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Identification of a DNA Segment That Is Necessary and Sufficient for α-Specific Gene Control in *Saccharomyces cerevisiae*: Implications for Regulation of α-Specific and a-Specific Genes

ERIC E. JARVIS, DAVID C. HAGEN, AND GEORGE F. SPRAGUE, JR.*
Department of Biology and Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

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***STE3* mRNA is present only in *Saccharomyces cerevisiae* α cells, not in a or a/α cells, and the transcript level increases about fivefold when cells are treated with a-factor mating pheromone. Deletions in the 5′ noncoding region of *STE3* defined a 43-base-pair (bp) upstream activation sequence (UAS) that can impart both modes of regulation to a *CYC1-lacZ* fusion when substituted for the native *CYC1* UAS. UAS activity required the α1 product of *MATα*, which is known to be required for transcription of α-specific genes. A chromosomal deletion that removed only 14 bp of the *STE3* UAS reduced *STE3* transcript levels 50- to 100-fold, indicating that the UAS is essential for expression. The *STE3* UAS shares a 26-bp homology with the 5′ noncoding sequences of the only other known α-specific genes, *MFα1* and *MFα2*. We view the homology as having two components—a nearly palindromic 16-bp "P box" and an adjacent 10-bp "Q box." A synthetic *STE3* P box was inactive as a UAS; a perfect palindrome P box was active in all three cell types. We propose that the P box is the binding site for a transcription activator, but that α1 acting via the Q box is required for this activator to bind to the imperfect P boxes of α-specific genes. Versions of the P box are also found upstream of a-specific genes, within the binding sites of the repressor α2 encoded by *MATα*. Thus, the products of *MATα* may render gene expression α or a-specific by controlling access of the same transcription activator to its binding site, the P box.**

Cell specialization is vital to the sexual life cycle of the yeast *Saccharomyces cerevisiae*. In *S. cerevisiae*, three types of cells occur. The two haploid cell types, **a** and α, are specialized for mating, whereas the **a**/α diploid cell type formed by mating of **a** and α cells is specialized for meiosis and sporulation. Each of the two haploid cell types expresses a specific set of genes that allows it to mate with cells of the opposite type. Among the cell-type-specific gene products are the small peptide pheromones, **a** factor and α factor, and the receptors for these pheromones. For example, only α cells secrete α factor and produce the cell surface receptor for **a** factor. These mating pheromones and receptors are the major components of a system that enables efficient mating of haploid cells of opposite type (for reviews, see references 20, 21, 41, 47).

How is this cell specialization controlled? The ultimate determinant of the cell type (mating type) of yeast cells is the *MAT* locus. Haploid α cells contain the *MATα* allele, which encodes two regulators, α1 and α2 (45). α1 is an activator of α-specific genes, which include the α-factor structural genes, *MFα1* (26, 39) and *MFα2* (39), and the gene that encodes the **a**-factor receptor, *STE3* (3, 16, 27, 34). Conversely, the α2 product represses **a**-specific genes, which include the **a**-factor structural genes, *MFa1* and *MFa2* (7), the α-factor receptor gene, *STE2* (19, 23, 27, 34), and two genes whose products are involved in pheromone metabolism, *STE6* (9, 50) and *BAR1* (42). For both α- and **a**-specific genes, regulation is at the level of RNA production; that is, transcripts from α-specific genes are found only in α cells (10, 11, 43; R. Jensen, K. Wilson, and I. Herskowitz, personal communication), and transcripts from **a**-specific genes are found only in **a** cells (7, 25, 34, 50; S. Michaelis and I. Herskowitz, personal communication). The mechanism by which α2 represses **a**-specific gene transcription is becoming clear; a 33-base-pair (bp) sequence has been shown to function as an operator for α2 in vivo, and an α2-β-galactosidase fusion protein has been shown to bind specifically to this sequence in vitro (24). The mechanism by which α1 activates transcription of α-specific genes, however, is less well understood and is the subject of this report.

We have sought to identify the site(s) of action of α1 and other regulators of α-specific genes. To this end, we used the *STE3* gene. *STE3* transcripts are abundant in α cells but are below the level of detection in **a** cells or **a**/α diploid cells (43). As an added feature of this system, the transcript level is increased four- to eightfold when α cells are grown in the presence of **a** factor (17). Such induction by pheromone is a property of several other mating-type-regulated genes as well (18, 25, 29, 48). Our approach to identifying the sites of action of α1 and the regulator that responds to reception of **a** factor was based on the presumption that the organization of sequences involved in the transcription of *STE3* would be similar to the organization of transcription signals from other yeast genes. Two elements are usually required for transcription, a TATA region and an upstream activation sequence (UAS) (for a review, see reference 12). The UAS element is required for transcription and typically confers sensitivity to the regulators that influence transcription of a particular gene. Thus, our goal was to delimit the *STE3* UAS.

By deletion analysis, we have defined an element upstream of the *STE3* gene that is both necessary and sufficient for the regulation of its expression. Sequence homologies among α-specific genes and the properties of chemically synthesized DNA segments suggest that the upstream element from *STE3* contains the sites of action of two regulatory proteins, α1 and a second protein that is found in all three cell types. We propose that the role of α1 is to allow

* Corresponding author.

this second protein to bind to *STE3* DNA and thereby activate transcription.

MATERIALS AND METHODS

Strains and media. Yeast strains HR125-5d (*MAT***a**), SY816 (*MATα*), and SY817 (*MAT***a**/*MATα*) are isogenic, except at the *MAT* locus (strains provided by R. Jensen and I. Herskowitz); other markers are *leu2-3 leu2-112 his3 his4 trp1 ura3-52 gal2*. In all cases, except for the experiments presented in Table 1, these strains served as standard **a**, α, and **a**/α genetic backgrounds in which β-galactosidase activity was measured after the introduction of *lacZ* fusion plasmids. Yeast strains for experiments presented in Table 1 were EG123 (*MAT***a**), 246-1-1 (*MATα*), 23α189 (*matα1-189*), and 23α182 (*matα2-182*), which are isogenic, except at the *MAT* locus (strains provided by K. Tatchell via A. Mitchell); other markers are *leu2 ura3 trp1 his4-519 can1*. Strain YY921 is identical to SY816 but has a 14-bp deletion in the *STE3* UAS region (see below). YY921 was derived from SY816 by two-step gene replacement (37) with plasmid pSL707. Other yeast strains used were DC5 (*MAT***a** *leu2-3 leu2-112 his3 gal2 can1*; from J. Strathern) and 227 (*MAT***a** *cry1 lys1*; from J. Hicks and I. Herskowitz). *Escherichia coli* SB69 was used for plasmid propagation (17).

The media used were the following: YEPD (same as YPD [38]), SD-Leu, and SD-Ura (SD supplemented with adenine, L-tryptophan, L-histidine, L-arginine, L-methionine, L-tyrosine, L-lysine, L-phenylalanine, L-threonine, and uracil or L-leucine [38]).

Plasmids. Plasmids pSL24 (17) and pSL57 (Fig. 1), which are closely related 2μm *LEU2* shuttle vectors derived from pJDB207 (2) and pMC1403 (8), were used to construct *STE3-lacZ* fusions. *STE3* fragments were derived ultimately from pSL1 (YEp13:STE3-30 [43]). For all plasmids depicted in Fig. 3, the 3′ end of the *STE3* fragment was the *Sau*3AI site (coordinates +316 to +319 [16]) joined to the *Bam*HI site adjacent to *lacZ* of the recipient vector. For pSL35 and pSL713, the 5′ end of the fragment was the *Hin*dIII site of *STE3* (at −923) joined to the *Hin*dIII site of 2μm in pSL24. For pSL64, the 5′ end was the *Eco*RI site of *STE3* (at −111) joined to the *Eco*RI site of 2μm of pSL24. For all other plasmids, the 5′ ends were created by joining various naturally occurring restriction sites of *STE3* to the *Sma*I site of pSL57.

Plasmid pSL330 (Fig. 1) was constructed by introducing *CYC1* sequences (as a *Hin*dIII-to-*Bam*HI fragment) from pLG669-Z (provided by L. Guarente [15]) into pSL24. The sequence at the junction between *CYC1* and *lacZ* was modified by restricting with *Bam*HI, digesting minimally with BAL 31 exonuclease, and ligating. pSL330 was identified as a plasmid in which the reading frames of *CYC1* and *lacZ* were suitably aligned. The resulting fusion has the first codon of *CYC1* followed by three codons created from cloning manipulations joined to the ninth codon of *lacZ*.

CYC1-lacZ fusion plasmids depicted in Fig. 4 were constructed from pSL330 by replacing the *Hin*dIII-*Xho*I-*Xho*I fragment, which includes the *CYC1* UAS, with *STE3* sequences. For the plasmids depicted in Fig. 4A, a *Hin*dIII-to-*Xmn*I (*STE3* coordinates −415 to −118) fragment of pSL126 was the source of *STE3* DNA. The 3′ endpoints were varied by digestion with BAL 31 exonuclease and subsequent addition of *Xho*I linkers. pSL351 (see Fig. 1) was one BAL 31-generated deletion in which no *STE3* DNA remained. The new plasmids of Fig. 4C were made by ligating particular *STE3* segments from the plasmids shown in Fig. 3A into pSL572 at the *Taq*I site (coordinate −313); pSL682 was made as a 5′ deletion to the *Taq*I site. pSL661 and pSL663 of Fig. 4D were made from pSL118 (Fig. 3A) as 3′ deletions to naturally occurring restriction sites. pSL687 of Fig. 4E was formed by joining the 3′ endpoint of pSL571 to the 5′ endpoint of pSL682; the net result was a precise 14-bp deletion of part of the *STE3* UAS. The 14-bp deletions in pSL713 (Fig. 3B) and pSL707 were formed similarly. pSL707, which was used to introduce the 14-bp deletion into the chromosomal *STE3* locus, has the same *STE3* fragment as pSL713 joined to the *Hin*dIII and *Bam*HI sites of vector pSL87, a derivative of YIp5 (46) in which the *Eco*RI site has been removed.

Plasmids pSL653, pSL655, pSL706, and pSL704 depicted in Fig. 6A were constructed from pSL545, pSL352, pSL684, and pSL351, respectively, by deleting the *Xho*I-*Sph*I fragment of *CYC1*. There is some variability at the deletion point as a result of the cloning manipulations. Plasmids pSL751 and pSL747 depicted in Fig. 6B were constructed from pSL684 by inserting one and two copies, respectively, of a 63-bp segment of DNA between the *STE3* and *CYC1* sequences. The 63-bp segment was obtained as a *Xho*I-*Xho*I fragment from a derivative of M13mp19 (52) in which *Xho*I linkers (5′-CCTCGAGG) had been inserted at the *Eco*RI and *Hin*dIII sites (the sites having been restricted and made blunt by using T4 DNA polymerase). Likewise, pSL745 was derived from pSL351 by inserting one 63-bp segment. In all cases, the 63-bp segment had the same orientation, with the *Eco*RI sites located near the 5′ end. The 63-bp segment showed no significant homology to the P and Q boxes or to the sequence 5′-ATGAAACA implicated in pheromone induction (25, 49; see Discussion).

CYC1-lacZ fusions of groups B and E of Table 2 were

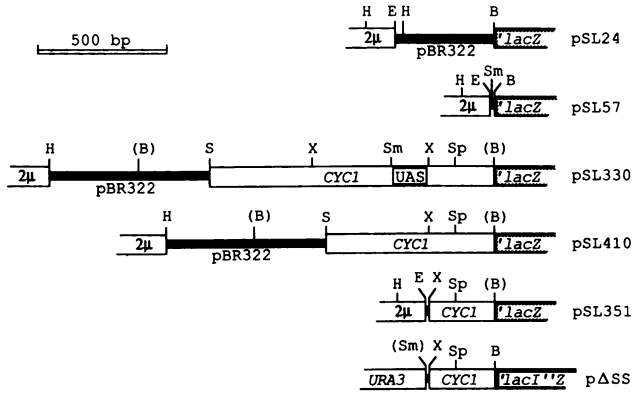


FIG. 1. Plasmids that served as recipients for *STE3* and *MFα2* DNA fragments and for synthetic oligonucleotides. Only the regions surrounding the site of insertion of *STE3* or other DNA fragments are shown. Plasmids pSL24, pSL57, pSL330, pSL410, and pSL351 are derivatives of pJDB207 (2) and differ from each other only in the region shown; plasmid pΔSS (24) is a derivative of YEp24 (6). DNA segments are labeled to indicate their origin. pΔSS has a different *lacZ* fragment from those of the other plasmids. The short solid black segments represent synthetic polylinkers. Bars labeled pBR322 represent only a short segment of pBR322 DNA. The positions of key restriction endonuclease sites are indicated by the following abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; Sm, *Sma*I; Sp, *Sph*I; X, *Xho*I. Restriction sites enclosed in parentheses were mutated or destroyed by cloning operations. See Materials and Methods for details of insertion of *STE3*, *MFα2*, and synthetic oligonucleotide sequences into these recipient plasmids. 2μ, 2μm plasmid.

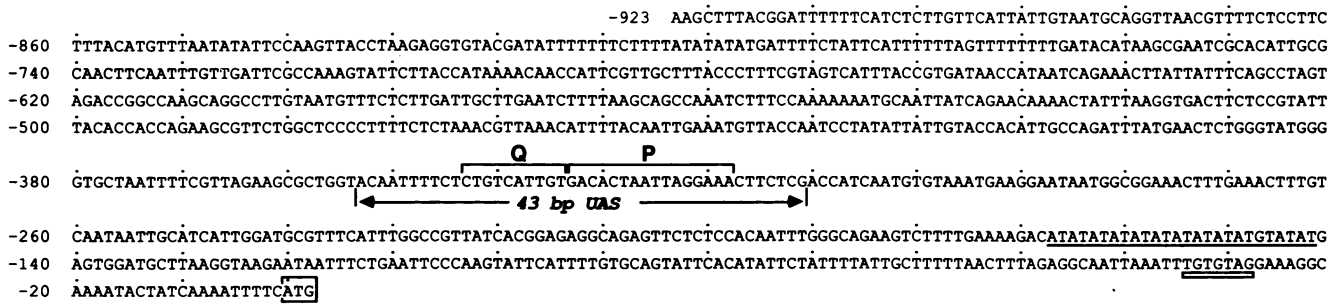


FIG. 2. The *STE3* upstream sequence. Coordinates are relative to the initiation codon, which is boxed. A putative TATA-like sequence is underlined; the major region of mRNA initiation is indicated by an open bar (16). The 43-bp UAS refers to the minimal fragment defined by deletion analysis sufficient to impart α-specific expression (see text). The P and Q homologies to other α-specific genes are indicated.

derived from pΔSS (24) (Fig. 1). pΔSS is a 2μm *URA3* shuttle vector related to YEp24 (6). For pSL691 and pSL695, an *Eco*RI-*Xho*I fragment (including the 43-bp *STE3* UAS region) was excised from pSL650 and ligated into the *Xho*I site of pΔSS; *Xho*I linkers were used to convert the *Eco*RI site to a *Xho*I site. pSL699 and pSL700 were made by ligating the synthetic fragment P(*STE3*) (see below) into the *Xho*I site of pΔSS. pSL701 was made by ligating the synthetic fragment P(PAL) (see below) into the *Xho*I site of pΔSS. pS1-Δ19-4 (24) is pΔSS harboring a synthetic version of the α2-binding site of *STE6* at the *Xho*I site. pΔSS and pS1-Δ19-4 were provided by A. Johnson and I. Herskowitz. Plasmids pSL703 and pSL702 of group C of Table 2 were constructed from pSL351 by insertion of a 44-bp fragment of *MFα2* DNA derived from pBR-5′ (provided by S. Fields and I. Herskowitz). A restriction fragment of *MFα2* was first cloned in both orientations into the *Sma*I site of M13mp18 (52), excised as an *Eco*RI-*Sal*I fragment, and then cloned into the *Eco*RI and *Xho*I sites of pSL351. Plasmid pSL410 of group D of Table 2 was constructed from pSL330 by eliminating the *Xho*I-*Xho*I fragment that encompasses the *CYC1* UAS. pSL597 and pSL595 were made by ligating P(*STE3*) (see below) into the *Xho*I site of pSL410. pSL593 was made by ligating P(PAL) (see below) into the *Xho*I site of pSL410.

Plasmid pSL615, which contains *STE3* and *URA3* sequences in pSP65 (Promega Biotec), was used to make SP6 polymerase-generated RNA probes for *STE3* and *URA3* mRNA. Plasmid SF9, which contains *STE5* sequences cloned into a pBR322-related vector, was provided by V. MacKay and was used to make DNA probes for pBR322 (5) and *STE5* DNA.

DNA manipulations. Standard methods (28) were used for DNA preparations, bacterial transformations, and plasmid constructions. All new junctions formed during plasmid construction by the ligation of blunt-ended DNA fragments were confirmed by DNA sequencing. Yeast transformations were by the spheroplast method of Beggs (1). The DNA oligonucleotide P(PAL) was synthesized as the self-complementary fragment 5′-TCGACTTCCTAATTAGGAAG at the University of Oregon with a Biosearch synthesizer (phosphotriester method); P(*STE3*) was synthesized as two complementary fragments 5′-TCGACACACTAATTAG GAAG and 5′-TCGACTTCCTAATTAGTGTG at Oregon State University with an Applied Biosystems model 380A synthesizer (phosphoramidite method).

DNA sequencing and DNA sequence analysis. The upstream region of *STE3* (Fig. 2) was sequenced by the dideoxy method of Sanger et al. (36) with overlapping subclones of *STE3* DNA in standard M13 sequencing vectors and with commercial synthetic oligonucleotide primers. Both strands of the DNA were sequenced. The sequence for *STE3* was identical to that of Nakayama et al. (33) obtained independently. The DNA sequence of the upstream region of *MFα1* was provided by M. Flessel, A. Brake, and J. Thorner; the DNA sequence of the upstream region of *MFα2* was provided by A. Singh; the DNA sequence of the upstream region of *CYC1* was provided by M. Smith. DNA sequence analysis was facilitated by software purchased from the University of Wisconsin Genetics Computer Group and installed on a VAX 11/750 computer at the University of Oregon Institute of Neuroscience and by software provided by J. Lis, Cornell University, Ithaca, N.Y., and installed on an Apple IIe computer.

β-Galactosidase assays. Plasmid-bearing strains were grown overnight to saturation in SD-Leu or SD-Ura selective medium. The cultures were centrifuged, and the cells were suspended in YEPD broth, inoculated to a density of about 10^7 cells per ml, and grown at 30°C for one doubling (3 to 4 h). These cultures were then divided, equal volumes of prewarmed YEPD or **a**-factor preparation (see below) were added, and the cultures were incubated with agitation for an additional 2.5 h.

Cells were assayed for β-galactosidase activity as previously described (17) except that Z buffer (32) was used for cell suspension and in the reaction mixtures. The units of β-galactosidase activity (modified from Miller [32]) are $(1{,}000 \times OD_{420})/(t \times v \times OD_{600})$, where t is the reaction time in minutes, v is the volume in milliliters, OD_{600} is the optical density at 600 nm of the processed cells actually added to the reaction, and OD_{420} is the optical density at 420 nm of the reaction mixture after termination and clarification by centrifugation. Optical densities were measured using a Beckman DU-6 spectrophotometer.

a-Factor preparations were simply the culture filtrates of strain DC5 (*MAT***a**) grown to saturation in YEPD medium at 30°C. Cells were removed by filtration through a 0.2-μm-pore-size membrane (Nalgene filter unit).

RNA analysis. RNA was extracted from yeast cells as described previously (43). Hybridization analysis was performed as previously described (43), except that poly(A)$^+$ RNA was isolated by using Hybond-mAP paper of Amersham Corp. and the recommended procedures of the manufacturer. RNA probes for *STE3* and *URA3* mRNA were made with plasmid pSL615 and a Riboprobe transcription system of Promega Biotec. Bands visible on autoradiograms were quantitated by using a Biomed Instruments scanning densitometer. Values for *STE3* mRNA were standardized to the *URA3* mRNA in the same lane.

Mating tests. The efficiency of mating was determined as

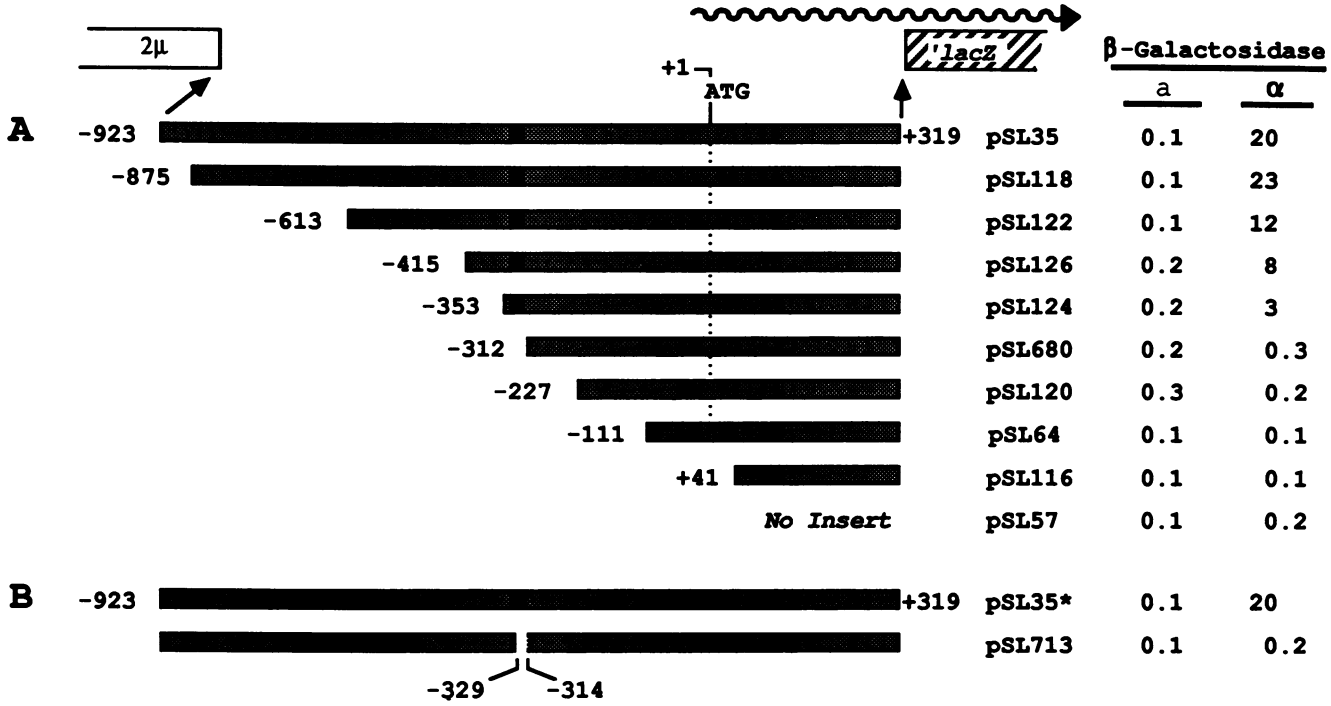


FIG. 3. *STE3-lacZ* fusions. *STE3* DNA segments (▨) have been inserted between 2μm (2μ) (▭) and the ninth codon of *lacZ* (▨), creating an in-frame fusion of *STE3* and *lacZ*. Coordinates are relative to the *STE3* ATG and indicate nucleotides included in the fragments. The QP homology (■), discussed in the text, and mRNA (⇝) are indicated. Each pSL plasmid was introduced into *MAT***a** and *MAT*α cells and assayed for β-galactosidase activity. Values reported are in modified Miller units (32; see Materials and Methods) and represent the means of assays on three different transformants. The standard deviations were typically 30% of the mean for values above 0.3 U. (A) 5′ deletion analysis. (B) A 14-bp deletion within the *STE3* UAS. A construction from panel A, pSL35, was repeated for clarity (*).

follows (modified from Hartwell [19]). Cells were grown in YEPD broth to a density of 3×10^7 cells per ml. The α cell cultures to be tested were mixed 1:1 with an **a** cell culture, strain 227. Portions (1-ml volumes) of the mixtures were filtered through 25-mm-diameter, 0.45-μm-pore-size nitrocellulose filter disks (Millipore Corp.). The filters were applied to prewarmed YEPD agar and incubated at 30°C for 5 h. After incubation, the cells were washed off into 1.0 ml of SD medium, subjected to sonication to disrupt clumps, diluted in SD medium, and plated on SD (to titer **a**/α cells) and on YEPD (to titer total cells). The mating efficiency was expressed as the titer of **a**/α cells divided by the titer of total cells.

Plasmid copy number determinations. Plasmid copy number was estimated by Southern analysis (40). Total DNA was prepared as described previously (35) from plasmid-bearing yeast cells. Plasmid SF9, labeled by nick translation with a kit from Bethesda Research Laboratories, Inc., served as a probe both for plasmid DNA (by virtue of its pBR322 sequences) and for chromosomal DNA (by virtue of its *STE5* sequences). Relative copy number was determined by comparing the ratio of plasmid-to-chromosomal hybridization observed for strains bearing 2μm-based plasmids to that for the same strains bearing a single-copy plasmid, YCp50 (44).

RESULTS

5′ deletion of the *STE3* upstream control region. A fusion of *STE3* sequences to the *E. coli lacZ* gene resulted in regulated expression of β-galactosidase (17). The multicopy plasmid pSL35 carries *STE3* DNA from −923 to +319 (relative to the *STE3* ATG) fused in frame to the ninth codon of *lacZ*. (The *STE3* upstream sequence is shown in Fig. 2.) The *STE3-lacZ* fusion directed the synthesis of β-galactosidase levels that are more than 100-fold higher in α cells than in **a** cells (17; Fig. 3A). Deletions from the 5′ end of the *STE3* fragment to various restriction sites in the upstream region resulted in a gradual loss of activity (Fig. 3A). The deletion to −353 retained the ability to confer some α-specific expression, but the next deletion, to −312, conferred no activity. These deletions show that sequence elements sufficient for the α-specific expression of *STE3* can be found between −353 and +319 and suggest that the 5′ border of sequences required for expression lies between −353 and −312. Sequences farther upstream, between −875 and −353, are required for maximal expression of *STE3* in this plasmid context, but their effect is much smaller in the context described below.

Definition of a site sufficient for α-specific activation by use of hybrid promoter constructions. To define more accurately the element(s) responsible for control of *STE3* transcription, we sought to identify sequences that are sufficient to confer α-specific expression onto a heterologous gene, a *CYC1-lacZ* fusion. It has been shown previously that expression of *CYC1-lacZ* requires two components, a TATA region and a UAS (13, 14). The *CYC1* UAS is required for activity and also confers regulation characteristic of the native *CYC1* gene. We have determined whether sequences from *STE3* can impart α-specific activity to a *CYC1-lacZ* fusion in which the UAS has been deleted. *CYC1* sequences (provided by L. Guarente) were fused to *lacZ*, and the hybrid gene was

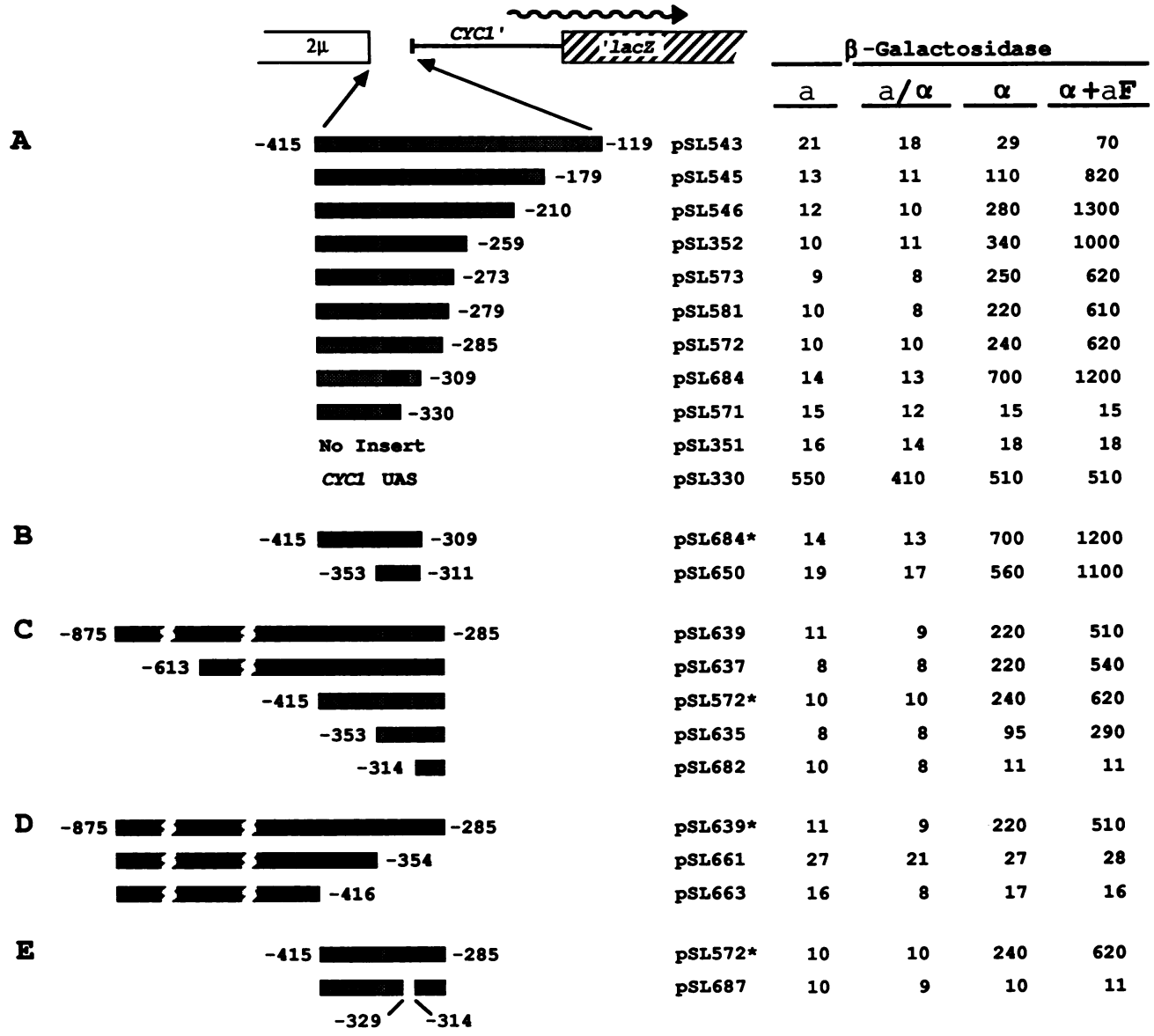


FIG. 4. *STE3-CYC1-lacZ* constructions. *STE3* DNA (▨) with the QP homology (■) is indicated (see text); coordinates are relative to the *STE3* ATG. Fragments were inserted between 2μm (2μ) (▭) and a 252-bp segment that carries the TATA boxes and the first codon of *CYC1* fused in frame to *lacZ*; the *CYC1* UAS had been deleted. mRNA (⇝) is indicated. Plasmids were introduced into *MAT***a**, *MAT***a**/*MAT*α, and *MAT*α cells and assayed for β-galactosidase activity. The α cells were assayed after growth with or without **a** factor (**a**F). Values reported are in modified Miller units (see Materials and Methods). For the diploid cells and pSL330-bearing cells, assays were performed one or more times on a single transformant; for the haploid strains, the values shown are the means of assays on three separate transformants. The standard deviations were typically 15% of the values reported. Parts A to E are different sets of deletions grouped together for comparison. pSL plasmids repeated between sets are also indicated (*).

placed in a 2μm *LEU2* plasmid, creating plasmid pSL330. The *CYC1* UAS was subsequently replaced with a family of upstream segments from *STE3*. Initially, we used the 5′ deletion to −415, which retains considerable α-specific expression, to create a series of 3′ deletions starting from position −119. All of these deletions, up to −309, conferred some α-specific expression onto *CYC1-lacZ*, although the precise levels of activity varied somewhat (Fig. 4A). The deletion to −330 (pSL571) abolished all UAS activity. Thus, the major conclusion from these data is that sequences required for activity of the *STE3* UAS reside in the interval between −330 and −309.

Several factors may contribute to the variation in β-galactosidase activities seen for the deletions. For example, removal of a negative element or improved spacing between the *STE3* UAS we have defined and the *CYC1* TATA region may be responsible for the threefold increase in activity seen upon deleting from −285 to −309. Likewise, the wide spacing between the UAS and TATA may be the cause of the low activity conferred by the largest fragment (−415 to −119, pSL543), as discussed further below. It should be noted that this particular fragment also contains the *STE3* TATA region, which may lead to transcript start points upstream of the *CYC1* TATA. Such transcripts would contain AUG codons that are terminated before *lacZ* and thus would be unproductive for translation of β-galactosidase.

TABLE 1. Dependence of UAS activities on the mating-type locus (*MAT*)

Plasmid	Fragment[a]	β-Galactosidase activity[b]			
		*MAT***a**	*MATα*	*matα1*	*matα2*
Group A					
pSL351	None	13	13	12	14
pSL650	*STE3* 43-mer	15	700	14	1,100[c]
pSL703	*MFα2* 44-mer	12	63	10	110[c]
Group B					
pSL410	None	47	52	57	51
pSL593	P(PAL)	510	530	600	600

[a] See text for descriptions of fragments.

[b] Plasmids were transformed into isogenic *MAT***a**, *MATα*, *matα1*, and *matα2* cells (EG123, 246-1-1, 23α189, and 23α182, respectively; see Materials and Methods). One transformant of each pSL351- or pSL410-containing strain and three transformants of each of the remaining strains were assayed for β-galactosidase activity. Values are in modified Miller units (see Materials and Methods); mean values presented had standard deviations of about 5%.

[c] Elevation of activity in these *matα2* mutant cells was probably the result of induction by endogenously produced **a** factor.

We have not determined the precise 5′ endpoints of the transcripts.

The 3′ deletions tested above have a common 5′ endpoint at −415. Because the 5′ deletion to −353 (pSL124; Fig. 3A) retained significant activity in the *STE3-lacZ* system, we next determined whether a fragment created by combining this 5′ deletion with a 3′ deletion to −311 retained UAS activity. The resulting 43-bp fragment conferred α-specific expression onto *CYC1-lacZ* (pSL650; Fig. 4B), and the activity was essentially the same as that of the larger fragment with coordinates −415 to −309 (pSL684).

α-Specific expression of the chromosomal *STE3* locus requires the α1 product of *MATα1* (43). To determine whether α-specific expression conferred by the 43-bp segment also requires the α1 product, we introduced pSL650 into a *matα1* mutant strain. In this mutant, β-galactosidase levels were reduced to those seen in an **a** strain, confirming that α1 is required for the α-specific expression we observe (group A of Table 1). As expected, the *MATα2* product was not required for expression.

A single UAS in *STE3*. The gradual diminution of β-galactosidase activity seen for the initial set of 5′ deletions suggested that there could be redundant UAS elements distal to the 43-bp UAS defined above. To test this possibility directly, we performed three experiments. First, we created a new set of 5′ deletions in the *CYC1-lacZ* system. These deletions have a common 3′ terminus at position −285. In this new set of deletions, those with 5′ endpoints −875, −613, and −415 directed comparable levels of β-galactosidase (Fig. 4C). Thus, in this plasmid context, unlike the original context for the 5′ deletions (Fig. 3A), the distal *STE3* sequences from −875 to −415 did not influence the level of expression. In agreement with the original 5′ deletion series, *STE3* sequences from −415 to −353 were required for maximal expression, and sequence elements within the coordinates −353 to −314 were essential for α-specific expression. (It is puzzling to note that the region from −415

TABLE 2. Activities of UAS fragments and synthetic oligonucleotides[a]

Plasmid	Fragment	Orientation	Recipient	β-Galactosidase activity			
				a	**a**/α	α	α + a**F**
Group A							
pSL351	None			16	14	18	18
pSL650	*STE3* 43-mer	Natural	pSL351	19	17	560	1,100
Group B							
pΔSS	None			0.2	0.1	0.2	0.2
pSL691	*STE3* 43-mer	Natural	pΔSS	0.3	0.2	11	20
pSL695	*STE3* 43-mer	Reversed	pΔSS	0.2	0.4	3.5	6.1
Group C							
pSL351*	None			16	14	18	18
pSL703	*MFα2* 44-mer	Natural	pSL351	17	14	55	130
pSL702	*MFα2* 44-mer	Reversed	pSL351	12	10	21	31
Group D							
pSL410	None			56	51	55	55
pSL597	P (*STE3*)	Natural	pSL410	51	50	54	53
pSL595	P (*STE3*)	Reversed	pSL410	65	59	64	59
pSL593	P (PAL)		pSL410	1,300	1,200	1,300	1,300
Group E							
pΔSS*	None			0.2	0.1	0.2	0.2
pSL699	P (*STE3*)	Natural	pΔSS	0.2	0.1	0.2	0.2
pSL700	P (*STE3*)	Reversed	pΔSS	0.2	0.1	0.2	0.2
pSL701	P (PAL)		pΔSS	4.7	3.6	5.5	5.4
pS1-Δ19-4	α2 site	Natural	pΔSS	3.8	0.2	0.2	0.1

[a] Plasmids were transformed into *MAT***a**, *MAT***a**/*MATα*, and *MATα* cells. Three transformants of each haploid and one transformant of each diploid strain were assayed for β-galactosidase. The α cells were assayed after growth in the presence or absence of **a** factor (a**F**). Values are in modified Miller units (see Materials and Methods). The mean values presented for the haploid strains had standard deviations of about 10% for groups A, C, and D and of about 25% for groups B and E. Rows presented elsewhere in the table that are repeated for clarity are indicated (*). Values for pSL351 and pSL650 are taken from Fig. 4. See text for descriptions of fragments; α2 site refers to the synthetic *STE6* version of the *MATα2*-binding site. Plasmids pΔSS and pS1-Δ19-4 were provided by A. Johnson and I. Herskowitz.

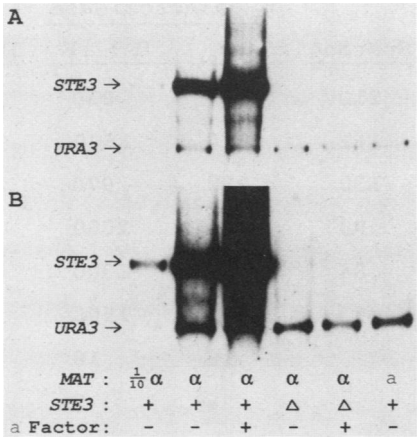


FIG. 5. RNA analysis of a strain harboring a 14-bp deletion within the *STE3* UAS. *STE3* DNA carrying the 14-bp deletion was used to replace the chromosomal copy (see Materials and Methods). Poly(A)$^+$ RNA was prepared from wild-type (+) and deletion-carrying (Δ) strains that were grown in the presence (+) or absence (−) of **a** factor. RNA was fractionated by electrophoresis, blotted to nitrocellulose, and probed for *STE3* and *URA3* transcripts. The same quantity of RNA (2.5 μg) was loaded in all lanes except 1/10α, which contains only 0.25 μg of RNA. Panel B is the same as panel A, except that the film was exposed nine times longer.

to −353 had more influence on fragments ending 3′ at −285 [Fig. 4C] than on fragments ending 3′ at −309 or −311 [Fig. 4B].)

In a second effort to detect redundant UAS activity, we tested distal *STE3* segments alone for such activity. Two segments were used, one extending from −875 to −354, the other extending from −875 to −416. Neither was able to confer expression to the UAS-deleted *CYC1-lacZ* gene (Fig. 4D).

A final experiment demonstrates that there are not redundant UAS elements at *STE3*. As shown below, deletion of 14 bp within the UAS defined in Fig. 4A and B abolished expression even when that deletion mutation resides at the chromosomal *STE3* locus.

Information in the 43-bp segment that is necessary for the expression of *STE3*. To determine whether the 43-bp segment shown to be sufficient for α-specific transcription is also required for expression, we made a short deletion within this segment and tested the activity of the UAS in three contexts. (i) Part (14 bp) of *STE3* sequence (from −328 to −315) was removed from the original *STE3-lacZ* fusion carrying *STE3* sequences from −923 to +319. When a plasmid harboring this deletion (pSL713) was introduced into *MAT*α cells, no β-galactosidase activity was detected (Fig. 3B). (ii) This same 14-bp deletion also abolished UAS activity when this mutant UAS was used in the *CYC1-lacZ* system (Fig. 4E). (iii) Finally, the 14-bp deletion was used to replace the chromosomal copy of the *STE3* gene. *STE3* RNA levels from α cells harboring this deletion were reduced 50- to 100-fold (Fig. 5). In addition, the ability of these cells to mate with **a** cells was reduced 100-fold (data not shown). Thus, the sequences disrupted by the 14-bp deletion are absolutely required for expression of *STE3*.

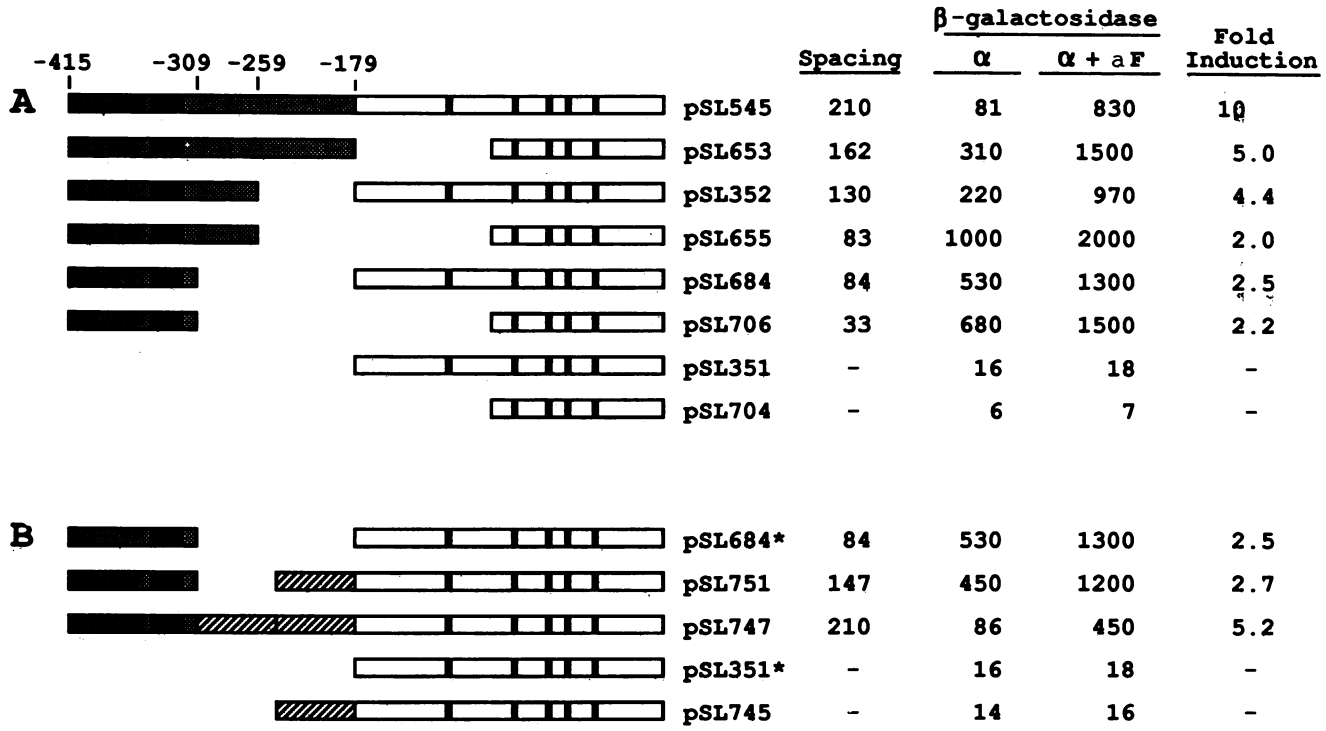
Orientation independence of the 43-bp UAS. A fragment carrying the 43-bp sequence was removed from plasmid pSL650 and put into plasmid pΔSS (provided by A. Johnson and I. Herskowitz [24]) in both orientations. Levels of expression were reduced in this new background, but both orientations of the 43-mer were capable of activating expression of *CYC1-lacZ* (group B of Table 2). The natural orientation (with respect to the TATA region) was slightly more active than the inverted orientation.

The cause of the large difference in expression between the two plasmid types is unclear, but there are at least three possibilities. (i) The original (pSL330-based) plasmids have a higher copy number; they were present at about 25 copies per cell, whereas the pΔSS plasmids were approximately sixfold less plentiful (data not shown). (ii) The fusion proteins were not identical (Fig. 1). Perhaps the protein encoded by the pΔSS-based plasmids is less active or less stable than the protein encoded by the pSL330-based plasmids. (iii) The plasmid sequences upstream from the *STE3* insert are different and perhaps could influence the expression levels. The pSL330-based plasmids had 2μm sequences adjacent to *STE3*, whereas the pΔSS-based plasmids had *URA3* sequences (Fig. 1). It should be noted here that with one pSL330-based construct (pSL572), we have experimentally altered the vector sequences immediately upstream of *STE3* (by inverting a large segment of 2μm DNA) and have found the activity to be essentially unchanged (data not shown).

Increased transcription in response to a-factor treatment. As noted in the introduction, transcription of *STE3* increases four- to eightfold when α cells are treated with **a** factor (17). To identify the site(s) of action of the regulator(s) that responds to the signal generated by **a**-factor reception, we used the same set of *STE3* fragments linked to *CYC1-lacZ* that were used to define the segment sufficient for α-specific transcription (Fig. 4). α Cells harboring these constructions were exposed to **a** factor, and β-galactosidase activities were measured. All *STE3* segments that allowed α-specific expression of *CYC1-lacZ* also conferred some sensitivity to induction by **a** factor. *CYC1-lacZ* coupled with large *STE3* segments (e.g., pSL545 [Fig. 4A]) exhibited the same magnitude of induction by **a** factor as was seen for *STE3* RNA from the chromosomal locus. As the *STE3* segment was shortened from the 3′ end, the magnitude of induction diminished gradually to a minimum of about twofold (e.g., pSL684 and pSL650 [Fig. 4A and B]).

The diminution of induction as *STE3* sequences were trimmed from the 3′ end could indicate that sequences responsible for maximum induction had been deleted. On the other hand, the diminution could simply reflect the change in spacing between the *STE3* UAS and the *CYC1* TATA region. To distinguish between these two possibilities, we selected representatives of the constructions shown in Fig. 4A and created derivatives in which the spacing between the *STE3* UAS and *CYC1* TATA was altered without deleting or adding *STE3* sequences. In one set of these derivative constructions, approximately 100 bp of *CYC1* DNA, located between the *STE3* segment and the *CYC1* TATA region, was deleted. (These constructions remove one of the five *CYC1* TATA boxes [30].) These *CYC1* deletions reduced the fold induction conferred by the large *STE3* segments (Fig. 6A). In the second set of derivative constructions, fragments of foreign DNA 63 bp in length were inserted between the *STE3* segment and the *CYC1* DNA. The original *STE3* 3′ deletion to position −309 resulted in only a two- to threefold induction of β-galactosidase, whereas a derivative that has a 126-bp insertion (two copies of the 63-bp fragment) showed a fivefold induction (Fig. 6B).

Taken together, these results indicate that the magnitude of induction conferred by a particular *STE3* segment is affected by the spacing between the *STE3* UAS and the *CYC1* TATA region. Constructions that spaced the *STE3*



	Spacing	β-galactosidase α	β-galactosidase α + aF	Fold Induction
pSL545	210	81	830	10
pSL653	162	310	1500	5.0
pSL352	130	220	970	4.4
pSL655	83	1000	2000	2.0
pSL684	84	530	1300	2.5
pSL706	33	680	1500	2.2
pSL351	-	16	18	-
pSL704	-	6	7	-
pSL684*	84	530	1300	2.5
pSL751	147	450	1200	2.7
pSL747	210	86	450	5.2
pSL351*	-	16	18	-
pSL745	-	14	16	-

FIG. 6. Effects of spacing on inducibility by a factor. *STE3* DNA (▨) with the QP homology (▬) (see text) and *CYC1* sequences (▭) with TATA boxes (▮) (30) are indicated. The *STE3* coordinates shown are relative to the *STE3* ATG. The spacing indicated is from the 3′ edge of the QP homology to the 5′ edge of the nearest TATA box. Each pSL plasmid was introduced into *MAT*α cells, and the cells were grown with or without added a factor (aF). β-Galactosidase activities are in modified Miller units (see Materials and Methods) and are the average of assays on three separate transformants; the standard deviations were about 15% of the values reported. Assays of strains harboring pSL545, pSL352, pSL684, and pSL351 are also presented in Fig. 4; the assays reported in this figure were performed independently and yielded comparable values with those of Fig. 4. (A) *CYC1* deletions. Each pair represents a plasmid from Fig. 4 and the corresponding plasmid in which approximately 100 bp of *CYC1* DNA has been deleted (see Materials and Methods). The fourth pair is a control plasmid containing no *STE3* DNA. (B) Insertions of foreign DNA. One or two copies of a 63-bp DNA fragment (▨) (see Materials and Methods) were inserted between *STE3* and *CYC1*. The bottom two constructs again represent controls with no *STE3* DNA. Constructions repeated from part A for clarity are indicated (*).

UAS and the nearest *CYC1* TATA at a distance that approximated the natural spacing between the *STE3* UAS and its own TATA at *STE3* (a distance of about 150 bp) were inducible to nearly the same extent as was the chromosomal *STE3* locus (Fig. 6). Thus, these findings suggest that the 43-bp UAS contains sequences that are sufficient to allow essentially normal induction of transcription in response to a-factor treatment of α cells. The possibility still exists, however, that sequences between −309 and −179 are partially responsible for induction of the wild-type gene (compare, for example, pSL545 and pSL747 or pSL653 and pSL684 [Fig. 6]).

To confirm that the induction of β-galactosidase observed upon a-factor treatment reflected an increase in *CYC1-lacZ* mRNA, we performed RNA blot analysis on a and α strains harboring representative plasmids (pSL352, pSL650, pSL684, and pSL747) (data not shown). Production of *CYC1-lacZ* mRNA was α specific, as expected. Moreover, the quantity of *CYC1-lacZ* transcript increased in parallel with the increase in β-galactosidase activity. We further showed that there was no difference in plasmid copy number between a and α strains and that the copy number did not change upon a-factor treatment (data not shown). Thus, the induction of β-galactosidase activity reflects bona fide transcription induction directed by the *STE3* UAS element.

Homology of the *STE3* control region to upstream regions of other α-specific genes. The only other genes known to be expressed specifically in α cells are those encoding the α-factor mating pheromone. These genes, *MFα1* and *MFα2*, have been sequenced by other investigators (22, 26, 39; far upstream sequence of *MFα1* and *MFα2* provided by M. Flessel, A. Brake, and J. Thorner and by A. Singh, respectively.) We compared these sequences for homologies with *STE3*. The most striking homology that can be found between the upstream noncoding regions of each of these genes was a 26-bp sequence (Fig. 7A). *STE3* has a single 26-mer spaced 317 bp from the putative initiation codon; 22 positions match the consensus 26-mer. *MFα1* has two 26-mers with scores of 23 (*MFα1*A) and 21 (*MFα1*B) of 26 and spaced 286 and 337 bp, respectively, from the ATG. *MFα2* has one 26-mer (score, 21 of 26) spaced 291 bp from the ATG. The orientation of the 26-mer of *STE3* relative to the ATG is opposite to that of the 26-mers of *MFα1* and *MFα2*. We view the 26-mer sequence as having two components (discussed below), a 16-bp, partially palindromic P box and an adjacent 10-bp Q box.

The deletion analysis described above localizes an element sufficient for regulation of *STE3* to a 43-bp sequence. This sequence contains the *STE3* version of the 26-mer homology. In addition, the 14-bp deletion, which reduced

STE3 transcription by at least 50-fold, disrupted the P box region of the 26-mer (Fig. 7C). Thus, the deletion analysis is consistent with the idea that the 26-mer is responsible for α-specific transcription of *STE3*.

α-Specific expression also conferred by a homologous sequence from *MFα2*. To further test the significance of the sequence homologies, we determined whether the 26-mer from a second α-specific gene, *MFα2*, could confer regulated expression. A 44-bp fragment containing the *MFα2* 26-mer (Fig. 7) was placed upstream of *CYC1-lacZ*, creating plasmids pSL702 and pSL703. The *MFα2* DNA was able to direct α-specific expression of β-galactosidase (see group C of Table 2), although to a lesser degree than did the *STE3* 43-mer (see group A of Table 2). As was seen for the *STE3* segment, expression conferred by the *MFα2* segment required the wild-type product of *MATα1* but not *MATα2* (see group A of Table 1), and the *MFα2* 44-mer functioned better in one orientation (see group C in Table 2). In addition, the expression was induced about twofold by growing cells in the presence of **a** factor (see group C of Table 2). (The transcription of the chromosomal *MFα2* gene was stimulated about sixfold upon **a**-factor treatment [data not shown].) In light of the observation that *MFα2* transcript is substantially less abundant than *STE3* transcript (11; our unpublished observations), it is perhaps not surprising that the *MFα2* segment is less active as a UAS element than the *STE3* segment. In this regard, it may be significant that, among α-specific genes, the *MFα2* P box has the worst match to the consensus P box (Fig. 7).

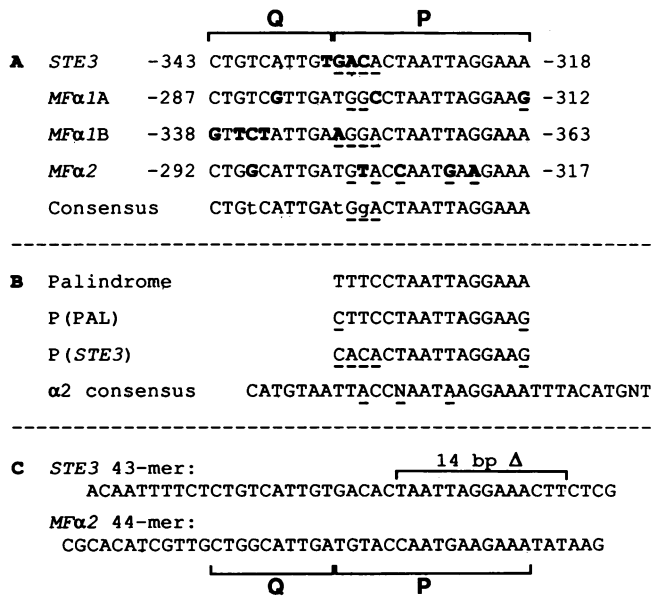


FIG. 7. The P and Q box homologies. (A) The 26-bp homology seen between the three α-specific genes, *STE3*, *MFα1*, and *MFα2*. Coordinates are given relative to the ATG of each gene. *MFα1* has two such sequences, labeled A and B. The reverse complements of the *MFα1* and *MFα2* sequences are shown to match the orientation of the *STE3* sequence. Boldface letters represent mismatches to the consensus; lowercase letters in the consensus indicate an agreement of only two of four, whereas capital letters indicate three or four matches. Underlined letters show mismatches to the perfect palindrome (part B). (B) The palindromic version of the 16-bp P box. Mismatches to the perfect palindrome are underlined. P(PAL) and P(*STE3*) show the relevant regions of the synthetic oligonucleotides tested for UAS activity (Table 2; see Materials and Methods for complete sequences of oligonucleotides); for technical and historical reasons, these sequences differ from the perfect palindrome at their end positions. α2 consensus, the site of binding of the *MATα2* protein, is derived from the **a**-specific genes, *STE2*, *STE6*, *BAR1*, *MFa1*, and *MFa2* (24, 31). The *STE6* version of this sequence, which was assayed for activity in the *CYC1-lacZ* system (Table 2), differs from the α2 consensus at only two positions. (C) Sequences of the *STE3* and *MFα2* UAS fragments. The sequences are aligned by their P and Q boxes; *MFα2* sequence is inverted. The location of the 14-bp deletion (14 bp Δ), which removes most of the *STE3* P box, is shown.

High constitutive transcription directed by a synthetic palindromic oligonucleotide. The P box in the 26-bp homology seen among α-specific genes was partially palindromic; however, none of the 26-mers contained a precise match to the perfect 16-bp palindrome (Fig. 7). Divergence from the palindromic sequence was seen primarily in the region adjacent to the Q box. To address the role of the P box elements, two oligonucleotides were synthesized. One corresponds to the 14-bp core of the *STE3* P box, termed P(*STE3*); the other, P(PAL), is the same sequence modified so that it is a perfect palindrome (Fig. 7B). P(*STE3*) was unable to confer significant activity onto the UAS-deleted *CYC1-lacZ* fusion (see groups D and E of Table 2). P(PAL) did confer high levels of expression onto the fusion, but surprisingly, this expression was unregulated; i.e., the same activity was seen in **a**, α, and **a**/α cells. The level of expression in α cells did not change in response to **a** factor (see groups D and E of Table 2), and expression did not require the *MATα1* product (group B, Table 1). Thus, the P box palindrome serves as a constitutive activator sequence.

The synthetic P(PAL) sequence, when incorporated into the *CYC1-lacZ* vector, creates a 28-bp palindrome due to contributions of the linkers used, as well as neighboring *CYC1* base pairs. The possibility that palindromes of this size have UAS activity in general was addressed by introducing other synthetic palindromes (of 20, 30, and 32 bp; each derived from the restriction site clusters of M13 cloning vectors) into the same site of the same vector (pSL410). None of the other palindromes displayed UAS activity, as judged by β-galactosidase activity of **a**, α, or **a**/α cells bearing these plasmids (data not shown). Thus, we believe that P(PAL) has UAS activity because of its specific sequence and not merely because it is a palindrome.

The noncoding upstream regions of *STE3*, *MFα1*, and *MFα2* were searched for additional segments homologous to the 16-bp palindromic P box. By using a score of 11 (of 16) or higher as a criterion, *MFα1* has one additional P box 462 bp from the ATG, *MFα2* has one additional P box 508 bp from the ATG, and *STE3* has no additional P boxes. Neither of these additional P boxes has associated Q box sequences. The significance, if any, of these P boxes that lack associated Q boxes is not clear.

The P box as a UAS for a-specific genes. Good matches to the P box can also be found upstream of **a**-specific genes (Fig. 7B; S. Fields and I. Herskowitz, personal communication). These homologies are not found in conjunction with Q boxes but rather are found within the operator sites to which the repressor *MATα2* binds (24). A 33-bp synthetic oligonucleotide corresponding to the *STE6* version of the operator (provided by A. Johnson and I. Herskowitz) contains a match (13 of 16) to the palindromic P box. This oligonucleotide was able to activate expression of the UAS-deleted *CYC1-lacZ* gene. Activity was limited to **a** cells, where the *MATα2* product (α2) is absent (see group E of Table 2; 24). Thus, the P boxes found upstream of **a**-specific genes may be

activator sites that can be occluded by the binding of α2. Models for the control of both α- and **a**-specific genes are discussed below.

DISCUSSION

Deletion analysis of the *STE3* upstream sequence has defined a single, small element that is both necessary and sufficient for α-specific expression. A 43-bp segment was able to confer activity onto a *CYC1-lacZ* fusion specifically in α cells; this segment could function in either orientation. Activity of this UAS was dependent on the product of the *MATα1* gene. Moreover, a deletion of 14 bp from this 43-bp UAS abolished the ability of the UAS to activate transcription. Most dramatically, when DNA carrying a 14-bp deletion was used to replace the wild-type sequence at the chromosomal *STE3* locus, transcription was reduced 50- to 100-fold. The *STE3* UAS, as defined by the 43-bp fragment, included a 26-bp segment homologous to sequences found in the control regions of other α-specific genes. The 14-bp deletion eliminated 11 bp of the *STE3* version of this 26-mer. The importance of the homology is confirmed by the ability of a 44-bp fragment containing the 26-mer from *MFα2* to confer α-specific expression onto *CYC1-lacZ*. Likewise, deletion analysis performed with the *MFα1* gene further supports the idea that the homologous regions are key components of an α-specific UAS (22; M. C. Flessel and J. Thorner, personal communication). That UASs can confer α-specific expression upon a heterologous gene implies that the *MATα1* product controls transcription initiation rather than transcript stability.

The presence of **a** factor in the growth medium enhances the transcription of wild-type *STE3* four- to eightfold (17). All *STE3* fragments that conferred α-specific activity onto the *CYC1-lacZ* fusion also conferred at least some capacity for increased transcription in response to **a** factor. In general, constructs that placed the *STE3* UAS close to the *CYC1* TATA region resulted in high basal levels of activity and low magnitudes of induction (about twofold). Constructs that placed the UAS far from the TATA region resulted in low basal levels and high magnitudes of induction (up to 7- or 10-fold). We believe that the lower inducibility of plasmids with the UAS close to the TATA region may not be due to a loss (by deletion) of *STE3* sequences but rather to differences in spacing, because higher magnitudes of induction can be restored by inserting foreign DNA fragments between the UAS and TATA regions. Thus, we conclude that information sufficient for induction is present on the 43-bp segment. What might this information be? Kronstad et al. (25) and Van Arsdell and Thorner (49) have each focused on a sequence that they propose to be responsible for induction by pheromone. Versions of the consensus sequence 5′-ATGAAACA (49) (or 5′-TGAAACA [25]) are found in two or more copies in the upstream regions of several genes known to be inducible by pheromone. Two matches (six of eight) are seen within the 43-bp *STE3* UAS segment; one overlaps the P sequence, and the other overlaps the Q sequence. However, other matches of this quality are present elsewhere in the 5′ noncoding sequences of *STE3*, in *CYC1*, and in 2μm. The 44-bp *MFα2* sequence, which also conferred inducibility in *CYC1-lacZ* fusions, has three such matches (six of eight). Whether these weak representatives of the putative induction sequence play a role in induction of *STE3* or *MFα2* is not known.

The 26-bp homologous segments found at the three known α-specific genes (*STE3*, *MFα1*, and *MFα2*) can be thought of as having two components, the P box (16 bp), which is nearly a palindrome, and the Q box (10 bp), which extends the homology on one side of the P box (Fig. 7). We found that a synthetic oligonucleotide corresponding to the core of the palindromic version of the P box, P(PAL), functioned as a UAS in all three cell types, whereas the *STE3* version of this sequence, P(*STE3*), was inactive as a UAS. Moreover, the *STE3* P box is essential for activity of the *STE3* UAS; the 14-bp deletion that removes the P box inactivated the *STE3* UAS. These findings suggest a model for transcription activation of α-specific genes (Fig. 8). We propose that P boxes are recognized by a transcription factor that we call PRTF (for P box recognition transcription factor or pheromone/receptor transcription factor, indicative of the genes identified thus far that probably use this factor). PRTF can bind to P boxes if they have the perfect palindrome sequence or very nearly that sequence, and binding of PRTF allows transcription to proceed. PRTF cannot bind to the imperfect P boxes actually present at α-specific genes, however, unless *MATα1* protein is present to aid its binding. We imagine that α1 makes specific contacts both with PRTF and with the Q box and thereby recruits PRTF to bind at α-specific genes,

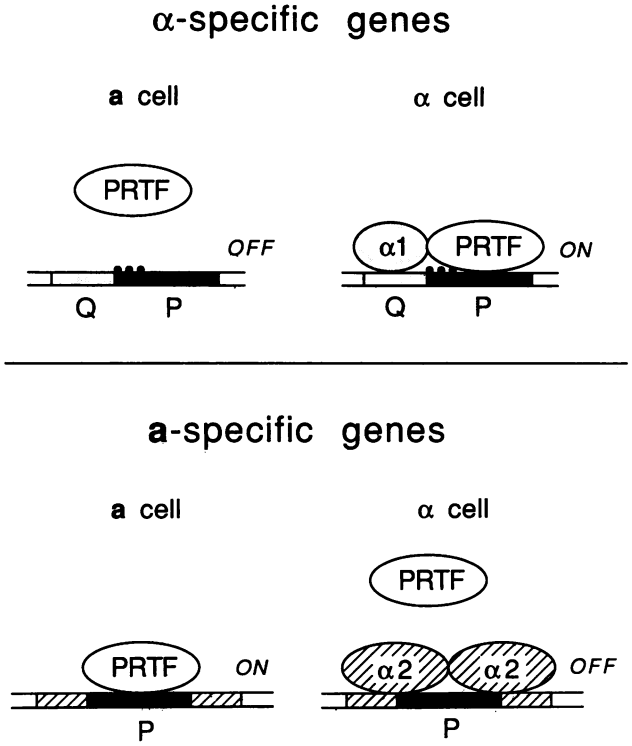


FIG. 8. Models for the regulation of α- and **a**-specific genes. The α-specific genes have imperfect P boxes; the region of deviation from the perfect palindrome is indicated ▪▪▪. The transcriptional activator protein PRTF (P box recognition transcription factor or pheromone/receptor transcription factor) is unable to recognize these imperfect P boxes without the help of α1 (the *MATα1* product) binding to the Q box. The **a**-specific genes have P boxes capable of binding PRTF to stimulate transcription, but in α cells, α2 (the *MATα2* product) covers the P box and prevents PRTF from binding. For the α-specific genes, Q (▩) and P (▬) box homologies are indicated. The α2 site of **a**-specific genes (▨), with the central region, (▬) which is homologous to the P sequence, is also indicated. The α2 protein is shown as a dimer, as suggested by Johnson and Herskowitz (24). The precise nucleotides that are required for binding of α2 within the large α2 site have not been determined.

where it promotes transcription. In this regard, it is perhaps significant that α-specific P boxes differ from the palindromic P box at the nucleotides adjacent to the Q box. In support of this model, we have recently found that α1 protein binds in conjunction with a second protein, found in all three cell types, to the P and Q box region of α-specific genes (4). The second protein is capable of binding alone to the palindromic P box but not to the *STE3* P box.

The model presented above can be extended to include transcription activation of **a**-specific genes. Versions of the P box are also found in the control regions of **a**-specific genes within the 33-bp sequence that has been shown to function as an operator for the *MATα2* product (Fig. 7B). The 33-bp operator has some activity as a UAS in **a** cells (24; also see group E of Table 2) suggesting that these **a**-specific P boxes may be functional. Thus, we propose that in **a** cells, PRTF binds to the P boxes of **a**-specific genes and contributes to their transcription activation (Fig. 8). In α cells, however, the binding of α2 to its operator presumably blocks the binding of PRTF to the P box; perhaps occlusion of the P box by α2 is part of the mechanism whereby α2 represses transcription of **a**-specific genes. Alternatively, PRTF may still bind in the presence of α2 but be unable to activate transcription. The **a**-specific P boxes may vary in the overall contribution they make to the expression of **a**-specific genes. For *BAR1*, deletion of the α2 operator decreases transcription at least 10-fold (25), whereas for *STE6*, more distal sequences (with respect to the TATA box) appear to be responsible for the bulk of expression (51).

The model presented above is conservative in that a single activator system—PRTF and the P box—is used by both α- and **a**-specific genes. The role of the mating-type-locus-encoded regulators, α1 and α2, is to control access of PRTF to the UAS elements of these two classes of genes. Because a palindrome P box can function as a UAS in all three cell types, we further speculate that this activator system may be used by other classes of genes as well.

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