STE12, a protein involved in cell-type-specific transcription and signal transduction in yeast, is part of protein–DNA complexes

Beverly Errede¹ and Gustav Ammerer²

¹Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599 USA; ²Cell Biology Section, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, UK

The *STE12* gene of *Saccharomyces cerevisiae* is essential for the expression of genes required for mating, such as those involved in pheromone response, and for genes unrelated to mating but regulated by the presence of an adjacent copy of the transposable element Ty1. We show that the STE12 protein is a component of specific DNA–protein complexes that form with transcriptional control elements from Ty1 and the α -pheromone receptor gene *STE2*. Although a sequence involved in pheromone-dependent transcriptional activation is protected in both complexes, competition experiments indicate that the complexes are intrinsically different from each other. We show that another factor involved in cell-type-specific transcription, PRTF/GRM, is a component of the complex with the *STE2* fragment but not the Ty1 fragment. We propose that the *STE12* product interacts with different transcription factors in different sequence contexts and that PRTF/GRM is one of these factors.

[Key Words: DNA–protein interactions; yeast mating response pathway; gene expression; epitope-tagging]

Received April 26, 1989; revised version accepted June 16, 1989.

The yeast *Saccharomyces cerevisiae* provides a model system for the study of events controlling cell-type determination and cell communication. The *a* and α haploid cells of this yeast are able to mate with each other to form an *a*/ α diploid cell that is mating incompetent. Recognition between the *a* and α cell types initiates and coordinates events that are a precondition for cell fusion. This recognition is mediated by peptide pheromones *a*- and α -factor, which are characteristic of each cell type and their respective receptors (for review, see Cross et al. 1988). It appears that all components necessary for the response elicited by pheromone receptor binding are the same in both cell types (Bender and Sprague 1986; Nakayama et al. 1987). Both pheromone receptors are predicted to be structurally similar to vertebrate receptors that are coupled to classical G proteins such as the β -adrenergic receptor (Nakayama et al. 1985; Dixon et al. 1986). Moreover, three genes in *S. cerevisiae* (*GPA1/SCG1*, *STE4*, and *STE18*) were found to encode proteins homologous to the α -, β -, and γ -subunits of mammalian G proteins. Genetic evidence has established a role for these three gene products in the mating response pathway (Dietzel and Kurjan 1987; Miyajima et al. 1987; Jahng et al. 1988; Whiteway et al. 1989).

The signal generated by the interaction between receptor and mating pheromone causes arrest of the mitotic cell cycle in the *G*₁ phase, synthesis of cell-surface agglutinins, and morphological changes associated with mating. It also leads to transcriptional induction of several genes, including the receptor and pheromone structural genes (Strazdis and MacKay 1983; Nakayama et al. 1985; Hartig et al. 1986; Jarvis et al. 1988). One target sequence for the signal causing pheromone-inducible transcription has been identified. The sequence element TGAAACA appears in the promoter region of all pheromone-responsive genes and has been shown to be necessary for induction by pheromone (Kronstad et al. 1987; Van Arsdell and Thorner 1987). We refer to this sequence as a pheromone response element (PRE). A *trans*-acting factor that binds to the PRE has not been identified yet.

Genetic approaches to dissect the signal transduction pathway have led to the identification of several genes (*STE4*, *STE5*, *STE7*, *STE11*, *STE12*, and *STE18*) that are necessary for mating competence in both cell types and may specify, directly or indirectly, components of the signal pathway (Hartwell 1980; Nakayama et al. 1988; Whiteway et al. 1989). Mutations of these genes prevent mating and response to pheromone, including inducible gene expression (Hartwell 1980). In addition, these mutations reduce basal transcription of genes required for mating, suggesting that the integrity of the signal

¹Corresponding author.

²Present addresses: Institut f. Allgem. Biochemie, A1090 Vienna, Austria; Institute for Molecular Pathology, A1030 Vienna, Austria.

pathway is important for transcription even under non-inducing conditions (Hartig et al. 1986; McCaffrey et al. 1987; Fields et al. 1988). The transcriptional defects caused by these mutations also can be observed for transcription associated with transposable elements such as Ty1 and σ (Errede et al. 1981; Van Arsdell et al. 1987). Although these *STE* gene products are necessary for full expression of genes required for mating, not all of the components identified by this group are likely to be transcription factors. For example, the predicted STE4 and STE18 proteins are related to the β - and γ -subunits of mammalian G proteins, respectively, and the predicted STE7 and STE11 proteins are related to protein kinases (Teague et al. 1986; Whiteway et al. 1989; B. Errede, unpubl.).

The aim of studies presented here was to determine the function of the *STE12*-encoded protein. A *cis*-acting regulatory element within Ty1 that requires the *STE12* product for activation of the *CYC7* promoter was described recently (Company et al. 1988). In the same study it was found that formation of a DNA-protein complex with the Ty1 regulatory sequence was dependent on the presence of the *STE12* gene product. In this report we describe a *STE12*-dependent regulatory element from the promoter region of the α -pheromone re-

ceptor gene *STE2*. We show that formation of a DNA-protein complex with the *STE2* regulatory sequence is dependent on the presence of the *STE12* gene product. The results with the Ty1 and *STE2* regulatory elements suggested that the *STE12* product may be a component of these DNA-protein complexes. The experiments described in this paper address this question.

Results

Identification of *cis*-acting *STE12*-dependent elements

A *cis*-acting regulatory element within Ty1 that requires the *STE12* product for activation of the *CYC7* promoter was described recently (Fig. 1A; Company et al. 1988). For comparison, we wished to identify a *STE12*-dependent regulatory element from the promoter region of another gene whose expression is decreased in a *ste12* mutant background. One such gene is the α -pheromone receptor gene *STE2* (Hartig et al. 1986; Fields et al. 1988). One of the *STE2* upstream activating sequences (UAS) that confers cell-type-specific gene expression was shown previously to be within a 97-bp *AvaII*–*HindIII* fragment (Miller et al. 1985). We tested this *STE2* UAS for the ability to cause *STE12*-dependent expression of

Figure 1. *STE12* dependent regulatory elements. (A) Nucleotide sequence of the Ty1 and *STE2* factor binding regions as determined by DNase I protection assays. Fragments used to demonstrate *STE12*-dependent regulatory and factor binding activity are the 94-bp Ty1 fragment A5 (*PvuII*–*Sau3A* fragment described by Company et al. 1988), the 97-bp *STE2* UAS (*AvaII*–*HindIII* fragment, nucleotide positions 266–362 according to Nakayama et al. 1985) and the synthetic 47-bp *STE2* UAS (nucleotide positions 305–351, according to Nakayama et al. 1985). Matches to the P box (solid underline) and PRE (hatched underline) are indicated. Base pair differences for a nonfunctional Ty1 or *STE2* regulatory element are given above the corresponding sequences. Methylation interference sites for the Ty1 fragment are indicated (*). (B) Structure and *STE12*-dependent expression for regulatory fragments inserted upstream of the *CYC1-lacZ* fusion gene. *CYC1* sequences (stippled) and the *lacZ*-coding region (open) of the fusion gene are indicated. Relevant features of the UAS are P boxes (solid) and PRE sequences (hatched). β -Galactosidase assays and units were determined as described by Breeden and Nasmyth (1987).

the *CYC1-lacZ* fusion. The 97-bp *STE2* fragment and a 47-bp subfragment were each inserted upstream of the fusion gene in the vector pLGΔ178 to give pGA1680 and pGA1702, respectively (Fig. 1). Each of these plasmids was used to transform a wild-type *STE12* yeast strain (G2, *STE12*) and an isogenic *ste12* deletion strain (G2,Δ12, *ste12Δ*). The *CYC1-lacZ* plasmid with a functional *CYC1* promoter (pLG669-Z) and the *CYC1-lacZ* plasmid with the disabled promoter (pLGΔ178) were introduced into the two yeast strains for reference. Expression of the fusion genes was quantified as the amount of β-galactosidase produced by each transformed strain (Fig. 1B). Both *STE2* UAS fragments allowed significant expression of the reporter gene in the *STE12* strain. In the *ste12Δ* strain, expression was reduced 21-fold for the 97-bp *STE2* UAS plasmid (pGA1680) and 96-fold for the 47-bp *STE2* UAS plasmid (pGA1702).

We attribute the residual activity of the *STE2* UAS fragments in the *ste12Δ* strain to the presence of a palindromic P-box element (Fig. 1). The P-box element is the binding site for a factor known as the pheromone/receptor gene transcription factor (PRTF) or general regulator of mating (GRM) (Bender and Sprague 1987; Keleher et al. 1988; Tan et al. 1988; Hayes et al. 1988). PRTF and GRM are the product of the same gene (*MCM1*) and are therefore the same factor (Passmore et al. 1988; G. Ammerer, in prep.). We will refer to this factor as PRTF. P-box elements from the promoters of the *BAR1*, *STE6*, and *STE2* genes have been shown to be sufficient for UAS activity (Kronstad et al. 1987; Jarvis et al. 1988; Keleher et al. 1988; G. Ammerer, in prep.). It is assumed that this activity is due to the ability of PRTF to bind to the P box and function as a transcription activator. (Evidence that PRTF binds to the *STE2* UAS is presented below.)

We expected that comparison of the Ty1 and *STE2* fragments would reveal a common sequence that would identify a recognition element for the *STE12*-dependent binding factor (Fig. 1A). The most noteworthy similarity between the two sequences is that both contain a match to the PRE (TGAAACA), which is important for pheromone-inducible transcription (Kronstad et al. 1987; Van Arsdell and Thorner 1987). It has been shown that α-pheromone increases steady-state amounts of *STE2* mRNA (Nakayama et al. 1985; Hartig et al. 1986). Under conditions of α-pheromone induction that cause an approximate fourfold increase in *STE2* steady-state mRNA, we found an approximate twofold increase in the steady-state amount of mRNA for a gene whose transcription is controlled by an adjacent Ty1 element (C. Adler and B. Errede, unpubl.). A P-box sequence, which is another feature of the *STE2* UAS, is not present in the Ty1 regulatory element.

STE12 dependent protein–DNA complexes

It was shown previously that the *STE12*-dependent regulatory sequence from Ty1 forms a specific protein–DNA complex and that its formation requires the presence of the *STE12* gene product (Company et al. 1988). To deter-

mine whether the same dependence is associated with the *STE2* UAS, we used a gel mobility shift assay with whole-cell extracts prepared from isogenic *STE12* and *ste12Δ* strains. When the 97-bp *STE2* fragment was used as a probe, two protein–DNA complexes (C1 and C2) were observed with extracts prepared from the *STE12* strain (Fig. 2A). Only complex C1 was *STE12* dependent, as evidenced by its absence from binding reactions using extract from a *ste12* deletion strain (*ste12Δ*).

Factor binding site(s) within the *STE2* regulatory element were mapped by a DNase I protection assay. Two different 3'-end-labeled probes were prepared for the top and bottom strands of the *STE2* fragment. Factor binding sites for both complexes C1 and C2 were identified (Fig. 2B). A summary of the protected sites on both strands is given (Fig. 2C). Both complexes C1 and C2 show an identical footprint located at the P box. The *STE12*-dependent complex C1 shows an additional footprint at the PRE. These results suggest that the P-box binding factor, PRTF, is present in both complexes C1 and C2 and that an additional factor or factors is present in complex C1.

STE12 nucleotide and predicted amino acid sequence

To begin investigations into the role of the *STE12* product, we determined the nucleotide sequence of the *STE12* gene that had been cloned in the plasmid pLB1214 (kindly provided by L. Bell, Zymogenetics, Inc.). The sequence of 3519 nucleotides that encompass the *STE12* locus is shown (Fig. 3). No DNA sequences that encode the consensus for RNA splicing in yeast were found. An ATG at position 1127 is followed by an open reading frame terminating with a TGA codon at position 3191. A protein of 688 amino acids with a net negative charge and a molecular mass of 78,000 daltons is predicted by the open reading frame.

We looked for features in the predicted *STE12* protein that are characteristic of known DNA-binding proteins because the *STE12* product is required for protein–DNA complex formation. We found no regions similar to known DNA-binding domains (Pabo and Sauer 1984; Shephard et al. 1984; Berg 1986). The predicted *STE12* protein was compared to the NBRF Protein Sequence Data Base [release 17, January 1989], using the Word-Search program from the Wisconsin Genetics Computer Group Sequence Analysis Software Package. No striking similarities with other proteins were identified.

Tagging the *STE12* gene product

To facilitate detection of the *STE12* product, we fused the coding sequence of a known epitope to the *STE12* gene. The chosen epitope consisted of 10 amino acids (EQKLISEEDL) from human c-myc for which a specific monoclonal antibody (Myc1-9E10) was available (Evan et al. 1985; Munro and Pelham 1987). Two different in-frame fusions of the *STE12* gene were constructed (Fig. 4). One fusion, designated *STE12-469M*, was made at *STE12* codon 469, using the *NcoI* site at nucleotide

Figure 2. Factor binding to the *STE2* regulatory element. (A) Protein–DNA complexes detected by gel mobility shift assays. Binding reactions contained extract prepared from yeast strains with *STE12* or *ste12Δ* genetic backgrounds, as specified. The positions of complex C1 (C1), complex C2 (C2), and free probe (F) are indicated at left. (B) DNase I protection analysis of factor binding sites in complexes C1 and C2. The top or bottom strand of the 97-bp *STE2* UAS was 3'-end-labeled and used as probe DNA. Extract used for factor binding was prepared from yeast with either *STE12* or *STE12-668M* genetic background, as indicated. Lanes display cleavage products of DNase-I treated binding reactions that were fractionated by gel electrophoresis into free (F), complex 2 (C2), and complex 1 (C1) probe DNA. Also shown are the cleavage products from purine-specific reactions with probe DNA (A + G). Regions showing DNase I-protected sites for complexes C1 and C2 are indicated by brackets at right. (C) Summary of footprint analysis. The nucleotide sequence of the footprint sites is given. The locations of hypersensitive (▽), strongly protected (●), and weakly protected (○) sites corresponding to complex C1 and C2 are indicated. The locations of P-box and PRE elements in the sequence are indicated by brackets (top).

position 2529. This fusion replaces the 219 carboxy-terminal amino acids of the normal *STE12* product with an 11-amino-acid peptide tag. The other fusion, designated *STE12-668M*, was made at *STE12* codon 668, using the *SacI* site at nucleotide position 3129. This fusion replaces the 20 carboxy-terminal amino acids of the normal *STE12* product with a 20-amino-acid peptide tag. We established that the monoclonal antibody Myc1-9E10 specifically detected the tagged *STE12* proteins produced in yeast by Western blot analysis (data not shown).

In vivo function of the tagged *STE12* products

We tested the ability of the *STE12-469M* and *STE12-668M* gene products to complement the mating defects of a *ste12* deletion mutation. A yeast strain with a *ste12* deletion (E929-6C-6, *ste12Δ*) was transformed with the *STE12-469M* gene carried on either a low-copy centromeric yeast plasmid (pNC204) or a high-copy 2- μ m yeast plasmid (pGA1635). The *ste12* deletion strain was also transformed with the *STE12-668M* gene carried on a low-copy centromeric yeast plasmid (pNC228). As a control, the same recipient strain was transformed with a plasmid carrying the normal *STE12* gene on a centromeric

plasmid (pSC4). The *STE12-469M*, *STE12-668M*, and *STE12*-transformed strains were mating competent when assayed by a nonquantitative patch test (Table 1).

The nonquantitative mating assay does not indicate whether the *STE12-M* products are functionally identical to the normal product. (Strains can differ by an order of magnitude in mating ability and still show equivalent mating by the patch test.) For this reason we tested the *STE12-M* products for their ability to promote *STE12*-dependent gene expression. The *CYC7-H2* gene is a convenient reporter for these tests. The *CYC7-H2* mutation was caused by insertion of a Ty1 element in the 5'-noncoding region of *CYC7*, which is the structural gene for iso-2-cytochrome *c*. The inserted Ty1 element causes iso-2-cytochrome *c* expression to come under mating type control and to be dependent on a functional *STE12* product (Errede et al. 1981). Therefore, *CYC7-H2* expression in strains with different *STE12* alleles was quantified on the basis of its gene product, iso-2-cytochrome *c*. The *STE12-M* and *STE12*-transformed strains produced amounts of iso-2-cytochrome *c* that were greater than the basal amount produced in the *ste12* deletion strain (Table 1). The results demonstrate that the *STE12-668M* product is fully functional with respect to its ability to promote Ty1-regulated transcription. However, a quantitative difference was found be-

10 30 50 70 90 110
 GATCCACCGTGGCAATCAGCGAATATGAGCTCTCGGATACTTGATGATGCTGAGCAGTTGATGTACTATACGTTGCTGGATACCGTCTGTACGATTGTTGGATCAGCAATAATTT
 130 150 170 190 210 230
 TCTGTGCTCAGATGCGCAATTCATGGAAATGTTACTCTTCCAGAAATTAAGTGGTTCGATAGTCACTTCCAGCGTAGAAAACTCTTTATTACAGAAGTTGTCGGCAAGAGTTTGA
 250 270 290 310 330 350
 AGATATCCCTAACAAAGCGGAACAAGTTCAGTTCGCGCATCGAGAAATCGTCAAGAGTATGCAAGGAGTTTATCACAACCATCACAACTCCGTTAATCAAGAGCAGGTCTTGAACTGTT
 370 390 410 430 450 470
 TGTTCGGAATAGCAAGCAAGTTCTTTTAGGTTTAAAGATATGTTGAAAGCGGCGCAACCCACTTACATCTGAAATTCAGATTACATTCTTTGTATAACGAACGTTAAGG
 490 510 530 550 570 590
 AACCCATAAAGCTAAAGACATTTGTTGAAACGAAATGTAAGAAATTCGTCAGTTTGCACAAAGACACCTGAAGAAGCTTCGTTCAATACACTTTCAAGCTGTAGTATGTAACGATAT
 610 630 XbaI 650 670 SacI orf stop 710
 AGATGAAGTTTTCGTGTGTATAAATATATGAACCTAGAGTGTTCATAAATTTGAAACACAGCAATTTCTTTTCGAGAGCTCGTTTCAAAATGAAACAAACGCCGTTTCTCGGTTTTCGTC
 730 750 770 790 810 830**
 TCAATAGAAAAGTGAACAGATAAAAAATGTTTAAAGAAAAGCAATTTGCAACATCTTAAGATATATCAAACTAATACAAAACAGCTTAAAAAGATTGAACAACTCTTCGCGGTCA
 850 870 890 910 930 950
 GGTCTCGACACATAAATCGAAGTACTCGTACGCTAGTTTCTCGCACATAGTACCCTACTGCTCTTTTACAATTAGATTACTTCTTTTAGTTGACTTTTTCGAGACGCTCGTGGCAT
 970 990 1010 1030 1050 1070
 TCATAAATAGGAAAAGATAACAGGTAAGCACTGAAGACTTGTTTTATAAGTGTCCCAAGCGAGACCTAGAGTGGATATTGATATTCTCAAAACAGACTCGTGGAGAAAACACACTTT
 1090 1110 1130 1150 1170
 TATAGCGAAACCGCTTCTTTATTGAATGTCTTGTTCACCAAGGATGAAGTCCAAATAACCAATAGTAGAACAGAGAAATCTTAAAGATTCAAGCTAATATGAAACCGATGAAGT
 1210 1230 1250 1270 1290 1310
 CAGTAAAGCTACTCCGGCGGAAGTTGAAGAATCGCTAAGGTTAATCGGCGATCTAAATTTCTTTTATGCCACAGCGCGTAAATTCGCAAGAAAACCAATTTATAAGCGGATACTATCT
 1330 1350 1370 1390 1410 1430
 GAATAGTGGCAAGGCTTGTCTCTGTGTATTTTGGAAACAATCTATACATATATACAGTACTGATATTGTCAAATGTTGTCTTTACAGAAATGCAAAAGTTTGGGAGAGAAGTAGTTCA
 1450 1470 1490 1510 1530 1550
 AAAGAAAATTTGAAGAGGCTATTTTTTCAGATTGAAGAAATCTCAAAATGCTGTATAGATGCAACTTTAGAACAAACCAAGTCCGAAATTTTTCGTTTCTATTCAAGAAATATGTCTCT
 1570 1590 1610 1630 1650 1670
 GAAACCCAAAAGAGCAGAAAGTATTTTTCGTTTCAGTACGACATGATAAGTTGTTTGCAGATCGCTTGAAGAGATTAAAAAGAGAAAGTTGAATCAGGCTTCAACGACTAA
 1690 1710 1730 1750 1770 1790
 GCGCGTTAAGAGCCCGCTTATCTTTTCATATGATTCCTCATCTGATAAGCCCTCTACGATCAGTTACTTCAACATTTAGATTCTAAGAGACCATCTAGTACAAATCAGATAA
 1810 1830 1850 1870 1890 1910
 TCCGCTCCAAAATTAGAAAGCGAGAAATTTAAGGATAATGAGTTCGTAACAGTAACATCAACCGCTTTTAGCGGTTGGCTCATGGATGCGATGCGGAGAAATCCCGCTCTCAAT
 1930 1950 1970 1990 2010 2030
 TAATGATTTTATCTCTCAGAAATGATTATGAAGCCCAATCTCGAATGAATGGTCTCAGAGAGAAAGCCGCTCATGACTACCCAGAAATACCGCTAAGGGCAGAGACGAAGA
 2050 2070 2090 2110 2130 2150
 TTTTCTCTCGCATATTTCTCTGTATCTGTTGAATACCTACGAGGAAATCGGTTTGATCCGTTCCCTCCACAGGCTTTTACGCGAGCTGCCCTTCCATGCGCTATTTCCTATGATAA
 2170 2190 2210 2230 2250 2270
 CGTGAATGAAGGATTCTATGCCCGTTAATCTCTCTTAATAGATCCCTATCAGTTATCAGTGGCAGCCACTTCCAGTGGCCACCATCATCGAGGCAAGATTTTATGACAAA
 2290 2310 2330 2350 2370 2390
 TCGGATTTTATTCATCTAACAATAACAGGAAAAATGCTATCTCCTAGCGCCCTACAGCTACATGAAGTATGACGAACCAAGTTCGATTTTGTGAAATCTCGGCAAAATGAAA
 2410 2430 2450 2470 2490 2510
 CTGTACAAATGCAAAATCTCACAACCTTCGCGAGCAAACTAAACAACCAATATATTTCTAACCACTTCAGCAATCTTACCCAAACGGAATGGTTCAGGATACCTCAAAATGCC
 2530 2550 2570 2590 2610 2630
 GTATAATCCCATGGGGGGGATCCTCTACTCGATCAAGCCCTTTATCGCGCGGACGATTTTCTTCCACCAAGGATGTGATAACAATATCCTGTCTCACAACCTGCAACTTCATG
 2650 2670 2690 2710 2730 2750
 GAATGTTTTCGCCCTCAAGCTATGCAACAGCTCCAACTATGTTGGAGACATACACACCGAATATAGATCGACACCAAGGTTCCGCGATGTTCCCATACATGCAAGTTCAAATTC
 2770 2790 2810 2830 2850 2870
 CATGCACTGCAACACTGCTGTTTCACTTATAGTTCGAGAGCAACCTACAACTGCTAAAACTATCTCTAGCACATTTTATCTCAAAATATAAATCAATACCCAGGGCGAAGAAC
 2890 2910 2930 2950 2970 2990
 TGTGGGATGAAGTCTTCAACAGAAATGTTTCAACAGGTAATAAACAATCTGTGGGCAAGTCTGCAAAATTTCAAGGCTTACATATTAGACAAGTGTCTTATCAGAGCAATACAA
 3010 3030 3050 3070 3090 3110
 AATCAACTTGGAAACGAAGCCAGGCCAAGTGTGCTGACGAAGATTTCTGCTCATCTGATAAGAACAAAGAAATTCGATGCTACTCCGGAATCCCAATCTTTGCTGCTCCAGTCAGA
 3130 3150 3170 3190 3210 3230
 AGAAGTGGAGCTCATTCACTTGAGGTAGATACCAATCGAAGCTCGATAAAAACCTTCCAGATGCAACCTGATATATATAATTTTGAATTTATGATACAAGAAATAAAAATGCGGGC
 3250 3270 3290 3310 3330 3350
 CAGAATTTAATATAAACAATCTCAGAAAGAAACAACAGGCAATCTGTTTATAAATAAACAATCTTATACAAAGACTAGAGCAAAACAAAGCAAGAAAAAGGTAATAATGT
 3370 3390 3410 3430 3450
 AACAAATCTATTACGTTAGAAAATAACAAAAAAGAAAAAGATAATATAATATACCTTTATATATGTGATTATCTAGCGTCACAGTATGTAATATAAATATCAATGACT
 3470 3490 3510
 TTCTTAACATGACCTCCAAAATACATGATCGCTTAGTA

Figure 3. Nucleotide sequence of the *STE12* locus. The nucleotide sequence of the coding strand is shown. The amino acid sequence for the open reading frame corresponding to the predicted *STE12* protein is shown below the nucleotide sequence (nucleotide positions 1127–3190). The standard single-letter amino acid code is used. The end of another open reading frame upstream of the *STE12* gene at position 567 is indicated (orf stop). The function of the protein encoded by the open reading frame is not known. The 5' map positions for the *STE12* mRNA are indicated by asterisks (*) above the nucleotide sequence at positions 831, 832, 839, and 840. Five short sequences homologous to the PRE (underlined or overlined) are found in the presumed promoter region. Restriction sites are indicated for those enzymes referred to in this study: *XbaI* at positions 634 and 1766, *NcoI* at position 2529, *BamHI* at position 2539, and *SacI* at positions 677 and 3129.

Figure 4. Restriction map of the *STE12* locus and diagrams of the predicted *STE12* and *STE12-M* products. (A) The thick line represents the longest open reading frame. (The positions of the first ATG codon and the TGA termination codon are indicated.) The bars above the restriction map indicate *STE12* fragments in the plasmids used for DNA sequence analysis and construction of the *STE12-M* fusion genes. Restriction sites shown are *Bam*HI (B), *Cla*I (C), *Nco*I (N), *Sac*I (S), *Sph*I (Sp), and *Xba*I (X). *Bam*HI sites shown in parentheses were generated during genomic DNA cloning. (B) The *STE12* product is represented by the stippled box; the peptide tag in *STE12-M* products is indicated by the solid box. The sequences for the peptide tags and for the oligonucleotides encoding the peptide tags are given below the diagrams. The epitope in the peptide tags is 10 amino acids (EQKLISEEDL) from human c-myc.

tween the *STE12*- and *STE12-469M*-transformed strains. The amount of iso-2-cytochrome *c* was 17-fold above the background when *STE12* was expressed on a low-copy centromeric plasmid (pSC4). By comparison, the amount of iso-2-cytochrome *c* was 13-fold above background when *STE12-469M* was expressed on the low-copy centromeric plasmid (pNC204) and only 8-fold above back-

ground when overexpressed on the high-copy plasmid (pGA1635).

The result with the *STE12-469M* fusion product is somewhat unexpected in light of observations reported by Fields and Herskowitz (1987). They found that a *STE12* fragment with a breakpoint at the *Bam*HI site located within the coding region was unable to comple-

Table 1. The *STE12-M* products are functional for mating and activation of gene expression in yeast

Strain	Plasmid	Relevant genotype	Mating ^a	Amount of cytochrome ^b
E929-6C-6	none	<i>ste12Δ</i>	—	1 (—)
E929-6C	none	<i>STE12</i> +	+	20 (3)
E929-6C-6	pSC4	<i>ste12Δ::LEU2 STE12</i>	+	17 (3)
E929-6C-6	pNC228	<i>ste12Δ::LEU2 STE12-668M</i>	+	23 (3)
E929-6C-6	pNC204	<i>ste12Δ::LEU2 STE12-469M</i>	+	13 (3)
E929-6C-6	pGA1635	<i>ste12Δ::LEU2 STE12-469M</i>	+	8 (2)

^aMating competence was determined by the patch test (Sprague and Herskowitz 1981). (+) Ability to mate and (—) inability to mate is indicated.

^bLow-temperature spectroscopic examination of intact cells was used to estimate the relative amount of iso-2-cytochrome *c* (Sherman and Slonimski 1964). In the case of transformed strains, values are the average of determinations on at least 10 independent transformation isolates. Values given in parentheses are the standard deviations. One unit is the amount produced by standard *CYC7* + strains.

ment the mating defect of a *ste12* mutation. This break-point is only 10 bp 3' to the *NcoI* site used for the *STE12*–469M fusion junction (Figs. 3 and 4). One can explain the different results by assuming that an inactive fusion protein is generated fortuitously when vector sequences are joined with the open reading frame of *STE12*. In fact, we observed that a *STE12*–*lacZ* fusion, which used the same *Bam*HI junction, was unable to complement a *ste12* mutation (data not shown).

In vitro function of the tagged *STE12* products

The ability of protein extract prepared from *STE12*–469M and *STE12*–668M strains to form specific complexes with the *STE12*-dependent regulatory elements was determined by a gel mobility shift binding assay. In one set of experiments, the *CYC7*–*H2* Ty1 fragment A5, which was previously shown to form a specific *STE12*-dependent protein complex, was used as a probe for the binding assay (Company et al. 1988). No complex formed with the Ty1 probe when extract from a *ste12Δ* strain was used in the binding reaction. The *STE12*-dependent complex C1 formed with the Ty1 probe when extract from the *STE12*-, *STE12*–469M-, or *STE12*–668M-transformed strains was used in the binding reactions (Fig. 5, No Ab). It is evident that the complex formed with the *STE12*–469M extract migrated with a slightly greater mobility than did the complex with the *STE12* extract. This observation is consistent with the hypothesis that the *STE12*–469M product is a component of the protein–DNA complex. The DNase I footprint (one strand) for the Ty1 DNA complex that forms with *STE12*–469M extract was compared with that for the *STE12* extract (data not shown). The protected sites observed with the two different extracts were essentially the same as those published previously (Company et al. 1988). This result indicates that truncation of the *STE12* product does not alter protein contacts on the Ty1 regulatory element.

In a second set of experiments, the 97-bp *STE2* UAS was used as a probe in binding assays with extracts from strains producing the normal or tagged *STE12* proteins (Fig. 6, No Ab). The *STE12*-dependent complex C1, as well as complex C2, was observed when extracts prepared from the *STE12* or *STE12*–668M strains were used in the binding reactions. Furthermore, the DNase I-protected sites observed for complexes C1 and C2 were identical in extracts prepared from *STE12* or *STE12*–668M strains (Fig. 2B). However, only the *STE12*-independent complex C2 was observed when extracts from the *ste12Δ* or *STE12*–469M strains were used in the binding reactions. Because the *STE12*–469M fusion complements the mating defect of a *ste12* deletion, it is possible that the conditions of the *in vitro* assay are not sufficiently sensitive to detect interactions that may still occur *in vivo*. Nonetheless, the finding that the *STE12*–469M product does not support the *in vitro* formation of complex C1 with the *STE2* UAS suggests that requirements for this *STE12*-dependent complex are different from those necessary for formation of the complex with the Ty1 probe.

Figure 5. *STE12*–M complex formation with the Ty1 regulatory element. Probe DNA in each binding reaction was ~0.5 ng of end-labeled Ty1 fragment A5. Prior to DNA-binding reactions, extract prepared from yeast with *ste12Δ*, *STE12*, *STE12*–469M, or *STE12*–668M genetic background, as indicated, was incubated without antibody (No Ab), with 2 μg of mouse monoclonal antibody Myc1-9E10 (Anti-myc), or with 1.5 μl of anti-PRTF serum at a 1 : 25 dilution (Anti-PRTF).

The STE12 product is a component of DNA–protein complexes

The *STE12* product is critical for formation of a DNA–protein complex with the *STE12*-dependent elements from Ty1 and *STE2*. The simplest interpretation of the above results is that the *STE12* product is a component of the DNA–protein complex. If this hypothesis is correct, the antibody specific for the peptide tag could be used to determine whether the *STE12*–469M and *STE12*–668M products are a component of the protein–DNA complexes that form with the Ty1 and *STE2* UAS fragments. If an antibody recognizes a DNA-binding protein, it is expected to cause an alteration in the gel mobility shift pattern. The antibody–protein interaction could interfere with complex formation and abolish the signal. Alternatively, the antibody–protein interaction may not interfere with complex formation but will lead to a further retardation of the complex during electrophoretic fractionation.

Results consistent with the latter possibility were obtained with the Ty1 probe (Fig. 5). Preincubation of extract containing either *STE12*–469M or *STE12*–668M product with the Myc1-9E10 antibody specific for the

Figure 6. STE12-M complex formation with the STE2 UAS. Probe DNA in each binding reaction was ~0.5 ng of the end-labeled 97-bp STE2 UAS. DNA-binding reactions were performed with extract prepared from yeast with *ste12Δ*, *STE12*, *STE12-469M*, or *STE12-668M* genetic background, as indicated. Protein-DNA complexes were formed under one of the following conditions: Binding reactions were performed in the absence of antibody (No Ab); after protein-DNA-binding reactions were performed, the mixtures were incubated with 2 μ g of mouse monoclonal antibody Myc1-9E10 (Anti-myc); extracts were preincubated with 1.5 μ l of anti-PRTF serum at a 1 : 25 dilution prior to protein-DNA-binding reactions (Anti-PRTF).

peptide tag (Anti-myc) decreased the mobility of the Ty1 probe to a greater extent than extract not incubated with antibody (Fig. 5, No Ab). Preincubation of extract containing the unmodified STE12 product with the antibody did not affect the mobility of the complex. In addition, preincubation of extracts with another monoclonal antibody (Myc1-3C7) that does not recognize the peptide tag did not affect probe or complex mobility (data not shown) (also see Fig. 5, anti-PRTF). The same results were obtained if the protein-DNA complexes were allowed first to form and then were incubated with the antibody (data not shown). From these results we conclude that the STE12-469M and the STE12-668M products are a component of the Ty1 protein complex.

The antibody specific for the peptide tag also was used to determine whether the STE12-668M product is a component of the STE12-dependent protein-DNA complex C1 that forms with the STE2 UAS. When the antibody specific for the peptide tag was preincubated with

extract, formation of complex C1 was abolished with STE12-668M extract but not with STE12 extract (data not shown). When the protein-DNA complexes first were allowed to form and then incubated with the anti-myc antibody, the complex C1 that formed with the STE12-668M extract was detectable but was present in lower amounts. This suggests that complex C1 still was disrupted significantly by the antibody interaction. The important observation is that the residual amount of complex C1 was retarded to a greater extent than when extract was not incubated with antibody (Fig. 6). In contrast, the amount and mobility of complex C1 that formed with extract containing the normal STE12 product was not affected by incubation with the anti-myc antibody. Incubation of binding reactions with the anti-myc antibody did not affect the mobility of the STE12-independent complex C2 that formed with each of the extracts tested. We conclude that the STE12-668M product is a component of the complex C1 that forms with the STE2 UAS.

The DNase I footprint at the P box of the STE2 UAS suggests that PRTF is a component of both complexes C1 and C2. To test this, a rat polyclonal antibody produced against purified PRTF (anti-PRTF) was used in the gel shift assays. In these experiments, extracts from *ste12Δ*, *STE12*, and *STE12-668M* strains were incubated with or without anti-PRTF prior to the DNA-binding reactions. In the case of the STE2 DNA probe, preincubation of extracts with the anti-PRTF antibody severely retarded both complexes C1 and C2 relative to extracts without antibody (Fig. 6). The results are also consistent with the possibility that complex C2 is retarded, whereas complex C1 is disrupted by interaction of PRTF with the antibody. According to either interpretation, these results demonstrate that PRTF is a component of both complexes C1 and C2 that form with the STE2 UAS. In the case of the Ty1 DNA probe, anti-PRTF antibody had no effect on complex mobility (Fig. 5). We conclude that PRTF is not a component of the complex that forms with the Ty1 regulatory sequence.

Cross competition analysis

If the STE12 product binds directly to DNA, e.g., at the PRE-binding site common to the Ty1 and STE2 regulatory elements, the two fragments are expected to compete with each other for protein-DNA complex formation. However, we found no evidence for such competition (Fig. 7). As expected, the signal corresponding to complex C1 using STE12 extract with the labeled Ty1 probe was inhibited progressively by addition of a 5- to 150-fold molar excess of the unlabeled Ty1 DNA fragment. The same results were obtained for complexes C1 and C2 when the STE2 probe was competed with a 5- to 150-fold molar excess of the unlabeled STE2 fragment. In contrast, the unlabeled STE2 fragment did not inhibit complex formation with the Ty1 probe even when present in amounts that completely abolished the signal when the Ty1 fragment was used as competitor. Similarly, no inhibition was observed when the Ty1 frag-

Figure 7. Competition analysis for Ty1 and *STE2* factor binding. Probe DNA in each binding reaction was ~0.5 ng of end-labeled Ty1 fragment A5 (Ty1) or the 97-bp *STE2* UAS (*STE2*), as indicated. The same Ty1 and *STE2* fragments were added as competitors at the indicated (0–150) molar excess. Competitor DNAs were added to binding reactions prior to addition of extract from a *STE12* yeast strain.

ment was used as competitor for complex C1 and C2 formation with the *STE2* probe. The lack of cross competition indicates that the *STE12*-containing complex that forms with the Ty1 probe is intrinsically different from that which forms with the *STE2* probe.

Discussion

The STE12 protein is a component of two distinct DNA–protein complexes

The aim of these experiments was to define the function of the protein encoded by the *STE12* gene. For this purpose, we constructed two fusion genes, *STE12–469M* and *STE12–668M*, in which the *STE12* product was tagged with an immunologically recognizable peptide. One concern was whether the *STE12–M* products could substitute for the normal *STE12* product. Our results showed that both of these fusion genes carried on extrachromosomal plasmids were sufficient to restore the biologically relevant function of mating in a *ste12* deletion strain. No difference between the normal and *STE12–668M* proteins was detected for their ability to promote expression of the Ty1 reporter gene, *CYC7–H2*. Only a quantitative difference (~25%) in *CYC7–H2* expression was detected for the *STE12–469M* protein in which the carboxy-terminal 219 amino acids of the normal protein are deleted.

The results of gel shift assays in the presence of specific antibodies clearly show that the *STE12–469M* and *STE12–668M* products are components of specific protein–DNA complexes that form with Ty1 and *STE2* transcriptional control elements. These experiments do not distinguish between the possibilities that the *STE12* product binds on its own to DNA or that it requires cooperative interaction with other proteins to form the complex. It is not even clear that *STE12* makes direct contact with DNA. Nonetheless, *STE12*-dependent

complex formation in vitro correlates with gene expression in vivo, so we conclude that the *STE12* protein constitutes an integral part of active transcription complexes on the Ty1 and *STE2* regulatory elements (Figs. 1 and 6; Company et al. 1988). However, our results indicate the *STE12* protein complex that forms with Ty1 DNA is different from that which forms with *STE2* DNA. First, the two regulatory elements do not compete with each other for specific DNA-binding proteins. Second, the complex with the *STE2* UAS contains PRTF but that with Ty1 does not. Third, the truncated *STE12–469M* product supports complex formation in vitro with the Ty1 probe but not with the *STE2* probe. Finally, the anti-myc antibodies appear to interfere with the complex that forms with the *STE2* probe but not with the Ty1 probe.

Models for *STE12* interaction in protein–DNA complexes

The failure to observe competition between the Ty1 and the *STE2* UAS fragments for the formation of *STE12*-dependent protein–DNA complexes puts restrictions on any model to account for *STE12* interaction with DNA. Considering this result, we find it unlikely that the *STE12* protein binds independently to both the Ty1 and *STE2* regulatory elements (Fig. 8A). One would have to argue that the *STE12* protein contains DNA-binding domains with different sequence specificity. This situation has a precedent in the case of the HAP1 protein (Pfeifer et al. 1987). However, the absence of competition in our case imposes the restriction that such domain structures are locked into mutually exclusive and stable conformational states of the protein.

Another model is that the *STE12* protein interacts cooperatively with different DNA-binding proteins in different sequence environments (Fig. 8B). Here, we propose that *STE12* interacts at the PRE sequence common

Figure 8. Models for STE12-dependent complex formation. (A) Direct DNA-binding model. According to this model, STE12 (12) binds directly to the PRE sequences (hatched boxes) common to the STE2 and Ty1 elements. (B) Cooperative DNA-binding protein model. According to this model, STE12 requires cooperative interaction with another DNA-binding protein for DNA complex formation. The cooperative binding protein is proposed to be PRTF (P), which binds at the P box (solid box) in the STE2 UAS. The cooperative binding protein for complex formation with the Ty1 regulatory element is an unknown protein (X). (C) Non-DNA-binding model. According to this model, STE12 does not contact DNA directly but stabilizes complexes that form with different DNA-binding proteins on the Ty1 (X and Y) and STE2 (P and Y) elements.

to the Ty1 and STE2 regulatory elements. In the case of the STE2 UAS, the likely candidate for the cooperative DNA-binding protein is PRTF (see below). In the case of the Ty1 regulatory element, the factor proposed to interact with STE12 has not been identified. However, results with a base-substituted Ty1 fragment establish that sequences in addition to the PRE are essential for complex formation (Fig. 1; Company et al. 1988). Within the context of this model, we propose that the region defined by the base substitutions constitutes a recognition element for the cooperative binding factor. To explain the lack of competition between the STE2- and Ty1-binding sites, this model would require either that STE12 is not limiting for complex formation or that stable protein-protein complexes form in the absence of DNA and exchange of STE12 between these preformed complexes is rate limiting.

In an extension of this model, the STE12 protein could be involved in the stabilization of complexes with different DNA-binding proteins without contacting the DNA itself (Fig. 8C). This last model is reminiscent of the situation found with the viral *trans*-activator VP16. This protein is also necessary for the activation of *cis*-acting elements that appear unrelated by DNA sequence comparison. Subsequently, it was proposed that the VP16 protein interacts with different sequence-specific DNA-binding proteins without contacting DNA (Triezenberg et al. 1988a,b). Another example of protein-

protein interactions between transcription factors has been described for the ovalbumin promoter (Tsai et al. 1987). In this case a non-DNA-binding protein, S300-II, is proposed to stabilize a complex with the promoter and a sequence-specific DNA-binding protein. Their data also indicated that S300-II is necessary for *in vitro* transcription from several different promoters. Yet, as we found for Ty1 and STE2, the different promoter fragments did not compete for complex formation with specific DNA-binding proteins.

Does STE12 interact with PRTF?

If STE12 interacts with other DNA-binding proteins, PRTF is an obvious candidate for one such factor. It is the major factor responsible for DNA complex formation at the P-box element present in the promoter region of cell-type-specific genes. It has already been shown to bind cooperatively with two different proteins, the cell-type-specific regulators $\alpha 1$ and $\alpha 2$. In both cases, the factors interact directly with PRTF (Keleher et al. 1988; Tan et al. 1988). Although we have not proved the existence of direct interactions between the STE12 protein and PRTF, both are present in the complex C1 that forms with the STE2 UAS. A functional relationship between the two proteins is suggested by the finding that a double point mutation in the P box (Fig. 1) abolishes protein-binding activity of the 47-bp STE2 UAS and also destroys the *in vivo* activation functions of this highly STE12-dependent fragment (G. Ammerer, in prep.). In addition, the experiments with the truncated STE12-469M product suggest that the 219-amino-acid carboxy-terminal domain may be important for interactions between STE12 and PRTF at the STE2 UAS.

Is STE12 a distal element of the signal transduction pathway?

Genetic evidence established that the STE12 gene is necessary for physiological responses to pheromone and may specify, directly or indirectly, a component of the signal pathway that functions downstream of the α -subunit of G protein (Hartwell 1980; Nakayama et al. 1988). We propose that the STE12 protein is a nuclear target of the pheromone induction signal. Our data show clearly that the STE12 protein is related functionally to the transcriptional PRE. This sequence element is protected against DNase I digestion in the STE12-containing protein-DNA complex with the STE2 UAS. On the basis of DNase I protection and methylation interference analyses, the PRE sequence also is involved in complex formation with the Ty1 regulatory element (Fig. 5 in Company et al. 1988). As discussed above, either the STE12 protein binds to the PRE or it interacts with another factor that binds to this sequence. Because two other gene products (STE7 and STE11) that are required for response to pheromone show significant similarities to protein kinases (Teague et al. 1986; B. Errede, unpubl.), an obvious hypothesis is that the induction signal may affect STE12 function in transcription complexes by a

modification such as protein phosphorylation. The proposal rests partly on the observation that extracts from a *ste7* or *ste11* deletion strain show reduced binding activity with the Ty1 fragment (Company et al. 1988; C. Adler and B. Errede, unpubl.). Additionally, the *STE7*, *STE11*, and *STE12* gene products appear to act at the same point in the pathway. This view is supported by the observation that a deficiency in any one of the three gene products causes reduced expression of target genes and that there is not a more severe reduction in target gene expression when all three gene products are deficient (Fields et al. 1988).

The finding that STE12 is part of a DNA–protein complex also has implications for other responses to pheromone such as G_1 arrest. The STE12 protein plays a role in the pathway that leads to cell-cycle arrest induced by pheromone. This conclusion is based on the observation that loss of STE12 function relieves the growth defect caused by a mutation in the α -subunit of the G protein (Nakayama et al. 1988). Therefore, one prediction from our findings is that there is at least one gene required for G_1 arrest whose transcription is dependent on the STE12 product.

Materials and methods

Plasmids

Plasmid constructions to test STE12-dependent regulatory activity of STE2 UAS elements were made with the promoter-disabled, *CYC1-lacZ* vector pLGΔ178. pLGΔ178 was constructed from pLG669-Z (Guarente and Ptashne 1981) by deletion of the 433-bp *XhoI* fragment, which includes both UAS1 and UAS2 from the *CYC1* promoter region (Guarente et al. 1984). The 97-bp STE2 UAS fragment was isolated as an *AvaII*–*HindIII* fragment (nucleotides 266–362; Nakayama 1985). The fragment was end-repaired and blunt-end-ligated into the end-repaired *XhoI* site of pLGΔ178 to give pGA1680. Oligonucleotides corresponding to each strand of the 47-bp STE2 fragment (Fig. 1A) were synthesized with the addition of *XhoI*-compatible ends. The annealed oligonucleotides were inserted at the *XhoI* site of pLGΔ178 to give pGA1702.

The DNA sequence of the STE12 locus was determined from subclones of pLB1214 (Fig. 4A). The plasmid pLB1214 was a gift from L. Bell and is a yeast genomic DNA clone that was able to complement a *ste12* mutation. The 3.2- and 2.3-kb *Bam*HI fragments from pLB1214 were subcloned into the *Bam*HI site of pUC4 to give pLB204 and pLB205, respectively.

Two different STE12 fusion genes were constructed. For STE12–469M, the STE12 product was fused at amino acid 469 with an antigenic peptide tag using DNA fragments from pLB204 and pMCT61. The plasmid pLB204 carries a *Bam*HI fragment encompassing the amino-terminal 472 amino acids of STE12 (Fig. 4A). The plasmid pMCT61, provided by H. Pelham, contains a fragment of the human *c-myc* gene that codes for a 10-amino-acid antigenic peptide. The *c-myc* sequence is flanked by an *NcoI* site on the 5′-end which provides an in-frame ATG codon, and a filled-in *EcoRI* site on the 3′-end, which adds one amino acid and provides an in-frame termination codon (Fig. 4B) (Munro and Pelham 1987). In addition, pMCT61 contains ~700 bp of unspecified sequence from the herpes simplex thymidine kinase gene and SV40 DNA. The unspecified sequence is located downstream of the *c-myc*-coding segment, and its end point is defined by an *SphI* site. The 3.2-kb

EcoRI–*NcoI* fragment that encodes 469 amino acids of STE12 from pLB204 and the 700-bp *NcoI*–*SphI* fragment from pMCT61 were cloned into *SphI*–*EcoRI*-cut pUC19 to give the plasmid pGA1630. This STE12–469M fusion was transferred as a *Bam*HI–*HindIII* fragment into the vector pGA1618 to give the 2- μ m plasmid pGA1635. The vector pGA1618 is identical to pSEY101, except that the polylinker region contains a *HindIII* site (Douglas et al. 1984). The STE12–469M fusion gene also was transferred as an *EcoRI*–*SphI* fragment from pGA1635 to the vector YCp50 to give the centromeric plasmid pNC204.

For STE12–668M, the STE12 product was fused at amino acid 668 with the antigenic peptide tag using DNA fragments from the plasmids pSC4 and pNC212. The plasmid pSC4 carries the STE12 gene cloned at the *Bam*HI site of YCp50 and was provided by S. Fields (Fields and Herskowitz 1987). A 4.1-kb *SphI*–*SacI* fragment from pSC4 encompassing the amino-terminal 668 amino acids of the STE12 gene was cloned into the *SphI*–*SacI* site of pNC161 to give the plasmid pNC205 (Fig. 4A). The plasmid pNC161 is a centromeric yeast–*Escherichia coli* shuttle vector. It is the same as the previously published plasmid pNC98, except that the polylinker from M13mp19 replaces the *CYC7* sequences (Company and Errede 1987). Site-directed mutagenesis was used to insert an oligonucleotide encoding the *c-myc* epitope between the last codon and the stop codon of STE11 to give the plasmid pNC212 (C.N. Rhodes II and B. Errede, unpubl.). A 1.1-kb *PstI* fragment from pNC212 was isolated. This fragment includes codons for 9 amino acids from STE11, the 10-amino-acid peptide tag, and 1 carboxy-terminal amino acid. The coding segment is followed by a TGA stop codon and 3′-noncoding sequences from STE11. After appropriate end repair, the 1.1-kb fragment was inserted at the *SacI* site at codon 668 of STE12 to give the STE12–668M fusion in the plasmid pNC228.

The *ste12Δ::LEU2* plasmid pSUL16 was provided by S. Fields and has been described previously (Fields and Herskowitz 1987). The *XbaI*–*PstI* fragment from the plasmid pNC188 was the source of the Ty1 DNA (fragment A5) used as a probe for DNA-binding reactions (Company et al. 1988). The plasmid pGA1682 carries the *AvaII*–*HindIII* fragment from the 5′-noncoding region of STE2 (nucleotides 266–362) cloned into the *SmaI* site of pUC13. An *XbaI*–*EcoRI* fragment from pGA1682 was the source of the STE2 UAS, which was used as a probe for DNA-binding reactions.

Standard recombinant DNA procedures were used for all plasmid constructions and preparations (Maniatis et al. 1982).

Sequencing

The STE12 nucleotide sequence was determined on both strands by the dideoxy sequencing method (Sanger et al. 1977). A directed deletion strategy was employed with derivatives of pLB204 and pLB205 (Henikoff 1984).

Yeast strains and genetic procedures

Yeast strains used in these studies were E929-6C (MATa STE12+ *cyc1* *CYC7*–H2 *can1* *leu2*-3,112 *trp1*– Δ 1 *ura3*-52), E929-6C-6, G2 (MATa *leu2* *trp1* *ura3*), G2Δ12, and KZ8-1D (MATa *cyc1*-1 *CYC7* *his4* *ura1*). E929-6C-6 and G2Δ12 are isogenic *ste12Δ::LEU2* derivatives of E929-6C and G2, respectively. Each was constructed using the 6-kb *SacI*–*SphI* fragment from pSUL16 for gene replacement. E929-6C and E929-6C-6 carry the STE12-dependent reporter gene *CYC7*–H2 at its chromosomal locus. Media and genetic procedures were as described in Sherman et al. (1979).

Antibodies

Mouse monoclonal antibody Myc1-9E10 was raised against a synthetic peptide comprising residues 409–439 of human c-myc, and mouse monoclonal antibody Myc1-3C7 was raised against a synthetic peptide comprising residues 171–188 of human c-myc (Evan et al. 1985). Acites fluid for each monoclonal antibody was a gift from G. Evan. Saturated $(\text{NH}_4)_2\text{SO}_4$ (100%) in buffer [20 mM HEPES (pH 8.0), 5 mM EDTA] was added to the crude acites fluid to give a final $(\text{NH}_4)_2\text{SO}_4$ concentration of 40%. This mixture was incubated at 4°C with gentle agitation for 30 min. The protein precipitate was pelleted by centrifugation at 4°C for 10 min at 13,000g. Each protein pellet was suspended in 20 mM HEPES (pH 8.0), 5 mM EDTA, to a final protein concentration of 10 $\mu\text{g}/\mu\text{l}$. Protein concentrations were determined using the Bio-Rad Laboratories (Richmond, California) protein assay kit. The rat polyclonal antibody anti-PRTF was raised against the DNA affinity-purified PRTF protein (G. Ammerer, in prep.). The anti-PRTF serum was used without $(\text{NH}_4)_2\text{SO}_4$ fractionation at a 1 : 25 dilution.

Preparation of yeast cell extracts

Cultures of E929-6C-6, pGA1635, or pNC228 were grown in appropriate synthetic omission medium to maintain selection for the plasmids encoding the *STE12-469M* or *STE12-668M* products. Cultures of E929-6C or E929-6C-6 without plasmid were grown in complete synthetic medium. Extracts were prepared according to procedures described previously (Company et al. 1988). The protein precipitate from the 40% ammonium sulfate fraction was used for both DNA-binding reactions and immunoblot analysis.

Gel electrophoresis, DNA-binding assays, and DNase I protection analysis

Protein–DNA-binding reactions were carried out in a 20- μl volume containing ~0.5 ng of probe DNA and 10–30 μg of protein extract in binding buffer [20 mM Tris (pH 8), 40 mM NaCl, 4 mM MgCl_2 , 1 mM dithiothreitol, 5% glycerol, 2 μg of salmon sperm DNA]. Binding reactions were incubated for 7–10 min at room temperature. Conditions for competition assays were exactly the same as those used for binding assays, except that competitor DNAs were added to the reaction mix before addition of the protein extract. For experiments in which antibodies were used, either of two protocols was employed. In one, antibody was preincubated with cell extract for 7 min at room temperature. DNA-binding buffer and probe DNA were added to initiate the DNA–protein-binding reaction, and the mixture was incubated for an additional 8 min. In the other protocol, the standard DNA–protein-binding reactions were prepared and incubated for 8 min at room temperature. Then the antibodies were added, and the mixtures were incubated for an additional 7 min at room temperature. Gels for resolving protein–DNA complexes were 5% 19 : 1 polyacrylamide : bis-acrylamide gel in 0.5 $\times$ TBE. (1 $\times$ TBE is 89 mM Tris, 89 mM Borate, and 2.4 mM EDTA). Gels were run in 0.5 $\times$ TBE at 150 V for 4–4.5 hr without pre-electrophoresis. After electrophoresis, the gels were dried and autoradiographed with an intensifying screen at –70°C. Procedures for DNase I protection assays were as described previously (Company et al. 1988), except that the experiments in this study used 3'-end-labeled DNA fragments and standard DNA-binding reactions were scaled up 10-fold.

Acknowledgments

We acknowledge F. Grant (Zymogenetics, Inc.) for his contribution in determining the majority of the *STE12* nucleotide se-

quence. We are grateful to P. Sorger for initiating this collaboration and to C. Price, A. Bird, S. Lord, and R. Swanstrom for comments on the manuscript. We also thank K.A. Nasmyth for providing space in his laboratory. G.A. was a recipient of a long-term fellowship from European Molecular Biology Organization. This research was supported, in part, by U.S. Public Health Service grant GM-30619 from the National Institutes of Health.

Note

Sequence data described in this paper have been submitted to the EMBL/GenBank Data Libraries.

References

- Bender, A. and G.F. Sprague, Jr. 1986. Yeast peptide pheromones, α -factor and α -factor, activate a common response mechanism in their target cells. *Cell* **47**: 929–937.
- . 1987. *MAT α 1* protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. *Cell* **50**: 681–691.
- Berg, J.M. 1986. Potential metal binding domains in nucleic acid binding proteins. *Science* **232**: 484–487.
- Breeden, L. and K.A. Nasmyth. 1987. Cell cycle control of the yeast HO gene: cis- and trans-acting regulators. *Cell* **48**: 389–397.
- Company, M. and B. Errede. 1987. Cell-type-dependent gene activation by yeast transposon Ty1 involves multiple regulatory determinants. *Mol. Cell. Biol.* **7**: 3205–3211.
- Company, M., C. Adler, and B. Errede. 1988. Identification of a Ty1 regulatory sequence responsive to *STE7* and *STE12*. *Mol. Cell. Biol.* **8**: 2545–2554.
- Cross, F., L.H. Hartwell, C. Jackson, and J.B. Konopka. 1988. Conjugation in *Saccharomyces cerevisiae*. *Annu. Rev. Cell. Biol.* **4**: 429–457.
- Dietzel, C. and J. Kurjan. 1987. The yeast *SCG1* gene: A $\text{G}\alpha$ -like protein implicated in the α - and α -factor response pathway. *Cell* **50**: 1001–1010.
- Dixon, R.A.F., B.K. Kobilka, D.J. Strader, J.L. Benovic, H.G. Dohlman, T. Frielle, M.A. Bolanowski, C.D. Bennett, E. Rands, R.E. Diehl, R.A. Mumford, E.E. Slater, I.S. Sigal, M.G. Caron, R.J. Lefkowitz, and C.D. Strader. 1986. Cloning the gene and cDNA for mammalian β -adrenergic receptor and homology with rhodopsin. *Nature* **321**: 75–79.
- Douglas, M.G., B.L. Geller, and S.D. Emr. 1984. Intracellular targeting and import of an F1-ATPase β -subunit- β -galactosidase hybrid protein into yeast mitochondria. *Proc. Natl. Acad. Sci.* **81**: 3983–3987.
- Errede, B., T.S. Cardillo, G. Wever, and F. Sherman. 1981. Studies on transposable elements in yeast. I. ROAM mutations causing increased expression of yeast genes: Their activation by signals directed toward conjugation functions and their formation by insertion of Ty1 repetitive elements. *Cold Spring Harbor Symp. Quant. Biol.* **45**: 593–602.
- Evan, G.I., G.K. Lewis, G. Ramsay, and J.M. Bishop. 1985. Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol. Cell. Biol.* **5**: 3610–3616.
- Fields, S. and I. Herskowitz. 1987. Regulation by the yeast mating type locus of *STE12*, a gene required for cell-type-specific expression. *Mol. Cell. Biol.* **7**: 3818–3821.
- Fields, S., D.T. Chaleff, and G.F. Sprague Jr. 1988. Yeast *STE7*, *STE11*, and *STE12* genes are required for expression of cell-type specific genes. *Mol. Cell. Biol.* **8**: 551–556.
- Guarente, L. and M. Ptashne. 1981. Fusion of *Escherichia coli*

- lacZ* to the cytochrome *c* gene of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **78**: 2199–2203.
- Guarente, L., B. Lalonde, P. Gifford, and E. Alani. 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. *Cell* **36**: 503–511.
- Hartig, A., J. Holly, G. Saari, and V.L. MacKay. 1986. Multiple regulation of *STE2*, a mating-type-specific gene of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **6**: 2106–2114.
- Hartwell, L.H. 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. *J. Cell. Biol.* **85**: 811–822.
- Hayes, T.E., P. Sengupta, and B.H. Cochran. 1988. The human *c-fos* serum response factor and the yeast factors GRM/PRTF have related DNA-binding specificities. *Genes Dev.* **2**: 1713–1722.
- Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**: 351–359.
- Jahng, K., J. Ferguson, and S.I. Reed. 1988. Mutations in a gene encoding the α subunit of a *Saccharomyces cerevisiae* G protein indicate a role in mating pheromone signaling. *Mol. Cell. Biol.* **8**: 2484–2493.
- Jarvis, E.E., D.C. Hagen, and G.F. Sprague Jr. 1988. 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. *Mol. Cell. Biol.* **8**: 309–320.
- Keleher, C.A., C. Goutte, and A.D. Johnson. 1988. The yeast cell-type-specific repressor $\alpha 2$ acts cooperatively with a non-cell-type-specific protein. *Cell* **53**: 927–936.
- Kronstad, J.W., J.A. Holly, and V.L. MacKay. 1987. A yeast operator overlaps an upstream activation site. *Cell* **50**: 369–377.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- McCaffrey, G., F.J. Clay, K. Kelsay, and G.F. Sprague Jr. 1987. Identification and regulation of a gene required for cell fusion during mating of the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7**: 2680–2690.
- Miller, A.M., V.L. MacKay, and K.A. Nasmyth. 1985. Identification and comparison of two sequence elements that confer cell-type specific transcription in yeast. *Nature* **314**: 598–603.
- Miyajima, I., M. Nakafuku, N. Nakayama, C. Brenner, A. Miyajima, K. Kaibuchi, K. Arai, Y. Kaziro, and K. Matsumoto. 1987. *GPA1*, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. *Cell* **50**: 1011–1019.
- Munro, S. and H.R.B. Pelham. 1987. A C-terminal signal prevents secretion of luminal ER proteins. *Cell* **48**: 899–907.
- Nakayama, N., A. Miyajima, and K. Arai. 1985. Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. *EMBO J.* **4**: 2643–2648.
- . 1987. Common signal transduction system shared by *STE2* and *STE3* in haploid cells of *Saccharomyces cerevisiae*: autocrine cell-cycle arrest results from forced expression of *STE2*. *EMBO J.* **6**: 249–254.
- Nakayama, N., Y. Kaziro, K. Arai, and K. Matsumoto. 1988. Role of *STE* genes in the mating factor signaling pathway mediated by *GPA1* in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **8**: 3777–3783.
- Pabo, C.O. and R.T. Sauer. 1984. protein-DNA recognition. *Annu. Rev. Biochem.* **53**: 293–321.
- Passmore, S.G., G.T. Maine, R. Elble, C. Christ, and B. Tye. 1988. *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating of *MAT α* cells. *J. Mol. Biol.* **204**: 593–606.
- Pfeifer, K., T. Prezant, and L. Guarente. 1987. Yeast HAP1 activator binds to two upstream activation sites of different sequence. *Cell* **49**: 19–27.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Shepherd, J.C.W., W. McGinnis, A.E. Carrasco, E.M. DeRobertis, and W.J. Gehring. 1984. Fly and frog homoeo domains show homologies with yeast mating type regulatory proteins. *Nature* **310**: 70–71.
- Sherman, F. and P.P. Slonimski. 1964. Respiration-deficient mutants of yeast. II. Biochemistry. *Biochim. Biophys. Acta* **90**: 1–15.
- Sherman, F., G.R. Fink, and J.B. Hicks. 1979. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Sprague, G.F., Jr. and I. Herskowitz. 1981. Control of yeast cell type by the mating type locus. I. Identification and control of expression of the *a*-specific gene *BAR1*. *J. Mol. Biol.* **153**: 305–321.
- Strazdis, J.R. and V.L. MacKay. 1983. Induction of yeast mating pheromone α -factor by α -cells. *Nature* **305**: 543–545.
- Tan, S., G. Ammerer, and T.J. Richmond. 1988. Interactions of purified transcription factors: binding of yeast *MAT α 1* and PRTF to cell type-specific, upstream activating sequences. *EMBO J.* **7**: 4255–4264.
- Teague, M.A., D.T. Chaleff, and B. Errede. 1986. Nucleotide sequence of the yeast regulatory gene *STE7* predicts a protein homologous to protein kinases. *Proc. Natl. Acad. Sci.* **83**: 7371–7375.
- Triebenber, S.J., R.C. Kingsbury, and S.L. McKnight. 1988a. Functional dissection of VP16, the *trans*-activator of herpes simplex virus immediate early gene expression. *Genes Dev.* **2**: 718–729.
- Triebenber, S.J., K.L. LaMarco, and S.L. McKnight. 1988b. Evidence of DNA-protein interactions that mediate HSV-1 immediate early gene activation by VP16. *Genes Dev.* **2**: 730–742.
- Tsai, S.Y., I. Sagami, H. Wang, M.J. Tsai, and B.W. O'Malley. 1987. Interactions between a DNA-binding transcription factor (COUP) and a non-DNA binding factor (S300-II). *Cell* **50**: 701–709.
- Van Arsdell, S.W. and J. Thorner. 1987. Hormonal regulation of gene expression in yeast. In *Transcriptional control mechanisms*, (ed. D. Granner, M.G. Rosenfeld, and S. Chang), pp. 325–332. Alan R. Liss, New York.
- Van Arsdell, S.W., G.L. Stetler, and J. Thorner. 1987. The yeast repeated element sigma contains a hormone-inducible promoter. *Mol. Cell. Biol.* **7**: 749–759.
- Whiteway, M., L. Hougan, D. Dignmard, D.Y. Thomas, L. Bell, G.C. Saari, F.J. Grant, P. O'Hara, and V.L. MacKay. 1989. The *STE4* and *STE18* genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. *Cell* **56**: 467–477.

STE12, a protein involved in cell-type-specific transcription and signal transduction in yeast, is part of protein-DNA complexes.

B Errede and G Ammerer

Genes Dev. 1989, **3**:

Access the most recent version at doi:[10.1101/gad.3.9.1349](https://doi.org/10.1101/gad.3.9.1349)

References

This article cites 46 articles, 21 of which can be accessed free at:
<http://genesdev.cshlp.org/content/3/9/1349.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).