-16 (*mat***a***1*, 30°C); lane 5, VN33 (*matα1*, 30°C). (B) Lane 1, XT1177-S47c (**a**, 23°C); lane 2, XT1177-S47c (**a**, 36°C); lane 3, XCL632-2-2 (*matα2-34*, 36°C); lane 4, XCL632-2-2 (*matα2-34*, 23°C); lane 5, 23α75 (*matα2*, 23°C); lane 6, 23α75 (*matα2*, 36°C).

Poly(A)-enriched RNA from the transformants was probed with the intact *Pst*I-*Bam*HI fragment to determine whether the 1.4-kb transcript was affected by any of the gene disruptions. The 1.4-kb RNA is absent only in the pZV63 transformant (Fig. 2B, lanes 2 through 4). Similar results were obtained when total RNA from two transformants of each plasmid type was analyzed (data not shown). Since the absence of the 1.4-kb transcript corresponds to the loss of the *STE2* gene function by gene disruption, this RNA is encoded by *STE2*.

Regulation of *STE2* transcription. Analysis of poly(A)-enriched RNA from a variety of yeast strains showed that transcription of the *STE2* gene is regulated by the mating-type locus (Fig. 3). The 1.4-kb *STE2* RNA was found in **a**, *mat***a***1*, and *matα2* strains, but not in α, *matα1*, or **a**/α cells. These results support the *α1*-*α2* hypothesis (53) that **a**-specific genes are negatively regulated by the *MATα2* gene product. To confirm *MATα2* regulation of *STE2* at the transcriptional level, we examined RNA samples from four different *matα2* mutants grown at 23 and 36°C. Three of these are nonmaters at both temperatures, and all had the 1.4-kb band (see, e.g., Fig. 3B, lanes 5 and 6). The fourth *matα2* mutant is temperature sensitive (32); at 23°C it mates as an α, and the 1.4-kb transcript was not detected (Fig. 3B, lane 4). However, at 36°C, mating is very poor (<0.1% of mating at 23°C), and a low level of the *STE2* transcript was found (approximately 20% of the wild-type **a** at 36°C; Fig. 3B, lane 3; Table 2). Thus, the *MATα2* gene product directly or indirectly represses transcription of the *STE2* gene.

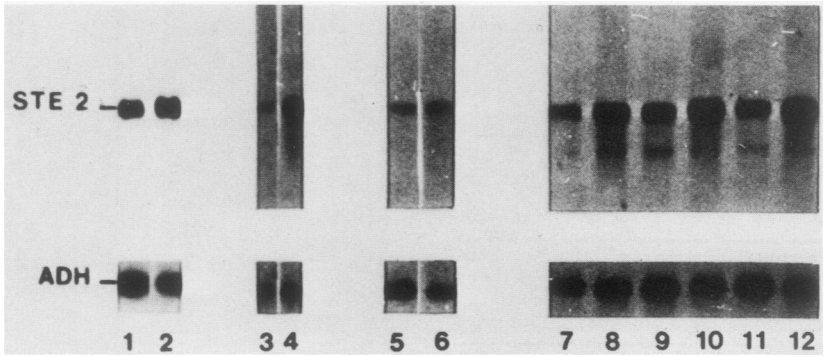


FIG. 4. *STE2* RNA levels in wild-type and temperature-sensitive *ste* mutant strains. Poly(A)-enriched RNA samples from various *ste* mutants and their parent strains grown at the indicated temperature were isolated, fractionated by electrophoresis, transferred to nitrocellulose, and probed with nick-translated fragments from pAH1 (either the 1.3-kb *Pst*I-*Hin*dIII fragment or the 1.1-kb *Pvu*II-*Pvu*II fragment) (see Materials and Methods). After hybridization and autoradiography, the blots were cleaned and rehybridized with nick-translated pY9T6 which contains the *ADH1* gene. (A) Lane 1, XT1177-S47c (**a**, 36°C); lane 2, XT1177-S47c (**a**, 23°C); lane 3, VZ4 (**a** *ste4-1*, 36°C); lane 4, VZ4 (**a** *ste4-1*, 23°C); lane 5, VAC1 (**a** *ste5-1*, 36°C); lane 6, VAC1 (**a** *ste5-1*, 23°C); lane 7, 381G-79A (**a** *ste7-2*, 36°C); lane 8, 381G-79A (**a** *ste7-2*, 23°C); lane 9, 381G-41A (**a** *ste11-1*, 36°C); lane 10, 381G-41A (**a** *ste11-1*, 23°C); lane 11, 381G-59C (**a** *ste12-1*, 36°C); lane 12, 381G-59C (**a** *ste12-1*, 23°C). Strain 381G, the parent of the*ste7*, *ste11*, and *ste12* mutants, gave results similar to those of XT1177-S47c (data not shown).

Poly(A)-enriched RNA samples from the nonspecific *ste* mutants were also probed for the *STE2* transcript (Fig. 4); for all temperature-sensitive mutants, RNA was isolated after growth at 23 and at 36°C. Most of the temperature-sensitive nonspecific *ste* mutations had little if any effect on *STE2* mRNA levels (however, see Discussion); the threefold less RNA was found in most of the mutants at 36°C relative to 23°C, as compared to a twofold level in the wild-type **a** parent at 36°C (Fig. 4; Table 2). This reduction might reflect a general temperature effect on transcription of conjugation-specific genes. In contrast, in the *ste4-1* mutant the *STE2* transcript level was at least eightfold lower at 36 than at 23°C (Fig. 4, lanes 3 and 4). This result was confirmed by analysis of poly(A)-enriched RNA isolated from other *ste4* mutants, specifically from strains carrying a nonsense mutation or gene disruption in *STE4* (Fig. 5). In all cases, *STE2* RNA was detected at approximately 10% of the level seen in the wild-type parent. The *STE4* gene product is therefore not absolutely required for *STE2* transcription but is necessary for the synthesis or maintenance of normal levels of the RNA. In contrast, gene disruptions in the *STE5* gene had very little effect on *STE2* RNA levels (Fig. 5, lane 4), indicating that this gene product may have little or no role in *STE2* expression.

Preliminary experiments suggested that *STE2* transcript levels were higher in **a** cells that had been incubated for 2 h with α-factor (data not shown). This possibility was more directly examined by analysis of RNA samples isolated from **a** cells incubated with α-factor for increasing periods (Fig. 6). After only 15 min of incubation with α-factor, *STE2* RNA levels increased about three- to fourfold relative to those of the *ADH1* control; in this experiment, longer incubations with α-factor did not seem to lead to greater stimulation of *STE2* RNA accumulation.

Sequence of the 5′ regulatory region. Transcriptional regulation of *STE2* appears to be identical to that of *BAR1* (V. L. MacKay and T. R. Manney, manuscript in preparation), and detailed analysis of the latter gene has demonstrated that all

TABLE 2. Quantitation of autoradiograms in Fig. 3B and 4 by scanning densitometry[a]

Strain	*STE2*/ADH ratio at:		
	36°C	23°C	36°C/23°C
XT1177-S47c (**a**)	0.51	1.00	0.51
XCL632-2-2 (*matα2-34*/*matα2-34*)	0.10	0	
VZ4 (**a** *ste4-1*)	0.20	1.60	0.125
VAC1 (**a** *ste5-1*)	0.31	1.00	0.31
381G-79A (**a** *ste7-2*)	1.00	3.09	0.32
381G-41A (**a** *ste11-1*)	1.72	4.65	0.37
381G-59C (**a** *ste12-1*)	1.22	5.53	0.22

[a] The autoradiograms were scanned as described in Materials and Methods. The calculated areas of the *STE2* and ADH bands were normalized for each autoradiogram and used to determine the *STE2*/ADH ratio.

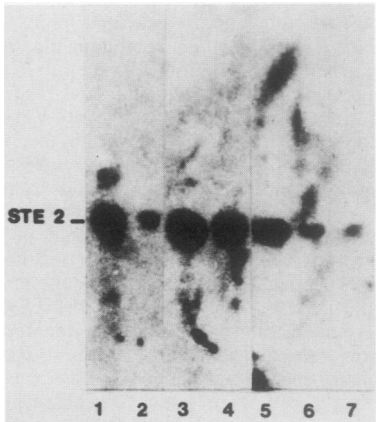


FIG. 5. *STE4* and *STE5* influence on levels of *STE2* RNA. Poly(A)-enriched RNA samples were obtained from wild-type **a** strains X2180-1A and G2, from derivatives of G2 in which either the chromosomal *STE4* or *STE5* gene has been disrupted with *LEU2* or *URA3* (strains kindly provided by L. Bell, ZymoGenetics), or from strains bearing nonsense mutations in the *STE4* gene (see Materials and Methods). The RNA was subjected to electrophoresis, transferred, and hybridized with radiolabeled mp11-ZV36 (the *Pst*I-*Bam*HI fragment from pAH1 subcloned into M13mp11). Lane 1, G2; lane 2, G2 *ste4Δ1*; lane 3, G2; lane 4, G2 *ste5Δ5*; lane 5, X2180-1A (**a**); lane 6, XL4-S47 (**a** *ste4-4*); lane 7, XL4-S118 (**a** *ste4-4*). Similar results were obtained with RNA isolated from a G2 derivative bearing a different *STE4* gene disruption (data not shown).

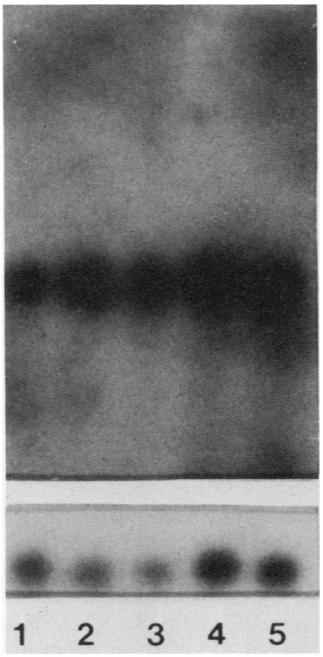


FIG. 6. Time course of α-factor stimulation of *STE2* transcription. Log-phase cultures of X2180-1A (**a**) were incubated in YEPD medium with α-factor for the time indicated. Poly(A)-enriched RNA samples were fractionated by electrophoresis, transferred, and hybridized with nick-translated pZV37 (top) and pY9T6 (bottom) (see Materials and Methods). Lane 1, 0 min; lane 2, 15 min; lane 3, 30 min; lane 4, 60 min; lane 5, 90 min.

the sequence elements needed for its transcription and regulation are located within 500 base pairs 5′ to the translational start (J. Kronstad and V. L. MacKay, unpublished data). A short fragment of the 5′ noncoding sequence of *STE2* was sequenced previously and shown to have a 33-base-pair sequence that has been implicated as a *MATα2* binding site (21, 34), although the precise location of this sequence relative to the translational start site was not established. We have therefore sequenced approximately 1.8 kb of DNA encompassing the *STE2* gene. The only long open reading frame extends at least 1,107 base pairs (Fig. 7A), a size that is consistent with that predicted for *STE2* from the RNA blots. This probable *STE2* coding sequence is immediately adjacent to the *Hin*dIII fragment that was deleted in the nonfunctional *LEU2* insertion (pZV63; Fig. 2). The DNA sequence of 540 base pairs of the 5′ noncoding region that presumably contains the promoter and regulatory sequences is shown in Fig. 7B. The *MATα2* binding site is located 200 to 230 base pairs 5′ to the translational start; deletion and mutational studies are necessary to prove the function of this sequence, including possible position effects, and other regions of the 5′ sequence. (It should be noted that our sequence is in agreement with sequences recently published by other groups [6, 37].)

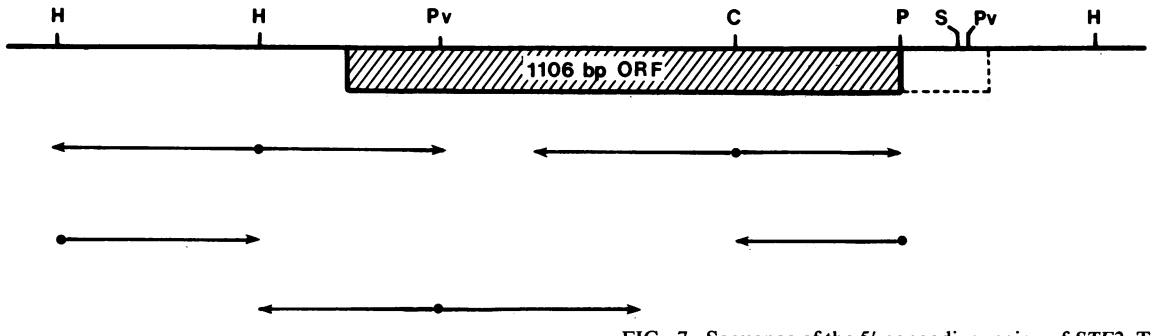
DISCUSSION

Although the mating type of a yeast cell segregates as a single gene, the phenotypic differences among **a**, α, and **a**/α cells indicate that selective expression of several different genes is required to establish the manifested cell type. Our results have demonstrated multiple ways in which the expression of a single gene required for conjugation is regulated.

Transcriptional regulation of *STE2*. *MATα2* regulation of genes expressed specifically in **a** cells was predicted in the *α1-α2* hypothesis and is confirmed for the specific cases of *STE6* (59), *BAR1* (31; MacKay and Manney, in preparation), and *STE2* (this work). For all of these genes, there was no detectable homologous RNA in cells that contained a functional *MATα2* gene. Although *MATα2* regulation of **a**-specific genes could occur by rapid turnover of the encoded RNAs, it is more likely that the *MATα2* protein acts by blocking transcription of these genes. Recent work from other groups has identified a consensus sequence in *STE6*, *BAR1*, *STE2*, and the **a**-factor structural gene *MFa1* that is responsible for *MATα2* repression (21, 34). In all of these genes, the consensus sequence is approximately 200 to 300 base pairs 5′ to the translational start and probably is part of a binding site for the *MATα2* protein in the nucleus (12). That the *MATα2* product is sufficient for the repression at least of *STE2* was indicated by the insensitivity to α-factor of *mata1* mutants transformed with a plasmid in which the *MATα2* coding sequence had been joined to the constitutive *ADH1* promoter (G. Ammerer, personal communication).

Other factors also influence *STE2* expression at the transcriptional level, although our data cannot discriminate between transcription initiation and RNA stabilization. The three- to fourfold stimulation of stable *STE2* RNA levels is similar to the α-factor enhancement observed for both secreted Barrier activity (31) and *BAR1* RNA (MacKay and Manney, in preparation) and for secreted **a**-factor activity (55). (It should be noted that none of these genes appears to be represented among the hormone-responsive genes described by Stetler and Thorner [52].) Similarly, the abundance of the α-specific RNA encoded by *STE3* (proposed to be the **a**-factor receptor gene) has also recently been reported to be increased approximately fourfold by incubation of α cells with **a**-factor (11).

A third level of *STE2* regulation involves the products of at least some of the nonspecific *STE* genes. Using temperature-sensitive *ste* mutations, we could detect the effect of the *ste4* (and possibly the *ste12*) mutation on *STE2* RNA levels, and the role of the *STE4* gene product was subsequently confirmed with both a *ste4* nonsense mutation and gene disruptions in *STE4*. Although the *STE4* protein is not absolutely required for the synthesis or accumulation of *STE2* RNA, its deficiency effects a 10-fold reduction in *STE2* transcript levels. Relative to a wild-type control, the temperature-sensitive *ste12* mutation led to a two- to threefold decrease in *STE2* RNA at the restrictive temperature (Table 2); however, using tighter *ste12* mutations, Fields and Herskowitz have recently shown that the *STE12* gene product is also required for normal levels of *STE2* RNA (10). Since gene disruptions in the *STE7* and *STE11* genes have likewise been shown to cause decreased levels of *STE3* RNA in α cells (D. Chaleff and G. Sprague, personal communication), it is possible that a similar role for these gene products in the accumulation of *STE2* RNA can be demonstrated with the use of the gene disruption strains. The slight effect of a disruption in the *STE5* gene on *STE2* RNA levels was somewhat surprising, since high levels of *STE5* gene product can suppress the mating defect of strains carrying the temperature-sensitive *ste4-1* allele used in the experiments shown in Fig. 4 (28; L. Bell, K. Nasmyth, and V. L. MacKay, manuscript in preparation). Perhaps the expression of other genes required for conjugation is affected by both the *STE4* and *STE5* gene products. From the data accumulated in all of these studies, it is tempting to hypothesize that the products of the nonspecific *STE* genes may




```
        10          20          30          40          50          60
AAGCTTTTTA ATACACCAAA GATTCAAGAT AAGAGCATAG AACGAACTGT AGAATAGTCC

        70          80          90         100         110         120
GGATATGTTA TCCAATGCCT GCCAAAATGC ATTGTCACAC GCTGTAGTGC TCGAATAGGT

       130         140         150         160         170         180
GTTGCAATCC GTCAATATAC GTCTTGCTCT GTGGGTAAAT GTCTCGTGCA TTAAGACAGG

       190         200         210         220         230         240
CTAGTATAAA CGAGAAGAAG TATCCTGCTT TGCAATGAAA CAATAGTATC CGCTAAGAAT

       250         260         270         280         290         300
TTAAGCAGGC CAACGTCCAT ACTGCTTAGG ACCTGTGCCT GGCAAGTCGC AGATTGAAGT

       310         320         330         340         350         360
TTTTTCAACC ATGTAAATTT CCTAATTGGG TAAGTACATG ATGAAACACA TATGAAGAAA

       370         380         390         400         410         420
AAAGCTTTCC TACATATTCA AGATTTTTTT CTGTGGGTGG AATACTATTT AAGGAGTGCT

       430         440         450         460         470         480
ATTAGTATCT TATTTGACTT CAAAGCAATA CGATACCTTT TCTTTTCACC TGCTCTGGCT

       490         500         510         520         530         540
ATAATTATAA TTGGTTACTT AAAAATGCAC CGTTAAGAAC CATATCCAAG AATCAAAAATG
                                                              MET
```

FIG. 7. Sequence of the 5′ noncoding region of *STE2*. Top panel: diagram of the 5′ flanking region and portion of the coding region sequenced; arrows indicate the strand sequenced. Note that the orientation of the DNA is opposite to that shown in Fig. 1. Bottom panel: nucleotide sequence.

form a transcription complex that is specifically required for high-level expression of the mating-type-specific genes. Alternatively, although mutants with defects in any one of the nonspecific *STE* genes (*STE4*, *STE5*, *STE7*, *STE11*, or *STE12*) have as yet identical cellular phenotypes, the gene products could play quite dissimilar roles in the conjugation process.

Multiple levels of transcriptional regulation have also been described for the *S. cerevisiae HO* gene, which codes for an endonuclease that initiates mating-type switching in homothallic cells (24, 39). *HO* transcription is repressed by the combined action of the *MATa1/MATα2* products, is limited to the G1 phase of the cell cycle, occurs in mother but not daughter cells, and is dependent upon the products of at least five unlinked *SWI* genes (20, 39, 51). Thus, the products of the nonspecific *STE* genes and the *SWI* genes may serve analogous functions in the expression of two distinct classes of genes that serve specialized functions in yeast cells.

Function of the *STE2* gene product. The phenotype of *ste2* mutants originally suggested that this gene might encode all or part of a surface receptor on **a** cells for α-factor (13, 29; 30); analogously, *STE3* was proposed to code for the α-specific receptor for **a**-factor (11, 29, 30, 48). This role for the *STE2* gene product has been supported by more recent biochemical studies (18, 19), which also demonstrated that the α-factor binding sites exist in approximately 8,000 copies per cell (19; revised from the earlier estimate in reference 18). The 48,000-kilodalton *STE2* product predicted from the DNA sequence (Fig. 7) (6, 37) would therefore account for approximately 0.016% of total cellular protein if the protein was active as a monomer (calculated as in reference 18). Our RNA blots indicate that *STE2* RNA is more abundant than expected for this level of expression, i.e., approximately 25% as abundant as RNA encoding alcohol dehydrogenase, which is reported to represent ca. 1 to 2% of total poly(A)$^+$ RNA and total soluble protein (8). RNA stability and protein turnover could account for this apparent discrepancy.

In general, our results are in agreement with the hypothesis that *STE2* specifies the α-factor receptor; for example, the increase of *STE2* transcription during incubation of **a** cells with α-factor is consistent with the increase of receptor on the surface of **a** cells (18). Jenness et al. (D. Jenness, personal communication) also found that there was a loss of α-factor binding in temperature-sensitive *ste4* mutants grown at the restrictive temperature, a result that is consistent with our interpretation that the *STE4* protein is involved in *STE2* RNA accumulation. The loss observed for receptor activity was, however, substantially less than the decrease in *STE2* RNA levels in *ste4* mutants.

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