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The Yeast Repeated Element Sigma Contains a Hormone-Inducible Promoter

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A genomic clone (λScG7) from *Saccharomyces cerevisiae* encoded a 650-nucleotide poly(A)-containing [poly(A)$^+$] RNA that was about 50 times more abundant in *MAT*a cells that had been exposed to the peptide pheromone α-factor than in untreated cells. This RNA was transcribed from a cluster of repetitive sequences: both intact and truncated delta and sigma elements adjacent to a tRNA^{Trp} gene. Strand-specific probes indicated that this RNA initiated within an intact sigma element and contained sigma sequences at its 5′ end. *MAT*a cells produced two other prominent poly(A)$^+$ RNAs (500 and 5,300 bases) in response to α-factor that were homologous to the same strand of sigma but transcribed from other locations in the genome. Induction of the sigma-related transcripts was rapid, was not blocked by inhibition of protein synthesis, required a functional receptor (*STE2* gene product), and hence appeared to be a primary response to pheromone. Pulse-labeling confirmed that accumulation of sigma RNA following α-factor administration was accounted for by an increase in its rate of transcription. The sigma RNAs also were induced in *MAT*α cells that had been treated with a-factor, but were not present at significant levels in *MAT*a/*MAT*α diploids. In *MAT*a cells transformed with a plasmid in which the λScG7 sigma element was inserted just upstream of a gene coding for the intracellular form of invertase (*SUC2*) lacking its own promoter, a new poly(A)$^+$ RNA (2.2 kilobases) appeared in response to α-factor that hybridized to both sigma and *SUC2* probes, and intracellular invertase activity was elevated about 10-fold within 30 min. Primer extension showed that transcription from the hybrid gene initiated exclusively within the sigma sequence (117 nucleotides from the 3′ end of the element).

Regulation of cellular physiology and development in multicellular organisms is a complex process that often involves modulation of gene expression by peptides, growth factors, and other hormones (35, 41, 47). In the unicellular eucaryote *Saccharomyces cerevisiae*, mating between haploids of opposite cell type (*MAT*a cells and *MAT*α cells) is initiated by their exposure to mating pheromones. The mating pheromones are oligopeptides (*MAT*α cells secrete α-factor and *MAT*a cells produce a-factor) that elicit specific physiological changes in their target cells (including arrest of the cell cycle in the G1 phase, synthesis of cell surface agglutinins, and characteristic changes in cell shape) and are required for the mating response (for a review, see references 39 and 44). In addition to the genes encoding the pheromone precursors and the *MAT* locus which regulates their expression, other genetic loci have been identified that are required for mating competence. Two of these genes, *STE2* and *STE3*, probably encode cell surface receptors for α-factor and a-factor, respectively (27, 29).

We and others have demonstrated that the events of the mating process are preceded by rapid and dramatic changes in gene expression (28, 40). We report here our discovery that transcripts from a known repetitive element, sigma, are prominent among the sequences we previously identified as displaying a pronounced induction upon treatment of *MAT*a cells with α-factor. We show that α-factor increases the abundance of the sigma-related transcripts by elevating their rate of transcription and that a sigma element can drive the functional expression of a heterologous gene in a hormone-dependent manner. We conclude that at least a subset of the 25 to 30 sigma elements in the yeast genome (5) function as hormone-responsive promoters that permit expression of distal sequences, some of which may play a role in the mating process or in transposition of the sigma element.

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MATERIALS AND METHODS

Plasmid constructions. The 634-base-pair (bp) *Xho*I-*Xho*I fragment of λScG7 was inserted in either orientation into the M13 vector mp8 (33), yielding subclones XX634A and XX634B. The single-stranded form of XX634A contains the nontemplate strand of the sigma-650 element; XX634B contains the template strand. The 825-bp *Xho*II-*Bgl*II fragment of XX634A (containing yeast and M13 sequences) was inserted between the *Bam*HI and *Bgl*II sites of mp8 to yield XX158A. The yeast DNA sequences in XX158A correspond to the 158-bp *Xho*II-*Xho*I-B segment (Fig. 1). The insert in XX158A was excised by digestion with *Eco*RI and *Hin*dIII and inserted between the *Eco*RI and *Hin*dIII sites of mp9, yielding XX158B. The *Pst*I-*Hin*cII fragment of λScG7 was inserted into the vector pUC9 (33), yielding pGS75. The insert in pGS75 was excised by cleavage with *Eco*RI and *Xho*I and inserted between the *Eco*RI and *Sal*I sites of mp8 and mp9 to produce XH1000A and XH1000B, respectively. (The insert in XH1000A and B corresponds to the 1-kilobase [kb] *Hin*cII-*Xho*I-B segment; the single stranded form of XH1000B contains the nontemplate strand of the sigma-650 element.) The insert in pGS75 was cleaved with *Hin*dIII and *Xho*I and inserted between the *Hin*dIII and *Sal*I sites of mp9, yielding PX900. The 776-bp *Hin*dIII-*Bam*HI fragment of the yeast invertase gene (*SUC2*) (42) was inserted into mp19 (48) to create HB776.

The 634-bp *Xho*I-*Xho*I fragment of λScG7 was inserted in either orientation into the *Sal*I site of pSEY303 (18), yielding plasmids pSV17 and pSV19. In pSV17, the sigma-650 ele-

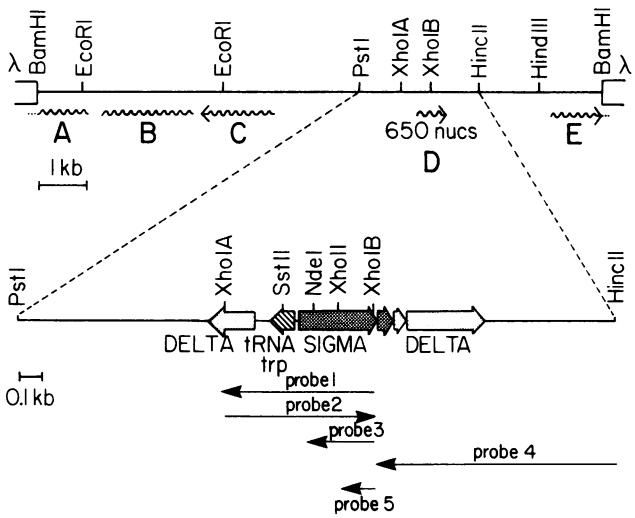

FIG. 1. Structure and transcription map of recombinant phage λScG7. Wavy arrows indicate the position of transcription units, as determined by Northern blot analyses with DNA subclones of the insert used as probes (see text for details). Synthesis of the 650-base transcript (D) is induced in haploid cells by mating pheromones. The lower restriction endonuclease map shows the location of repetitive sequence elements and the $tRNA^{Trp}$ gene contained within the *PstI-HincII* segment of the insert. Arrows at the bottom correspond to the single-stranded uniformly labeled DNA probes (see text) used in the majority of the experiments. An arrowhead denotes the 3′ end of a probe or transcript. nucs, Nucleotides.

ment (lacking 15 bp to the right of the *XhoI*-B site) is separated from the coding region of the *SUC2* gene by 8 bp of polylinker DNA. The *SUC2* sequence present lacks the promoter and first three codons of the secreted form of invertase, but contains the initiator methionine for the internal form of invertase, the remainder of the coding region, and the *SUC2* terminator. The 1.8-kb *BamHI-KpnI* fragment of pSV17 was subcloned into mp19 to produce BK1800.

DNA sequence analysis. M13 subclones containing DNA fragments of λScG7 (XX634A, XX634B, XH1000A, and PX900) were sequenced by the dideoxy chain termination method of Sanger et al. (38).

Preparation of hybridization probes. Single-stranded uniformly labeled DNA probes were prepared by the method of Ley et al. (32). M13 subclones were labeled by primer extension with [α-^{32}P]dATP and then digested with a restriction enzyme that cleaved 3′ to the labeled insert DNA. The unique single-stranded fragments so generated were purified by electrophoresis in 5% acrylamide gels containing 7 M urea, extracted from the gel by incubation overnight at 37°C in 2 ml of 0.5 M ammonium acetate–1 mM EDTA (pH 8.0), and concentrated by ethanol precipitation.

Subclones XX634A and XX634B were cut with *Eco*RI after primer extension, yielding probes 1 and 2, respectively (Fig. 1). Cleavage of primer-extended XX634A with *NdeI* yielded probe 3. A *SUC2* probe was made by primer extension of HB776 followed by cleavage with *Hin*dIII. Probes 4 and 5, synthesized by primer extension of XX158A and XH1000B, respectively, were not gel purified. A Ty probe was prepared by nick-translation of plasmid B301, which contains an intact Ty element, generously provided by B. Valent and G. R. Fink.

Preparation and electrophoretic analysis of RNA. Total RNA was isolated from cells grown in the absence or presence of synthetic α-factor (2.4 μM for wild-type cells and 0.24 μM for *sst1* mutants) (Serva Biochemicals or Peninsula Laboratories) by phenol extraction of cell lysates obtained by vigorous mixing in a Vortex mixer with glass beads (45). Poly(A)-containing [poly(A)$^+$] RNA was prepared by chromatography of total RNA samples on columns of oligo(dT)-cellulose (2).

Samples of poly(A)$^+$ RNA (5 μg) were denatured, fractionated by electrophoresis in 1.2 to 1.6% agarose gels containing 6% formaldehyde (30), and transferred to nitrocellulose or nylon filters by the method of Thomas (43). All filters were hybridized to labeled DNA probes (~10^5 cpm/ml) for about 18 h in a solution containing 5× SSPE, (1× SSPE is 0.126 M NaCl, 1 mM EDTA, 10 mM sodium phosphate, pH 6.5), 1% sodium Sarkosyl, and 100 μg of salmon sperm DNA (as carrier) per ml at 65°C. Blots were washed twice in 2× SSPE containing 0.1% sodium dodecyl sulfate for 15 min at 55°C.

Measurement of RNA synthesis in vivo. In two experiments, strain SEY2101 was grown to a cell density of 2 × 10^7/ml in SC medium containing uridine (82 μM) (instead of uracil) and buffered at pH 5 with 20 mM sodium succinate. Samples (2 ml) were exposed to α-factor at a final concentration of 2.4 μM for 10 min and then labeled with 300 to 500 μCi of [5,6-^{3}H]uridine for 15 min. Control cultures did not receive any pheromone. In two additional experiments, cells were grown to 2 × 10^7/ml as above, diluted to 7 × 10^6/ml in SC medium lacking any pyrimidine source, and grown to 1.4 × 10^7/ml prior to α-factor treatment and pulse-labeling. Total RNA was extracted by the method of Tuite et al. (45) and samples (5 to 40 μg, 0.5 × 10^6 to 1.7 × 10^6 cpm total, depending on the preparation) were hybridized for 24 h to either single-stranded M13mp9 DNA or single-stranded XX158B DNA (which corresponds to the template strand of sigma-650 in the M13mp9 vector) immobilized on nitrocellulose filter squares (1 cm; 5 to 15 μg of DNA per filter) essentially by the procedure of Zitomer et al. (49) except that alkali denaturation was not required. Hybridization and washing conditions were identical to those used for Northern blots, except that *Escherichia coli* tRNA (50 μg/ml) was used as the carrier. To calculate the fold increase in incorporation into sigma-specific RNA in each trial, the values obtained were normalized to take into account any difference in specific radioactivity between the RNA from pheromone-treated and control cells (see Table 1).

Measurement of invertase activity. Cultures (5 ml) of strain SEY2101 (*suc2-Δ9 ura3*), either untransformed or transformed with plasmid pSEY303, pSV17, or pSV19, were grown to mid-exponential phase (about 2 × 10^7/ml) in SC medium without uracil and buffered with 20 mM sodium succinate (pH 5), treated or not with cycloheximide at a final concentration of 15 μg/ml for 15 min, and finally exposed or not to α-factor at a final concentration of 2.4 μM for 30 min. The cells were then chilled on ice, harvested by centrifugation, washed two times in 3 ml of 10 mM NaN_3 and once in 0.1 M sodium acetate (pH 5.1), and frozen at −80°C for 30 min in glass tubes. The cell pellets were thawed and permeabilized in 0.1 M sodium acetate (pH 5.1) containing 0.1% Triton X-100 and assayed for invertase activity as described previously (17).

Primer expression for 5′-end mapping. The 22-base oligonucleotide complementary to the *SUC2* mRNA sequence (5′-GGCTGCAAAACCAGCCAAAAGG-3′) was assembled by an automated oligonucleotide synthesizer (Applied Biosystems). Primer extension by reverse transcriptase was performed by minor modifications of the method of Elion and Warner (16), using 20 μg of poly(A)$^+$ RNA per reaction.

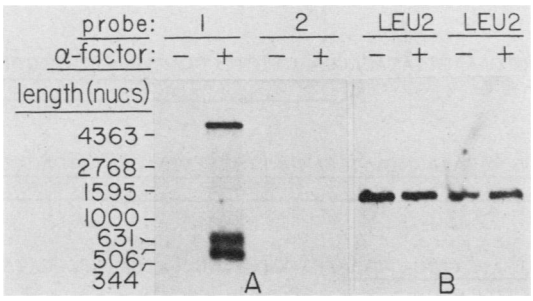

FIG. 2. One strand of the *Xho*I-*Xho*I fragment is homologous to three major poly(A)$^+$ RNAs induced by α-factor in *MAT***a** cells. Strain RC634 (*MAT***a** *sst1*) was grown to a cell density of 5×10^7/ml in SC medium buffered at pH 5 with 20 mM sodium succinate. The culture was split, and one half was exposed to α-factor at a final concentration of 0.24 μM. After 30 min, poly(A)$^+$ RNA was isolated from both the untreated and α-factor-treated cells and analyzed by electrophoresis and blotting as described in the text. (A) Filters were hybridized to probes (1 and 2) corresponding to either strand of the *Xho*I-*Xho*I fragment (see Fig. 1). (B) Same filters stripped in boiling water and rehybridized to a single-stranded probe (*Kpn*I-*Eco*RI) specific for the *LEU2* gene (1) (generous gift of R. Freedman). Length standards were a mixture of three separate digests (*Eco*RI, *Acc*I, and *Hin*fI-*Eco*RI) of pBR322 denatured by formaldehyde treatment. nucs, Nucleotides.

The cDNA products were fractionated on an 8% polyacrylamide gel in the presence of 7 M urea and compared with the products of a standard nucleotide sequencing reaction.

Computer analysis of DNA sequence. The BIONET programs maintained by Intelligenetics, Inc., were used for search and analysis of nucleotide sequences.

RESULTS

Mapping of hormone-induced transcripts. We previously isolated a recombinant phage, λScG7, that carries a segment of the yeast genome which hybridized to three prominent α-factor-induced transcripts and to four transcripts whose abundance in *MAT***a** cells was unaffected by α-factor (40). To map the location of each transcription unit within the 14-kb insert (Fig. 1), portions of the insert generated by restriction endonuclease digestion were subcloned and used as hybridization probes to analyze poly(A)$^+$ RNAs that had been separated by agarose gel electrophoresis and transferred to nitrocellulose paper (Northern blots). The four transcripts unaffected by α-factor (A, B, C, and E in Fig. 1) were localized to the left and right ends of the insert (data not shown); in contrast, the central region of the insert hybridized to three major α-factor-inducible RNAs of 500, 650, and 5,300 bases, respectively (Fig. 2). However, use of strand-specific probes derived from subclones of the insert in M13 vectors (see Fig. 1) demonstrated that only the 650-base transcript (D in Fig. 1) was actually encoded in the insert. All three α-factor-regulated RNAs hybridized to the same strand of the *Xho*I-*Xho*I fragment (probe 1) (Fig. 2), the *Nde*I-*Xho*I fragment (probe 3) (see Fig. 8), and the *Xho*II-*Xho*I fragment (probe 5) (Fig. 3), but only the 650-base RNA hybridized to the *Xho*I-*Hin*cII segment of the insert (probe 4) (Fig. 3). The *Xho*I-*Hin*cII probe also hybridized to Ty RNA, which was not induced by α-factor, because this region contained a full-length delta element, as will be described below. (None of the induced RNAs hybridized to restriction fragments located to the left of the *Xho*I-A site or to the right of the *Hin*cII site.) Hence, we conclude that the cloned DNA actually encoded only one of the three α-factor-induced RNAs, but had significant homology with the two other transcripts, which must be encoded elsewhere in the yeast genome.

Pheromone-induced RNA is transcribed from a cluster of repetitive elements. When probes from the central region of the insert in λScG7, for example the *Xho*I-*Xho*I fragment (probe 1, Fig. 1), were hybridized to blots of yeast DNA digested with a variety of restriction enzymes, a large number of bands (≥30) were observed, suggesting that this portion of the insert contained DNA sequences present at multiple positions in the yeast genome. To identify the nature of these repeated sequences, we determined the nucleotide sequence of 1,255 bp of this region (from left of the *Xho*I-A site to right of the *Hin*cII site) (Fig. 4). Search of the sequence by computer revealed homologies to known repetitive elements of yeast, namely both complete and partial copies of delta and sigma elements, and a tRNA^{Trp} gene (Fig. 1 and 4).

Normally, delta elements are the 333- to 337-bp direct repeats that flank the 5.3-kb retroviruslike transposon Ty (11, 24); however, so-called "solo" delta elements, which presumably arise from excision of Ty elements by recombination between its terminal delta repeats, are also found (36, 46). In the λScG7 insert, delta 1 was a truncated solo delta which has diverged considerably from Ty-associated deltas (72% homology to the 205-bp region, from nucleotides 110 to 314, of the delta elements flanking Ty912 [11]), and its transcriptional orientation was opposite that of the α-factor-induced 650-base RNA. The delta 2 fragment was a 52-bp sequence that was homologous to the first 52 bp of the 5′ end of other delta elements. It immediately abutted the 5′ end of a full-length (333-bp) element, delta 3. Delta 3 was more related to the delta elements flanking TyB10 (23) than to the delta elements flanking Ty912 (88 versus 77% homology). Both delta 2 and delta 3 were in the same transcriptional orientation as the 650-base α-factor-regulated poly(A)$^+$ RNA.

A complete sigma element (sigma 1), followed by a partial repeat of 67 bp that was 88% homologous to its 5′ end (sigma 2), was located between delta 1 and delta 2 (Fig. 1 and 4).

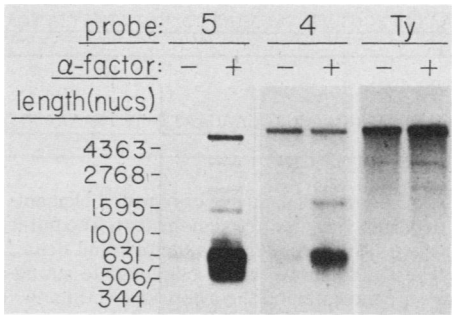

FIG. 3. All three α-factor-inducible poly(A)$^+$ RNAs contain homology to the repetitive element sigma. Poly(A)$^+$ RNA from α-factor-treated RC634 cells and an untreated control culture was isolated and subjected to gel electrophoresis and blotting as described in the legend to Fig. 2 and in the text. Three identical filters from the same gel were hybridized separately to probe 5 (single-stranded *Xho*II-*Xho*I-B segment containing only sigma sequences), probe 4 (single-stranded *Xho*I-B-*Hin*cII segment containing primarily delta sequences), and a Ty-specific probe (nick-translated plasmid B301) (see the text). Length markers are the same as in Fig. 2. Longer exposures showed that the Ty probe hybridized to the 650-base α-factor-induced RNA.

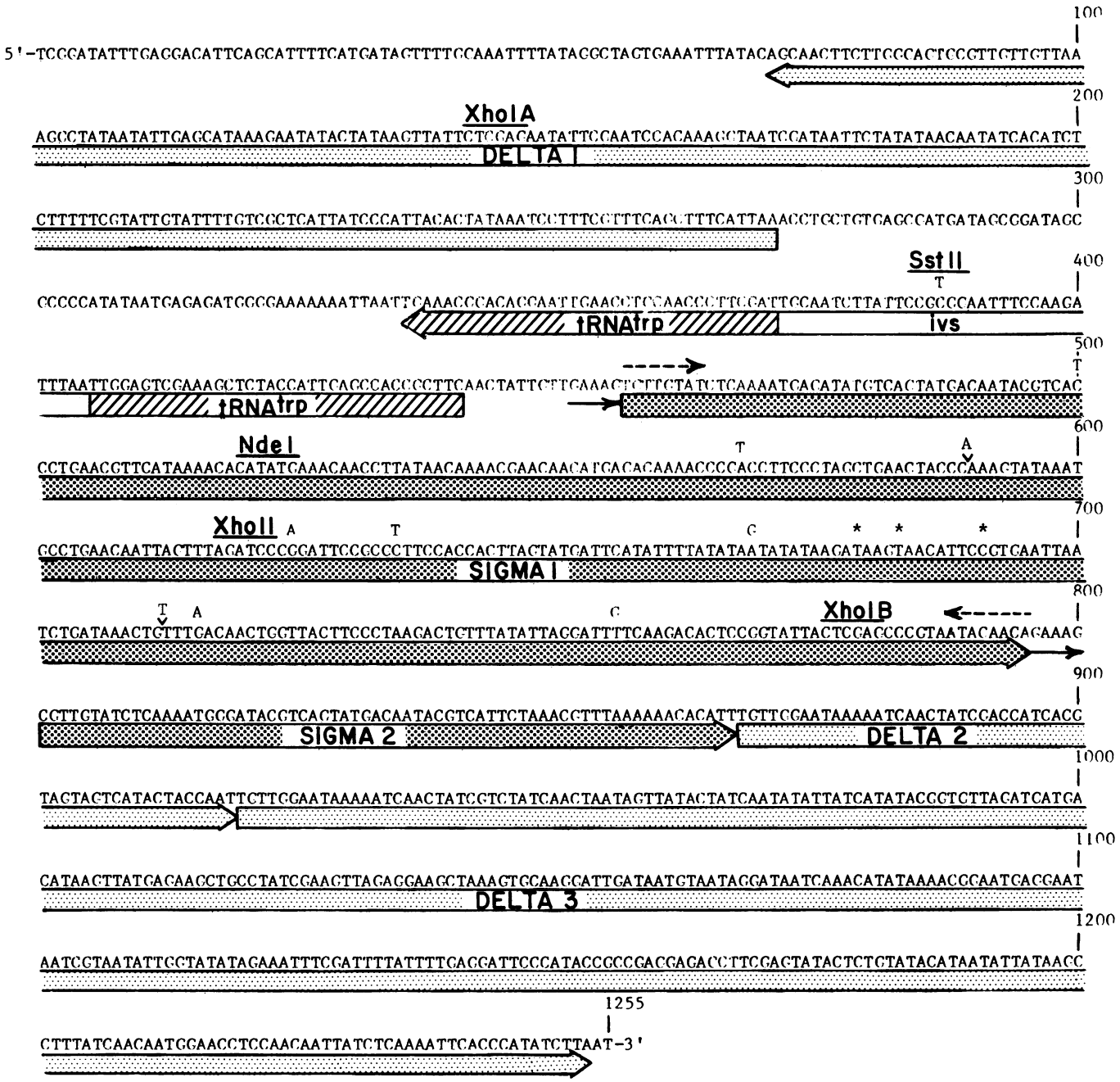

FIG. 4. λScG7 contains a cluster of repeated elements and a $tRNA^{Trp}$ gene. The nucleotide sequence of a 1,255-bp region of the insert in λScG7 was determined as described in the text. The full-length sigma-650 element (sigma 1) and a partial repeat (sigma 2), one full-length delta (delta 3) and two truncated elements (delta 1 and delta 2), and a $tRNA^{Trp}$ gene are indicated by stippled and hatched arrows beneath the sequences. The strand shown is the nontemplate strand and hence corresponds to the sequence found in the sigma-650 transcript, whose initiation sites are indicated by the asterisks (see Fig. 9). The 5-bp direct repeats that flank the intact sigma element are indicated by the thin arrows beneath the sequence; the 8-bp terminal inverted repeats of the sigma element are indicated by the dashed arrows above the sequence. Where the sequence of the sigma element located upstream of a $tRNA^{Lys}$ gene reported by del Rey et al. (15) differs by either base substitutions or insertions from the sequence of the sigma-650 element, it is indicated above the sequence. The single difference (in the intervening sequence [ivs]) between the $tRNA^{Trp}$ gene carried by λScG7 and that reported by Kang et al. (31) is indicated above the sequence.

Yeast sigma elements are repetitive DNA sequences of 340 to 341 bp that have many of the structural characteristics of eucaryotic transposable elements. First, sigma elements possess 8-bp inverted repeats at their ends that are homologous to the inverted repeats found at the ends of certain retroviruses and other transposable elements from a variety of different eucaryotic organisms (15). Second, sigma elements are flanked by 5-bp direct repeats of the target DNA sequence (37). Third, there are as many as 30 copies of sigma-related sequences in the yeast genome, and their genomic location varies from strain to strain (5). Another unusual feature of these elements is that all sigma sequences

isolated and analyzed at the nucleotide level to date have been situated 15 to 20 bp upstream of the 5′ end of a tRNA gene. The sigma element in λScG7 was no exception to these generalizations. It was 339 bp in length, had greater than 95% sequence homology with previously characterized sigma elements (15, 37), had 8-bp inverted repeats at its boundaries, was flanked by 5-bp direct repeats, and was situated 15 bp upstream of the 5′ end of a tRNA sequence with perfect homology to $tRNA^{Trp}$ (31) (Fig. 4). This $tRNA^{Trp}$ gene contained a 34-bp intervening sequence that differed at only one position from that reported for the $tRNA^{Trp}$ gene isolated by Kang et al. (31).

Pheromone-induced transcripts carry sequences homologous to sigma. Single-stranded probes that contained only sigma sequences, the 158-bp *Xho*II-*Xho*I-B fragment (probe 5, Fig. 1) and the larger *Nde*I-*Xho*I-B fragment (probe 3, Fig. 1), hybridized to the three prominent α-factor-induced poly(A)$^+$ RNAs (Fig. 3 and 8). Thus, these transcripts all contained sigma sequences in the same orientation. No probe to the left of the *Sst*II site (Fig. 1 and 4) hybridized to the RNAs (data not shown). Hence, at least for the 650-bp RNA encoded by λScG7, transcription is most likely initiated within the sigma sequence itself, and therefore the sigma-related sequences must be located at or very near the 5′ end of this RNA molecule.

The same three sigma-related poly(A)$^+$ RNAs were induced by α-factor in every *S. cerevisiae MAT***a** haploid tested, including X2180-1A, 381G, RC634, and SEY2101. We also observed, however, a variable number of other bands that hybridized more faintly to the sigma probes, which may reflect either their lower abundance or their weaker homology to the λScG7 sigma. The same three prominent sigma-related transcripts were found to be induced when poly(A)$^+$ RNAs from *MAT*α cells treated with a preparation of purified **a**-factor were examined by Northern analysis (data not shown). The sigma-related species were not present at detectable levels in poly(A)$^+$ RNA prepared from vegetatively growing *MAT***a**/*MAT*α diploid cells (data not shown). Thus, expression of the three major sigma-related transcripts appears to be haploid specific, as well as pheromone inducible.

Basal level of sigma transcription is very low. To ensure that the α-factor concentration remained constant during the period of pheromone treatment, in most experiments *MAT***a** strains carrying a mutation in the *SST1* gene, which encodes a protease responsible for extracellular degradation of α-factor (10; E. M. Ciejek, Ph.D. thesis, University of California, Berkeley, 1980), were used. *MAT***a** *sst1* cells are about 20 times more sensitive to α-factor than normal *MAT***a** cells (7, 8). We observed that in the absence of added α-factor, *MAT***a** *sst1* cells generally had a noticeably higher basal level of the sigma-related transcripts than *MAT***a** *SST1*$^+$ cells (Fig. 5). An even higher basal level of the sigma-related transcripts was observed in *MAT***a** cells carrying a mutation in the *SST2* gene, which confers an even greater degree of hypersensitivity to the action of α-factor (7, 8) (data not shown). We reasoned that these supersensitive mutants might display a higher basal level of sigma expression because they might be able to respond to a small amount of α-factor produced during growth by a small fraction of *MAT*α cells in the culture that arose from the low frequency ($\sim 10^{-6}$ per generation) of heterothallic mating type switching. To test this hypothesis, the abundance of the sigma-related RNAs, in the absence and presence of pheromone, was examined in a *MAT***a** strain (K624) incapable of undergoing mating type interconversion due to deletion of *HML* and *HMR*. The three prominent pheromone-induced sigma-related transcripts were virtually undetectable in poly(A)$^+$ RNA prepared from a culture of K624 prior to α-factor addition (Fig. 5). The same result was obtained when poly(A)$^+$ RNA from a *MAT***a** *HML***a** *HMR***a** *sst2* strain, which can never convert to the *MAT*α cell type, was analyzed before and after α-factor treatment (A. Axt and J. Thorner, unpublished results). (Interestingly, the abundance of those particular minor sigma-related RNAs that were not regulated by α-factor was also not affected by the inability of cells to switch mating type.) Thus, at least for the sigma-related transcripts under pheromone control, their apparent basal level seems to be attributable to autoinduction by other cells in the population that have undergone a mating type interconversion; hence, the true basal level of transcription of these sequences must be extremely low.

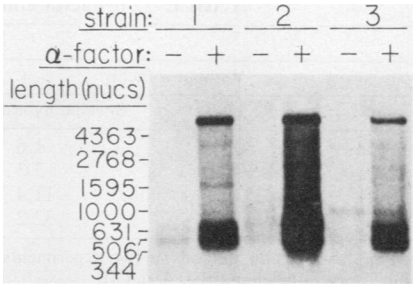

FIG. 5. Basal level of sigma transcripts is highest in mutants supersensitive to α-factor and lowest in mutants unable to switch mating type. RC634 (*MAT***a** *sst1-3 HML*α *HMR***a**) (strain 1), 381G (*MAT***a** *SST*$^+$ *HML*α *HMR***a**) (strain 2), and K624 (*MAT***a** *SST*$^+$ *hml*α-*Δ2 hmr***a**-*Δ4*) (strain 3) were grown to a cell density of 5×10^7/ml in SC medium buffered with sodium succinate (pH 5) (at 30°C for RC634 and 381G and 25°C for K624). A portion of each culture was then treated with α-factor (RC634, 0.3 μM for 45 min; 381G and K624, 2.5 μM for 30 min), and the remainder of each culture was left untreated. Poly(A)$^+$ RNA was isolated and analyzed by electrophoresis and blotting as described in the legend to Fig. 2 and in the text. The filters were hybridized to a sigma-specific probe (probe 3, Fig. 1). Length standards were as in Fig. 2.

Genes required for other physiological responses to α-factor are also required for induction of the sigma-related RNAs. A number of genes have been identified that are required for pheromone responsiveness. One gene, *STE2*, is transcribed only in *MAT***a** cells (34) and encodes the cell surface receptor for α-factor or an essential component thereof (29). Other genes, including *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*, are essential for mating competence, at least in part because they are required in either *MAT***a** or *MAT*α cells for full expression of cell type-specific genes such as *STE2*, essential for pheromone response (21; R. Freedman, S. Van Arsdell, and J. Thorner, manuscript in preparation).

Northern analysis of poly(A)$^+$ RNA demonstrated that the three major sigma-related RNAs were not induced by α-factor in a strain (JE105A *ste2*::*LEU2*) lacking a functional *STE2* product due to deletion of the gene (Fig. 6). Similar experiments with a strain (381G-50B) carrying a temperature-sensitive allele (*ste2-3*) showed that the sigma-related RNAs were induced by α-factor at the permissive temperature (25°C) but not at the restrictive temperature (37°C) (data not shown). The same kind of experiments demonstrated that a functional *STE5* gene is also required for induction of the sigma-related RNAs by α-factor (Freedman et al., manuscript in preparation).

Pheromone treatment increases the rate of transcription of

TABLE 1. α-Factor stimulates the rate of transcription of sigma-related RNA

Trial[a]	RNA bound (cpm/filter)						Increase[b] (fold)
	Without α-factor			With α-factor			
	mp9	XX158B	Specific hybridization	mp9	XX158B	Specific hybridization	
1	29.2	30.8	1.6	66.0	446.6	380.6	238
2	14.1	17.4	3.3	16.3	105.4	89.1	32
3	242.1	253.5	11.4	135.6	573.9	438.3	87
4	119.7	151.9	32.2	569.0	939.6	370.6	26

[a] As described in Materials and Methods, in two experiments cells were labeled in uridine-containing medium (trials 1 and 2) and in two experiments cells were starved for uridine prior to labeling (trials 3 and 4).

[b] Normalized to specific radioactivity of RNA sample (trial 1, 2×10^4 cpm/μg with and without α-factor; trial 2, 5×10^3 cpm/μg with α-factor, 6×10^3 cpm/μg without α-factor; trials 3 and 4, 4×10^5 cpm/μg with α-factor, 9×10^5 cpm/μg without α-factor).

the sigma-related RNAs. The previous results (Fig. 2, 3, 5, and 6) clearly demonstrated that exposure to α-factor increased the steady-state level of three prominent sigma-related poly(A)$^+$ RNAs. The accumulation of these species could be due either to an increase in their rate of synthesis or to a decrease in their rate of degradation. To distinguish between these alternatives, the total rate of transcription of this family of RNAs in the absence and presence of pheromone was measured. Strain SEY2101 in mid-exponential phase was either treated with α-factor for 10 min or not treated and then labeled with [^{3}H]uridine for 15 min. Total RNA was extracted and hybridized either to excess single-stranded M13mp9 DNA or to excess single-stranded XX158B DNA (containing the template strand of the *Xho*II-*Xho*I-B fragment of λScG7 inserted into M13mp9) that had been immobilized on nitrocellulose filters. In each of four separate trials (Table 1), the amount of sigma-specific radioactivity increased dramatically after exposure of the cells to α-factor. These data reflect an increase in the rate of transcription of these RNA species of 50- to 80-fold, assuming that the half-life of the sigma-related RNAs is, like that of most yeast poly(A)$^+$ mRNAs (13), in the same range as the labeling time of 15 min. The actual increase in transcription rate is probably even higher since, as described above, the true basal rate of transcription is difficult to measure. It is important to note, however, that these measurements reflect the total increase in a heterogeneous population of RNA species and not the induction of a single gene. Densitometric scanning of the regions of autoradiograms corresponding to the three major sigma-related RNAs indicated that each individual species was induced at least 20- to 50-fold within 30 min. Therefore, the apparent accumulation of these RNAs after pheromone treatment can be completely accounted for by an increase in their rate of synthesis.

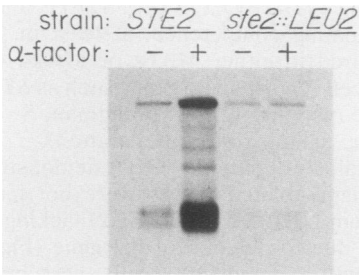

FIG. 6. Deletion of the α-factor receptor gene prevents induction of the sigma transcripts by α-factor. Strain RK511-7C (*MAT***a** STE2$^+$ sst1) and an isogenic receptor-deficient derivative, JE105A (*MAT***a** *ste2*::*LEU2 sst1*), were grown as described in the legend to Fig. 2, and a portion was treated with α-factor at a final concentration of 2.4 μM. Poly(A)$^+$ RNA was isolated from the pheromone-treated cells and from the controls and analyzed by electrophoresis, blotting, and hybridization with a sigma-specific probe (probe 3, Fig. 1). Length markers were as in Fig. 2.

Pheromone induction of a sigma-*SUC2* gene fusion. To determine what sequences are responsible for pheromone induction, the *Xho*I-*Xho*I fragment containing the sigma element (sigma-650) from the genomic region carried by λScG7 was excised and inserted in either orientation at the 5′ end of a *SUC2* gene lacking its own promoter region (42) in a plasmid vector (Fig. 7). Strain SEY2101, which carries a complete deletion of the chromosomal *SUC2* locus (17), was transformed with the parent plasmid and these two constructions. In cells containing the construction in which the sigma-650 element was in the same transcriptional orientation as the *SUC2* gene (pSV17), a new prominent poly(A)$^+$ RNA which hybridized to both sigma-specific and *SUC2*-specific probes (but did not hybridize to pBR322 DNA) appeared in response to α-factor treatment (Fig. 8). This species was not detectable in cells transformed with the parent plasmid (pSEY303) or with the construction containing sigma-650 in the reverse orientation (pSV19). The length of this transcript (2.2 kb) was completely consistent with that expected for a molecule initiating within the sigma-650 sequence and terminating at the normal *SUC2* terminator. In cells containing the construction in which sigma-650 was in the opposite transcriptional orientation from the *SUC2* gene, an α-factor-inducible 1.4-kb poly(A)$^+$ RNA that hybridized to sigma-specific and pBR322 probes but not to the *SUC2* probe was observed. This species most likely arose from initiation within the sigma-650 sequence and transcription through and termination in adjacent pBR322 sequences. These results demonstrate that the promoter for the sigma-650 element, and whatever other *cis*-acting regulatory sequences are required for pheromone induction, are contained within the *Xho*I-*Xho*I fragment. Furthermore, these signals are highly directional and are able to function on a multicopy plasmid as well as they do in single copy at their normal chromosomal locus.

A transcript (4 kb) that hybridized to the *SUC2* and pBR322 probes and another (1.9 kb) that hybridized only to the pBR322 probe were detected in cells transformed with pSEY303; however, neither of these transcripts was regulated by α-factor (Fig. 8). Because untransformed SEY2101 cells do not contain any poly(A)$^+$ RNAs homologous to *SUC2* or pBR322 (data not shown), these two species must be transcribed from vector sequences that fortuitously function as promoters in yeast. A region near the 3′ end of the *bla* gene of pBR322 is known to promote reasonably efficient transcription in the opposite direction in yeast (R. W. Davis, personal communication). Hence, the 4-kb transcript could

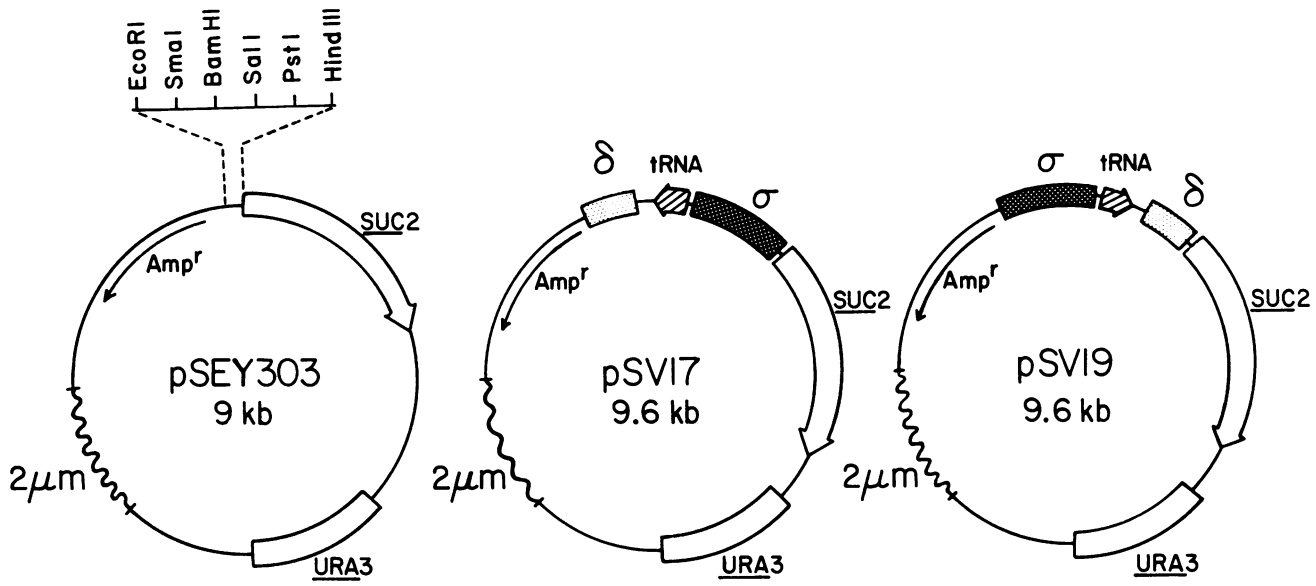

FIG. 7. Construction of a sigma-650-*SUC2* gene fusion. The 634-bp *Xho*I-*Xho*I fragment of λScG7 (Fig. 1) was inserted in both orientations into the *Sal*I site of the yeast-*E. coli* shuttle vector pSEY303 (18) to produce plasmids pSV17 and pSV19. The ampicillin-resistance (Amp^r), invertase (*SUC2*), and *URA3* genes are indicated. tRNA, tRNA^{Trp} gene; δ, delta sequence; σ, sigma sequence; and 2μm, origin of replication of the yeast 2μm plasmid.

arise from initiation at this site, transcription through the polylinker sequence, and termination at the *SUC2* terminator. Cells transformed with pSV17 and pSV19 lacked the 4-kb transcript but contained transcripts (~2 kb) homologous to just pBR322, presumably because insertion of the *Xho*I-*Xho*I fragment prevents readthrough of the larger transcript.

Transcription of the sigma-*SUC2* gene fusion initiates within the sigma element. Because the sigma-*SUC2* hybrid transcript possessed the unique *SUC2* sequence, it was possible to use primer extension of a synthetic oligonucleotide complementary to the *SUC2* sequence to determine the length of the sigma extension and to map precisely the 5′ end of the hybrid transcript. A 22-base oligonucleotide (5′-GGCTGCAAAACCAGCCAAAAGG-3′) complementary to the region of the *SUC2* gene located from 13 to 34 nucleotides downstream of the junction between *SUC2* and sigma-650 was synthesized, purified, and labeled at its 5′ end with [γ-^{32}P]ATP. The oligonucleotide was hybridized to poly(A)$^+$ RNA isolated from SEY2101 cells that had been transformed with pSV17 and treated with α-factor and used as a primer for synthesis of complementary DNA by reverse transcriptase. The extension products were fractionated by electrophoresis on a urea-polyacrylamide gel and compared directly with the products of standard nucleotide sequencing reactions. A major cDNA extension product of 146 nucleotides, and minor products of 142 and 134 nucleotides, were observed (Fig. 9). cDNAs of identical size were detectable in the products of RNA from control cells not treated with α-factor, but were at least 20 times less abundant, as estimated from densitometric scanning of the autoradiograms. Thus, pheromone induction does not affect the site of transcription initiation.

All three transcription initiation sites lay within the sigma-650 sequence. The two minor shorter cDNA species may be the result of premature termination by reverse transcriptase

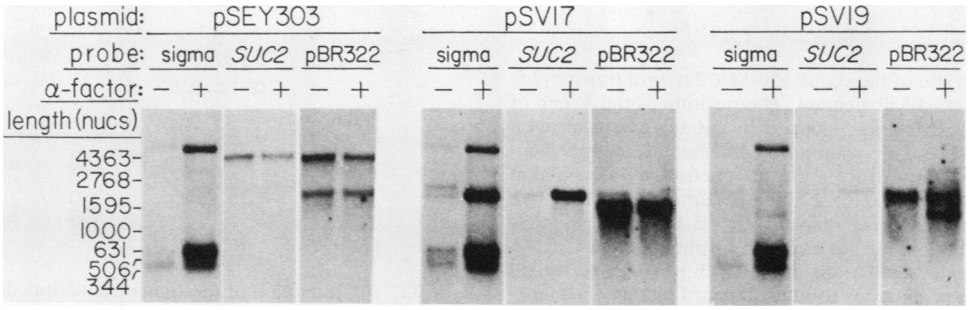

FIG. 8. Transcription of the *SUC2* gene is inducible by α-factor in *MAT***a** cells transformed with a plasmid carrying the sigma-650-*SUC2* gene fusion. Strain SEY2101 was transformed with plasmids SEY303, pSV17, and pSV19. Transformants were grown in SC lacking uracil as described in the legend to Fig. 2. A portion of each culture was exposed to α-factor at a final concentration of 2.4 μM. After 30 min, poly(A)$^+$ RNA was isolated from the α-factor-treated samples and from the untreated controls and subjected to electrophoresis and blotting as described in the text. Identical filters for each of the three strains were hybridized to three different DNA probes: sigma, probe 3 (Fig. 1); *SUC2*, primer-extended HB776 cleaved with *Hin*dIII (see the text); and pBR322, labeled by nick-translation. Length standards were as in Fig. 2.

TABLE 2. Pheromone induction of invertase expression from a sigma-650-*SUC2* gene fusion

Plasmid	Avg enzyme activity (nmol of glucose formed/min per A_{540} unit of cells) ± SD[a] (no. of expt)			
	Without cycloheximide		With cycloheximide	
	Without α-factor	With α-factor	Without α-factor	With α-factor
None	0.2 (1)	0.2 (1)	NT[b]	NT
pSEY303	1.3 ± 1.0 (2)	1.7 ± 0.7 (2)	NT	NT
pSV17	9.7 ± 3.4 (5)	76.7 ± 22.9 (6)	7.5 ± 0.1 (2)	7.5 ± 0.8 (2)
pSV19	13.0 ± 12.0 (2)	15.0 ± 11.0 (2)	NT	NT

[a] Values given represent the average and standard deviation for the number of independent experiments, each performed in duplicate, indicated in parentheses.
[b] NT, Not tested.

or could reflect the existence of multiple initiation sites. The major initiation site corresponded to nucleotide 223 of the sigma element (Fig. 4), if the element is numbered from the end closest to the $tRNA^{Trp}$ gene, and was 98 bp upstream of the *Xho*I-B site where the sigma element was fused to the *SUC2* sequence. If this transcription initiation site is designated +1, TATA-like sequences are found within the sigma element at positions −16 to −3 (TATATAATATATAA) and −85 to −80 (TATAAA).

Invertase activity is pheromone inducible in cells containing the sigma-*SUC2* gene fusion. Based on the results of the primer extension analysis, the first translational initiation codon in the transcript from the sigma-*SUC2* hybrid gene is the AUG that specifies the first methionine of the internal form of invertase (6). To determine whether the hybrid transcript was competent to produce functional invertase, SEY2101 cells transformed with pSV17 were grown to mid-exponential phase, treated with α-factor or left untreated, permeabilized, and assayed for invertase activity. Invertase activity was induced 5- to 10-fold within 30 min of being exposed to α-factor in *MAT***a** cells carrying pSV17 (Table 2). No elevation of invertase activity was observed in response to α-factor treatment in the same cells transformed with the parent *SUC2* vector lacking the sigma sequence (pSEY303) or with the plasmid in which the sigma element was inserted in the opposite orientation (pSV19). Hence, the α-factor-regulated promoter contained in the sigma-650 sequence was capable of driving the expression of a heterologous gene.

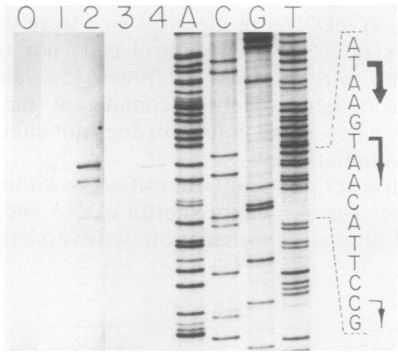

FIG. 9. Initiation site of the sigma-650-*SUC2* hybrid transcript is located within the sigma-650 element. The position of the 5′ end of the sigma-650-*SUC2* RNA was mapped by primer extension with reverse transcriptase and a synthetic oligonucleotide complementary to the *SUC2* sequence near the fusion junction, as described in detail in the text. Lanes: 0, no RNA added (control); 1 and 2, longer exposures of lanes 3 and 4, respectively; 3, RNA from cells transformed with pSV17 and not exposed to pheromone; 4, RNA from pheromone-treated cells transformed with pSV17 (see legend to Fig. 8). The same primer was used in standard dideoxy sequencing reactions (A, C, T, and G) to sequence M13mp9 subclone BK1800 (which includes the junction region and the entire sigma-650 element) (see the text), and the products were subjected to electrophoresis in the same gel as the cDNA extension products to permit direct comparison. The DNA sequence presented is that corresponding to the transcript and hence is complementary to the sequencing ladder. The major site of transcription initiation is indicated by the heaviest arrow; the two minor sites are indicated by smaller arrows.

Pheromone induction of sigma-related transcripts does not require new protein synthesis. Induction of sigma-related RNAs in *MAT***a** cells is rapid, occurring within 10 to 15 min after exposure to α-factor (40), suggesting that de novo protein synthesis is not required. The availability of the sigma-*SUC2* construction made it possible to test rigorously whether pheromone induction of transcription requires new protein synthesis, because it provided an internal control (invertase synthesis) for gauging the effectiveness of the treatment used to inhibit translation. Strain SEY2101 transformed with pSV17 was grown to mid-exponential phase, and prior to the addition of α-factor, cycloheximide was added to a portion of the culture. Northern analysis clearly indicated that the three major sigma-related transcripts and plasmid-encoded sigma-*SUC2* hybrid transcript were induced even in the presence of cycloheximide (Fig. 10); however, no elevation in invertase activity was observed in these cells, confirming that protein synthesis had been effectively blocked by the drug treatment (Table 2). Thus, sigma induction by α-factor is not dependent on new protein synthesis and hence represents a primary response to the pheromone.

DISCUSSION

We have shown here that a family of pheromone-inducible poly(A)$^+$ RNAs contains sequences homologous to the re-

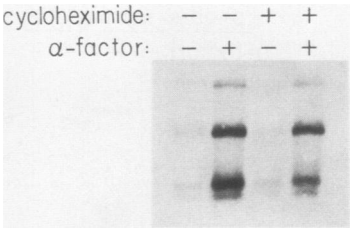

FIG. 10. Induction of the sigma transcripts does not require new protein synthesis. Strain SEY2101 transformed with plasmid pSV17 (Fig. 7) was grown as described in the legend to Fig. 8. A portion of the culture was treated with cycloheximide, and the remainder served as the control; then, one-half of each sample was treated with α-factor, all as described in the text. Poly(A)$^+$ RNA was prepared from the four samples, fractionated by electrophoresis, blotted to nitrocellulose, and hybridized to a sigma-specific probe (probe 3, Fig. 1).

petitive element sigma. We have demonstrated that transcription of one of these molecules initiates within a full-length sigma element (sigma-650) and hence carries sigma sequences at its 5′ end. Moreover, we have found that a gene not normally controlled by mating pheromones, the yeast invertase gene (*SUC2*), can be made α-factor-inducible by inserting the sigma-650 element in the proper orientation in place of the *SUC2* promoter. These data strongly suggest that at least a subset of the sigma elements in the genome represent directional pheromone-regulated promoters. Our results are the first demonstration that sigma elements are transcribed. Transcription of sigma elements in previous studies presumably went undetected because, as we have shown here, sigma-related poly(A)$^+$ RNAs are present in haploid cells at significant levels only after exposure to mating pheromones.

The sigma-650 element is embedded in a region of the genome that has an unusually complex structure. A $tRNA^{Trp}$ gene and a truncated, highly diverged delta element flank sigma-650 on its 5′ side; a partial repeat of a short segment of the 5′ portion of the sigma sequence and a partial and full-length delta element flank sigma-650 on its 3′ side. The 650-base transcript initiated in sigma-650 probably terminates in the most distal delta sequence.

If sigma elements represent hormone-inducible promoters, it is perhaps surprising that only three prominent sigma-containing poly(A)$^+$ RNAs were detected in our studies, since the yeast genome harbors approximately 30 sigma elements (5). One possibility is that only a small fraction of the sigma elements have retained the capacity to be hormone responsive, just as only a very few of the Ty elements in the yeast genome appear to be competent to catalyze their own transposition (22). Alternatively, most sigma elements may contain active pheromone-inducible promoters, but only sigma elements that are located proximal to functional terminators and poly(A)$^+$ addition sites would direct the synthesis of discrete, stable poly(A)$^+$ RNA species. The fact that longer exposures of Northern blots revealed numerous minor sigma-related bands, many of which were visible only after pheromone treatment, supports this latter view.

The function, if one exists, of sigma elements and the reason for their specific association with tRNA genes remain elusive. The finding that polymorphisms at the *SUP2* and *SUQ5* loci are due to the presence or absence of sigma elements provides the best evidence to date that sigma is tranposable (37). However, de novo transposition of sigma has not yet been observed. Some sigma elements could be the direct repeats flanking a larger transposable element, just as delta sequences flank Ty. However, clustering of sigma elements is rare (5); nonetheless, recent evidence indicates that there may be sigma-flanked retroviruslike elements in the yeast genome that encode a 5.2-kb transcript (S. Sandmeyer, personal communication). The 5.3-kb α-factor-induced sigma-containing RNA we have described here may correspond to the full-length transcript from such an element. Insertion of sigma elements 15 to 20 bp upstream of tRNA genes may reflect the preference of whatever transposition machinery is utilized by sigma elements for some structural feature of tRNA genes or the surrounding chromatin. Our findings, and the discovery of a cluster of delta, sigma, and tau elements upstream of a $tRNA_3^{Glu}$ gene (9, 25), suggest that if these short elements are capable of transposition, they tend to insert within or near each other. The association of all known sigma elements with tRNA genes has lead to speculation that sigma may play a role in the regulation of tRNA gene expression (15, 37); however, using a $tRNA^{Trp}$-specific probe, we observed no change in the level of $tRNA^{Trp}$ in total RNA from pheromone-treated and control cells (S. Van Arsdell, unpublished results). On the other hand, the number of $tRNA^{Trp}$ genes (≥4) in *S. cerevisiae* has not been definitively determined (26). The gene linked to sigma-650 may be responsible for only a minority of the $tRNA^{Trp}$ made in the cell, which would preclude detection of its hormone induction by Northern blots.

The biological role(s) of the three predominant sigma-related poly(A)$^+$ RNAs is unknown. Given their induction by pheromone, one reasonable supposition is that they encode functions involved in the mating process. At least one phenotypic trait of haploid cells that is highly pheromone inducible, and for which no gene(s) has yet been isolated, is their agglutinability (20). It is unlikely, however, that the 650-base sigma-related RNA encodes a polypeptide, because the sigma-650 element does not contain any open reading frame longer than 18 codons in the transcribed region. The only major sigma-related RNA large enough to encode a polypeptide of significant size is the 5,300-base transcript. We are currently trying to isolate the sequence encoding the 5,300-base sigma-containing RNA from a cDNA library that was enriched for sigma-related transcripts by hybrid selection of poly(A)$^+$ RNA from pheromone-treated cells with our cloned sigma-650 element.

Several genes required for mating are known to be induced by mating pheromones. Transcripts from the genes encoding both of the **a**-factor precursors, *MFa1* and *MFa2* (3), are significantly elevated in *MAT***a** cells exposed to α-factor (S. Van Arsdell, unpublished results). *STE2* mRNA also appears to be induced markedly in *MAT***a** cells by α-factor (34; S. Van Arsdell, unpublished results). Similarly, *STE3* mRNA is induced in *MAT***a** cells by **a**-factor (28). None of these genes is associated with a sigma element, however. On the other hand, we have preliminary evidence that these genes and the pheromone-responsive sigma-650 element have a common sequence motif that may be responsible for their hormonal regulation (S. Van Arsdell and J. Thorner, manuscript in preparation). As we have demonstrated here, the *cis*-acting transcriptional control signals required for α-factor regulation of sigma-650 expression were localized within the *Xho*I-*Xho*I fragment that contains over 222 bp of sigma sequence upstream of the primary transcription initiation site.

If the sigma-containing RNAs do not encode products with a role in mating, an alternative hypothesis is that they may be involved in transposition of the element. If so, it is possible that transposition of sigma to new locations in the genome is triggered in haploid cells by exposure to pheromones during mating. If specific sigma elements can be appropriately tagged and reinserted into the genome, this possibility can be tested directly.

A number of *cis*-acting regulatory sequences that respond to environmental and developmental signals and that are an integral part of other eucaryotic transposable elements have been identified in *S. cerevisiae* and other organisms. Ty elements contain an enhancer that can affect the expression of genes adjacent to the Ty insertion site and whose activity is dependent on the mating type of the cell (19). Expression of a transposable element (DIRS-1) in the slime mold *Dictyostelium discoideum* is induced during development and by stresses, including heat shock and high cell density, and its transcription initiates at a promoter located within the inverted terminal repeats of the element (12, 50). Integrated mouse mammary tumor virus DNA contains enhancer elements that are required for induction of viral gene expression

by glucocorticoid hormones in vivo and that are capable of binding purified glucocorticoid receptor protein in vitro (47). These examples and our finding that sigma elements contain a hormone-inducible promoter lend support to the view, first proposed by Britten and Davidson (4), that coordinate regulation of gene expression can be achieved by the interaction of *trans*-acting regulatory molecules with *cis*-acting repeated sequences flanking sets of unlinked genes (14). The location of regulatory sequences on potentially mobile genetic elements may facilitate the introduction of changes in established networks of coordinately regulated genes and thereby bring about evolutionarily significant alterations in regulatory patterns.

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