Coupling of cell identity to signal response in yeast: interaction between the $\alpha 1$ and STE12 proteins

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In *Saccharomyces cerevisiae*, the STE12 protein mediates transcriptional induction of cell type-specific genes in response to pheromones. STE12 binds in vitro to the pheromone response elements (PREs) present in the control region of α -specific genes. STE12 is also required for transcription of α -specific genes, but there is no evidence that it binds directly to these genes. Instead, the *MAT α* -encoded protein $\alpha 1$ and the *MCM1* product bind to the DNA element that is responsible for α -specific and α -factor-inducible expression. To explore the role of STE12 in the pheromone induction of α -specific genes, we cloned *STE12* and *MAT $\alpha 1$* homologs from the related yeast *Kluyveromyces lactis*. The *K. lactis* STE12 protein did not cooperate with the *S. cerevisiae* $\alpha 1$ protein to promote the overall mating process or the induction of transcription of an α -specific gene. However, introduction of both *K. lactis* STE12 along with *K. lactis* $\alpha 1$ did restore mating, suggesting that an interaction between STE12 and $\alpha 1$ is important for α -specific gene activation. We also show that bacterially expressed STE12 and $\alpha 1$ are able to form a complex in vitro. Thus, we demonstrate a coupling in α cells between a protein functioning in cell identity, $\alpha 1$, with a protein responsive to the pheromone-induced signal STE12.

[Key Words: *S. cerevisiae*; α mating type; cell identity; transcriptional induction; pheromone response elements]

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The yeast *Saccharomyces cerevisiae* provides an especially attractive model for understanding both the transcriptional regulation that gives rise to cell identity and the signal transduction processes that result in altered gene expression. Haploid yeast cells exist as either cell type *a* or α , which can mate with each other to form the *a*/ α diploid cell type. Phenotypically, the two haploid cell types differ principally in the combination of peptide mating pheromone and pheromone receptor that they express. This difference arises from cell type-specific transcription; α cells uniquely express α -specific genes, and *a* cells uniquely express *a*-specific genes. In addition, a set of haploid-specific genes is expressed in both *a* and α cells but not in *a*/ α cells (for review, see Herskowitz 1989; Sprague 1990; Dolan and Fields 1991). Genetically, *a* and α cells differ at only a single locus, *MAT*. The *MAT α* allele, present in *a* cells, plays no role in *a*-specific transcription; *a*-specific transcription is the default pattern and occurs in the absence of any information at *MAT*. The *MAT α* allele, present in α cells, encodes two transcriptional regulators: $\alpha 1$ activates the transcription of α -specific genes and $\alpha 2$ represses the transcription of *a*-specific genes. The *MAT α* allele encodes a repressor ($\alpha 1$) that is required to establish the *a*/ α diploid state because $\alpha 1$ in conjunction with $\alpha 2$ represses the transcription of the haploid-specific genes.

Binding of the appropriate pheromone to the surface of a haploid cell results in the activation of a signal transduction pathway that leads to numerous physiological changes. Among these changes are increased transcription of α -specific genes in α cells in response to *a*-factor and of *a*-specific genes in *a* cells in response to α -factor. In addition, many haploid-specific genes are transcriptionally induced in response to the appropriate pheromone. The signal pathway that mediates these changes in gene expression appears to be identical in both haploid cell types and includes a heterotrimeric G protein, the STE5 protein, five proteins with homology to protein kinases (STE7, STE11, STE20, FUS3, and KSS1), and a transcriptional activator (STE12) (for review, see Marsh et al. 1991; Dolan and Fields 1991; Sprague 1991; Kurjan 1992; see also Leberer et al. 1992; Ramer et al. 1993). STE12 binds to the pheromone response element (PRE), the DNA sequence responsible for α -factor induction of *a*-specific genes and for α - or *a*-factor induction of haploid-specific genes (Dolan et al. 1989; Errede and Ammerer 1989). A hybrid of STE12 with the GAL4 DNA-binding domain is rapidly phosphorylated with pheromone addition, and this modification correlates with an enhancement of its ability to mediate transcriptional activation (Song et al. 1991).

Cell identity in yeast must be coupled to signal re-

sponse. For example, binding of either pheromone activates a common response pathway, but this activation leads to induction of α -specific genes in α cells and α -specific genes in α cells. In addition, α and α cells modulate transcription of the cell type-specific genes, both in the absence and presence of pheromone, such that different genes vary significantly in their basal expression and in the magnitude of their pheromone induction. This coupling occurs at a number of levels. First, cell identity itself is functionally equivalent to the synthesis of the particular set of pheromone and receptor that allows a cell to be responsive to the opposite cell type (see Bender and Sprague 1989). Second, the transcription of the α - and α -specific genes, as well as of certain haploid-specific genes, is dependent on an intact pheromone response pathway (Hartwell 1980; Fields and Herskowitz 1985; Hartig et al. 1986; McCaffrey et al. 1987; Fields et al. 1988; Hagen et al. 1991). Third, in general, transcription of only cell type-specific genes is inducible by pheromone treatment. Fourth, a protein expressed in all three cell types, MCM1, functions in both cell type-specific transcription and pheromone response. MCM1 is required for activation of α -specific genes by $\alpha 1$ and for repression of α -specific genes by $\alpha 2$ (Bender and Sprague 1987; Keleher et al. 1988; Tan et al. 1988; Jarvis et al. 1989; Passmore et al. 1989; Ammerer 1990). MCM1 is also required for binding of STE12 to a PRE that lies adjacent to the MCM1-binding site in the upstream activation sequence (UAS) of an α -specific gene (Errede and Ammerer 1989). This requirement thus provides a link between a protein that functions in cell identity (MCM1) and one that functions in α -factor induction (STE12).

The mechanism by which α -factor induces α -specific gene transcription is less clear. The *cis*-acting DNA sequence for this induction is the sequence that binds $\alpha 1$ and MCM1 and is responsible for α -specific transcription (Jarvis et al. 1988). The protein responsive to the signal pathway appears to be $\alpha 1$, as demonstrated using LexA fusions (Sengupta and Cochran 1991). The *trans*-activation mediated by a fusion of $\alpha 1$ to the DNA-binding domain of LexA is inducible by α -factor and dependent on STE12 function, whereas that mediated by an MCM1–LexA fusion is not. Two different models can account for the role of the response pathway, and in particular STE12, in α -specific gene induction (see Dolan and Fields 1991; Sengupta and Cochran 1991). STE12 could be present in a complex with $\alpha 1$ and MCM1, with its binding perhaps mediated through a protein–protein interaction with $\alpha 1$. Alternatively, STE12 could bind to PRE sequences present in some unidentified gene whose product modulates the transcriptional activity of $\alpha 1$.

To analyze the roles of STE12 and $\alpha 1$, we have characterized their functional homologs from another budding yeast, *Kluyveromyces lactis*. We find that *K. lactis* STE12 can complement in *S. cerevisiae* for transcription and mating better in α cells than in α cells. However, *K. lactis* STE12 function in *S. cerevisiae* α cells is greatly improved by introduction of the *K. lactis* $\alpha 1$ homolog. These genetic results suggest that STE12 and $\alpha 1$ interact in vivo. In addition, we show that bacterially expressed

STE12 and $\alpha 1$ associate in vitro to form a readily detectable complex.

Results

Cloning and sequencing of the K. lactis STE12 homolog

K. lactis, a yeast related to *S. cerevisiae*, has been successfully used to identify several homologs of *S. cerevisiae* genes, including *GAL4* (Salmeron and Johnston 1986) and heat shock transcription factor (Jakobsen and Pelham 1991). To isolate a *K. lactis* STE12 homolog, we transformed a high copy library of *K. lactis* genomic DNA into an *S. cerevisiae* strain whose relevant genotype is *MATa ste12::LEU2*. Approximately 20,000 transformants were screened for restoration of mating ability by a replica-plating assay. In this assay, transformants are transferred to a lawn of α STE⁺ cells spread on a minimal plate. Neither haploid strain is capable of growth, owing to auxotrophic mutations, but diploids resulting from mating are prototrophs. Four transformants scored positive in this assay. Restriction analysis using several enzymes indicated that the inserts in three of these plasmids were overlapping, and these three were analyzed further. Transformation of subclones localized the complementing activity to a 5-kb *Xba*I fragment. This fragment on a high-copy plasmid restored mating in a *ste12* cells to a level comparable with that provided by the *S. cerevisiae* STE12 gene (see below).

DNA sequence analysis of this fragment revealed an open reading frame of 666 amino acids, which we designated Kl STE12 (Fig. 1). Comparison of this protein sequence with the 688-amino-acid STE12 protein of *S. cerevisiae* (Sc STE12) using the “local homology” algorithm of Smith and Waterman (1981) indicated that the two sequences overall have 40% identity and 58% similarity. Sequence conservation is significantly higher in four regions of the STE12 proteins. The largest of these regions corresponds to the DNA-binding domain, which is found at the amino terminus of the Sc STE12 protein (Yuan and Fields 1991). Residues 30–207 of Sc STE12 are 78% identical to residues 24–201 of Kl STE12. The other three regions of significant homology are short stretches of 12–13 residues (Fig. 2). It is notable that the most carboxy-terminal of these stretches also shows similarity to sequences in the microtubule-associated proteins Tau and MAP2 (Fig. 2) that contain one or two PXT motifs, respectively, which are phosphorylation sites for mammalian MAP kinase (Gonzalez et al. 1991). However, the PXT motif is not found in the Kl STE12 sequence.

To assay the DNA-binding activity of the Kl STE12 protein, we used the amino-terminal 217 residues in a DNase I footprinting experiment (Fig. 3). This fragment was overexpressed in *Escherichia coli* using a T7 polymerase expression system, and the DNA probe was a fragment containing two PREs [designated tail-to-tail double site in Yuan and Fields (1991)]. Both the protection pattern produced by the Kl STE12 fragment and its binding affinity for these sites were similar to that of the

1 AGATCAATTTCTACTGAATAAAGTACCGTGGTTTATTTGTCTCGCCCAATTTCATACAAAGTCTGTTTCATCCCATCTACTGAAAGGAAAAACAGAATTAGGTCG
 101 CTAGGCTGAACAATTGAAAGAAGAAAGAGACCAATCAGCCATTACGGGCTATAAGGTCCGAAGGAAAAGTACTGAGATTTACAATCGCAACAGAATTAACGA
 201 TAGGTTTGGCTGAAAAAGAAGAAGAGCATTCGGTCAATTTGATATTGATATTTTCAAAGTGGTTTAAAGAATACTTGTGATGATATAGCTAATTGGAAT
 301 ACTAATTGATACCTCTCGAAGAGGTTTCGCTCATTTATATAAGGTTTATAGTGGTTCTTTCTATAACCTGAAAAACTAAAGTTAATTAGACTGAAAAAAG
 401 AGACTGGATCTCAACATTAACACTATGGCAGGATCGATTGTAAGACTGAAGATATCAGTTCCATCTCTGGTAGAGGGGACACAGAGTCAATCGCCGGAAG
 M A G S I V K T E D I S S I S G R G D T S Q S P E E
 501 AAGTGGAGGAATCTCTCAGGCTCATTTGAAGATCTCAAAATTTTCTAGCGACACGTCGCCCAATTGGCAAGAAAATCAAGTAATCAGGCGGTATTATTT
 V E E S L R L I E D L K F F L A T R P A N W Q E N Q V I R R Y Y L
 601 GAGTAATGACGAAGGATTTGTATCGTGTGTGTTTGAAGCAATTTATACATATATTACAGGGACAGATATCGTCAGGTGCTGCGCATATAGGATGCAAAA
 S N D E G F V S C V F W N N L Y Y I T G T D I V R C C A Y R M Q K
 701 TTTGGTAGAGAGATTGTAGAAAGGAAAAATTTGAAGAAGGTATCTTTTCAGATCTACGAAACTTGAATGTGGCATTGATGCAACTCTTGAGAAACCTA
 F G R E I V E R K K F E E G I F S D L R N L K C G I D A T L E K P K
 801 AATCGGACTTACTTGCATTCTATATAAGAACATGTGTCTGAAGACACAAAAGAAGCAGAAGGTTTCTTTTGGTTCAGTGTACCACATGACAGATTATT
 S D L L A F L Y K N M C L K T Q K K Q K V F F W F S V P H D R L F
 901 TGCAGATGCACTGGAAGAGATTTGAAACGGTCATCTACTGGTCTCAGCCTACTACTAGGGCTGTAAGCGAACCCGCTTTATCGTTCAGATGGGAAGCT
 A D A L E R D L K R S S T G S Q P T T R A V S E P A L S F R W E A
 1001 AACTCAGATGTATCGCTATACGATCAAACTCAATCATGTAGATTCTCAAAGGACAGATTCAAGGCCGTCGACGGTGGTGGCAGGATGTGACGCAAA
 N S D V S L Y D Q I T N H V D S Q R T D S R P S T V G A Q D V T Q K
 1101 AGCAGAACAATCGTGTCTACTGACACTCCGGTAAGTCTTCAAGGACGTAGAACCATTGAATCTAATGTTGTCAGGATGAAGTCCAAATAGTAGATAA
 Q N N R V T D T P V S A S K D V E P F E S N V V E D E V Q I V D N
 1201 CAACAAGATGTGTACGGGCTGCCGCTCAGAGTGAACAACTATGTGCTCAACAATAATTGTCCTCAGTCTGATCTTGAAAGGAACGAACCTCACA
 N K M C Y G L P H S E S N N Y V P Q Q L I V P Q S D L E R N E L T
 1301 AATGAATTCGATGAATTGAACGCAGATTGAAACCGAGCGATATCTTACTTCCAACCAAGAAGATGATGATTTCCCATTTGGATTACTTCCAGTAGAGA
 N E F D E L N A D L K P S D I L T S N Q E D D D F P L D Y F P V E I
 1401 TAGAATACTCACAACAAGTATGGATAGTTCCTTACACATGGCCTCGCAAGGCGCTAAGCATCCTAGTCAATGATGTTCTACGAAGATATGGACGGAAT
 E Y S Q T S M D S S L H M A S Q G A K H P S Q M M F Y E D M D G M
 1501 GATGCTGGGCCCCAAATACCCAATCTCTGCTGGTATATGAAGATCCTTTTCCGTGAAGAAATGGCAGCTTCAAACGCTCTAAATATATGATGCCA
 M L G P K Y P I S A G I Y E D P F F R E E M A A S N A S K Y M M P
 1601 CCGCAATGTCTAGCTACTAGGGCGCACTTTATGACAAACAGAGAATATTACTCCAAGAGTAGAGAGGGTAAAAAGCATGGGAAATCGGGtaggcaacatg
 P P M S A T R A H F M T N R E Y Y S K S R E G K K H G K S G R Q H G
 1701 gacctgatgaTGGCAGAAGGACTACGATAATGAAACGTTGGTTCCTCCCATCTGAAAACCAAATCCTAATGAAGAACTCGATGCTACTGAAAATAA
 P D D G R R D Y D N E N V G S S P S E N Q N P N E E L D A T E N N
 1801 TCAAGATGCCGATGACAATCGTTCACCTGATATTTACCCGCCAAGTATGATGCAATGGCAATGACGAATATATACCAGCTTACAATAGAAGGATGCAATG
 Q D A D D N R S P D I Y P P T D A M A N D E Y I P A Y N R R M Q M
 1901 AGTGAATCTCTTGTGATCCGTACACTGGAATGATGCTGAACCTTACATGTTCTGTAATATGTTAGCGGTTGATCCATCGTTAAACATGGGAGTCAATC
 S E S L V H P Y T G M M L N P Y M F C N M L A V D P S L N M G V N P
 2001 CGGTAGTAGATCCCTTTTATGGACAGAATCATAGCATGGATGTGGCCACAGATGATATTTCTCTCAGGAGGTGTTTATCTCTTAACCTACAGATCTAC
 V V D P F Y G Q N H S M D V A H D V Y S P Q E V V Y P S N Y R S T
 2101 TCCAAAGGCGCTTACTTCAATATGAGATCGCCTTACGGTAGGAATTTCCACCACCCTCAGCAATGAACCCATATTACACTCCGTATCATCGCAGACAA
 P K A A Y F N M R S P Y G R N F P P P S A M N P Y Y T P Y H R R Q
 2201 CCCTCTTCTGCAACAAGAAGGTATTTCAAGGCAATCTTATCAACAGGAAATCCAAGTCTCCGCCAAGAAAGATGTGGTTTCCAAACCATCTCACA
 P S S A T R R Y F S K A N L I N R K S K S P P R K N V V S K P S H K
 2301 AAGCTCCTAAAAAGTCAATAGAACTAGACACGCTACTTCTATGCAGTGTGGCCTCGGTGGAAGGATTGAGAAACACTTCTTCAAGATCTCGTGATGA
 A P K K V N R T R H A T S M H V G L G G K D S G N T S S E S R D D
 2401 CTCGCAAAAGGAGGACTCTAATTAAGCATTAATTTCTGTAGCAGAATCTTTAAACCTCGCACCTTAGAGGCTATTCAIGTAACATTTTCAACAATTAT
 S T K E D S N *
 2501 TTAATAGACTTCATAATGACACTTTTTTATTTTACGCAACGCTGATCTTATTTGATTGAAAAGAATATCTTCTGCTATAGCAAACAGTTAAAGTTATTGC
 2601 AGAGCAAAAGTACAATATCTAGTACTGTATATACACTTTACCTATCTCCGCTTTCGACGCTGTGAGGTCGAC

Figure 1. Sequence of the *Kl STE12* gene. The DNA sequence of the coding strand is shown, and the deduced amino acid sequence is shown by the single-letter code. A short sequence in the 5' upstream region that matches the consensus sequence of the PRE of *S. cerevisiae* is underlined. (*) An asterisk represents the termination codon.

Sc STE12 amino-terminal fragment. This result confirms that the homologous DNA-binding domains can recognize the same sequence and suggests that the PRE in *K. lactis* is likely to be very similar, if not identical, to that in *S. cerevisiae*.

Complementation by the *K. lactis* STE12 gene for mating and transcriptional activation

To compare the functions of the two *STE12* genes, we introduced them on either a low-copy-number (cen-


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K1 STE12 DFPLDYFPVEIEY 328
      ||| ||| ||| : ||
Sc STE12 DFPLDYFPVSVEY 317

K1 STE12 RAHFMTNREYYS 410
      |. ||| ||| : ||
Sc STE12 RQHFMNTNRFYS 390

K1 STE12 VYPSNYRSTPKAA 563
      |.. ||| ||| . |
Sc STE12 PYTPNYRSTPGSA 535

      Tau  PGTPGSRSTPSPSLP
           * * * * *
      RATMAP2R PGTPSYPRTPGTP
           * * * * *

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Figure 2. The conserved regions between K1 STE12 and Sc STE12. In addition to the DNA-binding domain, three short regions are conserved between K1 STE12 and Sc STE12. The numbers at right correspond to the last amino acid residue in the respective STE12 proteins. The alignment was done with the BestFit command in the GCG programs.

tromere-containing) plasmid or a high-copy-number (2μ) plasmid into *S. cerevisiae* strains that were either a *ste12 Δ* or α *ste12 Δ* . Mating was assessed both by patch mating tests and quantitative assays (Fig. 4). The *Sc STE12* gene on the centromeric plasmid led to a high percentage of mating-competent cells, with the α cell type consistently mating at a somewhat higher frequency than the *a* cell type (Fig. 4). On the high-copy plasmid, the *Sc STE12* gene complemented somewhat less well than on the low-copy plasmid, with α cells again mating ~ 1.5 -fold better than *a* cells. In contrast, the *K1 STE12* gene on the low-copy plasmid complemented for mating very poorly in both cell types compared with the *Sc STE12* gene. In further contrast, the *K1 STE12* gene complemented ~ 14 -fold better in *a* cells than α cells. On the high-copy plasmid, the *K1 STE12* gene provided complementation similar to the high-copy *Sc STE12* gene and functioned only slightly more efficiently in *a* cells than α cells. However, we do not know the relative levels of the *Sc* and *K1 STE12* proteins expressed from these different vectors.

We also compared the ability of the two homologs to mediate transcriptional activation in *S. cerevisiae* on three different cell type-specific genes: *MFA2*, an *a*-specific gene encoding *a*-factor (Michaelis and Herskowitz 1988), *MFa1*, an α -specific gene encoding α -factor (Kurjan and Herskowitz 1982; Flessel et al. 1989), and *FUS1*, a haploid-specific gene necessary for cell fusion (McCaffrey et al. 1987; Trueheart et al. 1987). In each case the upstream regulatory region of the cell type-specific gene was linked to the *E. coli lacZ* gene. For *MFA2* and *FUS1*, the reporter genes were integrated into an *a* strain, and for *MFa1*, the reporter gene was integrated into an α strain. Each of these three yeast strains also contained a *ste12 Δ* allele, such that the plasmid-borne copy of the *S. cerevisiae* or *K. lactis* gene provided the only source of STE12 protein. We measured β -galactosidase activity as an assay of transcription under two conditions: in the

absence of pheromone, or in the presence of α -factor (for *MFA2* and *FUS1*) or *a*-factor (for *MFa1*). For the *FUS1* reporter, the two *STE12* genes on high-copy plasmids provided similar pheromone-inducible transcriptional activity (Table 1). On the centromeric plasmid, the two genes also functioned similarly, promoting virtually no uninduced transcription and mediating 8- to 16-fold induction. For the *MFA2* and *MFa1* reporter genes, there was transcriptional activity in the absence of any *STE12*

Figure 3. DNase I footprinting of the K1 STE12 amino-terminal 217-amino-acid fragment and the *Sc STE12* amino-terminal 215-amino-acid fragment on PRE-containing DNA. The DNA fragment used in this footprinting experiment contains two PREs in tail-to-tail orientation separated by a *SalI* site. Arrows indicate the PREs. (Lane 1) No extract; (lane 8) extract of cells carrying only the vector; (lanes 2–4) sequential 10-fold decreasing amounts of an extract containing *Sc STE12* fragment; (lanes 5–7) sequential 10-fold decreasing amounts of an extract containing K1 STE12 fragment. Lanes 2–4 and lanes 5–7 were normalized to contain equivalent amounts of the *Sc STE12* and K1 STE12 fragments.

Figure 4. Complementation of the *ste12Δ* mutation by the *Kl STE12* gene. (A) Patch mating assays. *Sc STE12*, *Kl STE12*, or vector on either a centromere-containing plasmid or a high-copy 2μ plasmid was transformed into *S. cerevisiae* strains that were either a *ste12Δ* or α *ste12Δ*. The transformants were transferred onto a minimal plate spread with cells of the opposite mating type. (B) Quantitative mating assays. Transformants were grown in selective liquid medium and serial dilutions were spread on YEPD plates to determine cell number and on minimal plates with a lawn of the opposite cell type to determine the number of mating-proficient cells. Mating efficiency is calculated as the number of cells that mated divided by the total number of cells.

gene (Table 1, vector controls). If this activity is taken into account, for the *MFA2* gene, the *Sc STE12* gene on a high-copy plasmid was 2.5- to 3-fold more active than the corresponding *Kl STE12* gene for uninduced and induced transcription. On a low-copy plasmid, the *Kl STE12* gene was inactive for uninduced *MFA2* transcrip-

tion and sixfold less active than the *Sc STE12* gene for induced transcription. The *MFA1* reporter gene behaved differently from the other two. Its transcription was only marginally activated by the *Kl STE12* gene under any conditions. Overall, the *Kl STE12* gene was 30- to 40-fold less active than the corresponding *Sc STE12* gene for either uninduced or induced transcription.

The inability of the *Kl STE12* protein to activate transcription of an α-specific gene suggests that this is the defect in α *ste12Δ* cells producing this protein that leads to low mating proficiency. It also suggests that the *STE12* protein may be functioning by a different mechanism in α-specific transcription than in a- or haploid-specific transcription. If the role of *STE12* in the transcription of α-specific genes was to bind to some gene containing PREs in its upstream region, then it might be expected that the *K. lactis* homolog would be as efficient in this process as in binding to the PREs of a-specific or inducible haploid-specific genes. However, because we observed that this homolog functions much less well in α-specific transcription, we suspected that the *Kl STE12* protein might be defective in its ability to interact with an *S. cerevisiae* α-specific protein.

The Kl STE12 sequence responsible for inefficient mating in α cells maps primarily to the activation domain

To localize the domain of *Kl STE12* that leads to a mating defect in *S. cerevisiae* α *ste12Δ* cells, we first constructed two hybrid genes (see Fig. 5). One of these encodes the amino-terminal 215 residues (DNA-binding domain) of *Kl STE12* and the carboxy-terminal 475 residues (activation domain) of *Sc STE12* (hybrid 1). The other encodes the DNA-binding domain (amino-terminal 215 residues) of *Sc STE12* and the activation domain (carboxy-terminal 451 residues) of *Kl STE12* (hybrid 2). Previously, we had defined the activation domain of the *Sc STE12* protein by mating and by transcriptional assays of a series of carboxy-terminal truncations and internal deletion mutants (Kirkman-Correia et al. 1993). The two hybrid proteins were expressed using the *Sc STE12* upstream region. In addition, we used this upstream region to express the intact *Kl STE12*. All of these constructions were carried on a centromere-containing vector and were introduced into a *ste12Δ* and α *ste12Δ* strains.

Table 1. Activity of *Sc* and *Kl STE12* genes for cell type-specific transcription

Reporter gene plasmid	<i>mfa2::lacZ</i>		<i>fus1::lacZ</i>		<i>mfa1::lacZ</i>	
	– α-factor	+ α-factor	– α-factor	+ α-factor	– α-factor	+ α-factor
2μ Kl STE12	90 ± 30	120 ± 50	5 ± 2	10 ± 4	32 ± 12	52 ± 20
2μ Sc STE12	180 ± 46	300 ± 50	2 ± 0.6	11 ± 4	360 ± 40	440 ± 53
2μ vector	30 ± 2	30 ± 4	0.5 ± 0.05	0.5 ± 0.1	35 ± 6	41 ± 1
CEN Kl STE12	30 ± 8	60 ± 25	0.5 ± 0.1	4 ± 1.6	42 ± 14	47 ± 16
CEN Sc STE12	90 ± 10	210 ± 16	0.5 ± 0.1	8 ± 1.2	260 ± 42	340 ± 30
CEN vector	30 ± 0.5	30 ± 1.5	0.5 ± 0	0.5 ± 0.2	34 ± 10	32 ± 3

Figure 5. The Kl STE12 sequence responsible for inefficient mating in α cells maps primarily to the activation domain of Kl STE12. (A) Schematic of the hybrid proteins. (P) The promoter region; (D) the DNA-binding domain, which is located in the amino-terminal 215 amino acids of Sc STE12 or Kl STE12; (A) the activation domain, which is residues 215–688 of Sc STE12 or residues 215–666 of Kl STE12. The shaded region indicates *S. cerevisiae*, and the open region indicates *K. lactis*. (B) Patch mating assays. Constructions were transformed into either an *a ste12Δ* or $\alpha ste12Δ$ strain, and mating was assayed by transferring patches to a lawn spread with cells of the opposite mating type. (C) Quantitative mating assays. Assays were performed as for Fig. 4. (% of ScSTE12) Calculated by dividing the mating efficiency of each construction by the mating efficiency of wild-type Sc STE12.

The *K. lactis* gene with the *S. cerevisiae* promoter resulted in similar mating relative to this gene with its own promoter (Fig. 5). These two genes also activated the *MFA2* reporter gene to similar levels (data not shown), indicating that the *K. lactis* promoter functioned as well as the *S. cerevisiae* promoter. Hybrid 1 led to a mating efficiency of ~30% of that produced by the Sc STE12 protein in both α and *a* cells. Therefore, although the hybrid with the *K. lactis* DNA-binding domain did not function for mating quite as well in *S. cerevisiae* as the intact Sc STE12, it did not display the cell-type bias of the intact Kl STE12. In contrast to the results with hybrid 1, the mating efficiency of hybrid 2 was ~3% of Sc STE12 in *a* cells but only 0.4% in α cells. These results suggest that the activation domain of Kl STE12 may be primarily responsible for the poor ability of the *K. lactis* protein to function in *S. cerevisiae* α cells.

Cloning and sequencing of the *K. lactis* MAT $\alpha 1$ homolog

To test the hypothesis that the *S. cerevisiae* α -specific protein defective in its interaction with the Kl STE12 protein is $\alpha 1$, we cloned the MAT $\alpha 1$ homolog from *K. lactis*. We reasoned that enhancement of the function of the Kl STE12 protein by the *K. lactis* $\alpha 1$ protein (Kl $\alpha 1$) would be consistent with the idea that there is a direct interaction between these two proteins. We introduced the *K. lactis* library into a *mata1* strain of *S. cerevisiae* and screened for restoration of mating ability. Of ~4,000 transformants, three plasmids complemented the mating defect, and restriction mapping of these plasmids in-

dicated that they contained independent but overlapping inserts (data not shown). We subcloned fragments from one of these inserts and found that the smallest complementing fragment was a 1.4-kb *HindIII* fragment. This 1.4-kb fragment, when present either on a CEN or 2 μ plasmid, efficiently complemented the mating defect of the *mata1* strain (Table 2).

DNA sequence analysis indicated that this fragment could encode a 262-residue protein (Fig. 6) that is homologous to the *S. cerevisiae* $\alpha 1$ protein (Sc $\alpha 1$) of 175 residues (Tatchell et al. 1981). The amino-terminal 192 residues of the predicted Kl $\alpha 1$ can be aligned with gaps with the Sc $\alpha 1$, with an additional nonconserved 70-residue carboxy-terminal tail on the Kl $\alpha 1$ protein (Fig. 7). This tail domain is acidic, in contrast to the region of overlap, which is basic. In this alignment, the two proteins are 30% identical and 47% similar in the conserved region. Analysis of the DNA sequence of the 1.4-kb fragment also revealed residues homologous to the *S. cerevisiae* $\alpha 2$ protein (Tatchell et al. 1981). As in *S. cerevisiae*, the orientation of the MAT $\alpha 1$ and MAT $\alpha 2$ open

Table 2. Complementation of a *mata1* mutation by the *K. lactis* MAT $\alpha 1$ gene

Plasmid	Mating efficiency (%)
Sc ADH $\alpha 1$ CEN	34
Kl ADH $\alpha 1$ CEN	15
Kl $\alpha 1$ CEN	5
Kl $\alpha 1$ 2 μ	11

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1  AAGCTTTCGCACCTTGCTGTGACCATGTCAATTTTGGTTGAAAGTTTCTAATCAACGACTCA
61  ATTTTATGTGAATCGTCATTATTTTCTAGCATTGAATAAAACATCTCTTGAAATGTAGTA
121  ATTACTTTTGCTCTCTCCTTGTGTAAGGTGCTTAAGTCTCTGTAATGTCTGGGTACATTA
181  CTGATGCTTTTACAACCTTCTGATGGGTTTAGCAATGAGTGTATGGGTATTCTACTCATT
241  TTTGCCTTGTGTTGGGGTTGTCCTTCACTGGAATTTATTTTAAGAATTTGGTATAATCTTTC
301  AGCTCAAATGCTCTTTTATACTATTTTTTTTTTAGACATGAAACTTTCTTGAGGCGACAGTGT
361  CAGTTTCATATAAATACCATGATTAATCAGAAGTGAAAGGTGCACAACCTCGACCATTTC
421  ATAGATCCTATCAAAGAAATACAAAAAGTAACGCCAAAAAATGAAATCGAATGCTCCAA
      M K S N A P T
481  CCTTTAAAGTGGCAGTCAGTAAAGAAGCTGTTTCATCGGTAAAGAAGACTTCAAAAAAGA
      F K V A V S K R S C S S V R K T S K K I
541  TTAGGTCTGGAATGATACGTAAAGCCAGTGTAGTTCAAGATATCGAAAACATGAAGGCG
      R S G M I R K P S V S S R Y R K H E G V
601  TAAACCTTTATATGTCAAAGGTTACACCAACGTCCATACCTGCTCCTCTCAAGTACTGG
      N L Y M S K V T P T S I P A P P Q V L V
661  TGGCCTACATAAAGGAGAAGGTAAAGACACTCAATAATCAGAGGTTTAAATGTGCTAG
      A Y I K E K V K T L N K S E V L M S L G
721  GAAATCTAACCAACTTTTCATCTAGGGATGTGAAGACGAAAACATAAAGAGCAGATAA
      N S N Q L S S R D V K T K T K K K Q I N
781  ATGATTTTATAGCTTTCCGAAGCTATTACTCCAGGCTACTGAATGGAATCTTACCCAAA
      D F I A F R S Y Y S R L L N G I L T Q T
841  CAGAGTTATCAACAATAATTTCTAAACACTGGACAGTAGATAAACAGACAAGAAAGATT
      E L S T I I S K H W T V D K Q T R K N W
901  GGGAAATTGATCGCACGGAATATACTGCGATGCCCTCAGGCAAGCATTTTAAATTGGT
      E L I A Q E Y N C D A S G K H F F N W L
961  TAGAAGTTAATATGGTATCGATAAACAGTGGTTGTATGAAATGTACAATACGAAGAGT
      E V N Y G I D K Q W L Y E I V Q Y E E C
1021 GCTTGACACCAACAACAAAAAGCCGTACGTGGAAAAACATTACAACAGCGGTTTAAAGC
      L T P T T K K P Y V E N I Y N S G F K Q
1081 AAAGAATAGATTCCCCAAACAAAAAGACATCAGTTCTCTCAATCTGATAATTCGGCAG
      R I D S P N Q K D I T F S Q S D N S A D
1141 ATTGGCTTGTGTGATCCCATATTTTGTGATCTAGTTCCTTGCCACCTCTTTGTAGAA
      W L V D P I F F D T E F L A T S F D R T
1201 CAAATCTAATCATTCTGATTGCTATTCTTGATGAACCTCAGTCTGAGTAACTTAATTTTC
      N L I I S D C Y S L M N S V *
1261 ACCAATTAACATAATGAATGATATATGTATATAACAATATATACGGTAAATAAAATGCA
1321 TCAAACCTTCATCACTATCACCATATCTGTAACATATTTGTCTAATTTTACCCAACTCTT

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Figure 6. Sequence of the *Kl MATα1* gene. The DNA sequence of the coding strand is shown, and the deduced amino acid sequence is shown by the single-letter code. An asterisk (*) represents the termination codon. Also shown (underlined) is the possible start of the open reading frame for the *MATα2* gene.

reading frames indicates that these genes should be transcribed in divergent directions. The *K. lactis* *HindIII* fragment could encode the amino-terminal 79 residues of a putative α2 protein (which is 211 residues in *S. cerevisiae*). The first 12 residues of the α2 homologs would be 67% identical, although the remainder of the available *K. lactis* sequence lacks significant homology to its *S. cerevisiae* counterpart. In addition, none of the three large inserts of *K. lactis* DNA identified as complementing the *mata1* mutation could complement a *mata2* mutation, suggesting that the α2 proteins may have diverged significantly.

The Kl MATα1 gene can enhance the mating efficiency of α ste12 cells expressing the Kl STE12 gene

We tested whether the *Kl MATα1* gene could increase the poor mating efficiency of *S. cerevisiae* α *ste12* cells

carrying the *Kl STE12* gene on a centromeric vector. For this purpose, we generated an *S. cerevisiae* strain whose relevant genotype is *mata1 ste12*, such that the only sources of α1 and STE12 proteins would be from genes introduced on plasmids. The coding sequence for the Kl α1 protein was placed under the transcriptional control of the *ADH1* promoter on a centromeric plasmid, similar to a construction used to express the Sc α1 protein (Ammerer et al. 1985). We compared the mating ability of the *MATα ste12* strain carrying only a *STE12* plasmid with the mating ability of the *mata1 ste12* strain carrying both *MATα1* and *STE12* plasmids (Fig. 8). Transformation of the *mata1 ste12* strain with the *Sc STE12* and *Sc MATα1* genes led to highly efficient mating (Fig. 8, line 1), as expected for the homologous genes. Similarly, the double mutant carrying the *Sc STE12* and *Kl MATα1* genes mated at a high efficiency (Fig. 8, line 1), which reflects the fact that the *Kl MATα1* plasmid comple-

Figure 7. Comparison of the amino acid sequences of Kl $\alpha 1$ and Sc $\alpha 1$. The alignment was done with the BestFit command in the GCG programs.

2). Mating promoted by hybrid 2 was stimulated >60-fold by the presence of the *Kl MAT α 1* gene, and only fivefold by the presence of the *Sc MAT α 1* gene (Fig. 8, line 3). These results therefore indicate a genetic interaction between the STE12 and α 1 proteins and are consistent with this interaction being mediated primarily through the carboxy-terminal sequences of the STE12 protein.

We sought to determine whether a physical interaction between STE12 and $\alpha 1$ could also be demonstrated. We synthesized both of the *S. cerevisiae* proteins in *E. coli*: STE12 as a fusion protein with glutathione S-transferase (GST) and $\alpha 1$ without any additional modifications. Extracts of the two bacterial cultures were prepared and

Figure 9. $\alpha 1$ binds to a GST-STE12 fusion protein in vitro. Western blot using an $\alpha 1$ -specific antibody. Lanes 1–8 show binding assays in which one or two *E. coli* extracts (as indicated) were incubated in the presence of glutathione–agarose, followed by washing of the agarose pellet and fractionation on a 15% polyacrylamide gel. Lane 7 contains a GST fusion with a protein that binds the human p53 protein, and lane 8 contains a GST fusion with the *Salmonella* InvE protein (provided by C.C. Ginocchio and J. Galan, State University of New York, Stony Brook). For lane 4, the incubation was carried out in the presence of 200 $\mu\text{g}/\text{ml}$ of ethidium bromide. Lanes 9 and 10 are markers for the $\alpha 1$ protein, lane 9 is the IPTG-induced *E. coli* extract containing the vector, lane 10 is the IPTG-induced *E. coli* extract containing pTAC $\alpha 1$.

incubated together in the presence of glutathione–agarose. The agarose-bound GST-STE12 was pelleted, the pellet was washed extensively, and the bound proteins were analyzed by SDS-PAGE. The presence of $\alpha 1$ in the pellet was detected by immunoblotting using an $\alpha 1$ -specific polyclonal antibody (a gift of G. Sprague) prepared against a carboxy-terminal peptide. This antibody also detected nonspecific bacterial proteins not dependent on the expression of either a GST fusion or the $\alpha 1$ protein.

$\alpha 1$ was found in the glutathione–agarose pellet only when the incubation mixture also included GST-STE12 (Fig. 9, lane 3). Neither the GST protein alone (lane 2) nor two unrelated GST fusion proteins (lanes 7,8) resulted in any detectable $\alpha 1$ on the beads. To ensure that the binding between STE12 and $\alpha 1$ did not reflect a DNA-mediated interaction as a result of contamination of DNA in the extracts (Lai and Herr 1992), we tested the effect of including 200 $\mu\text{g}/\text{ml}$ of ethidium bromide. The presence of this intercalating agent had no effect on the binding of $\alpha 1$ to the GST-STE12 (lane 4). These experiments indicate that STE12 and $\alpha 1$ can interact in vitro and that this interaction does not require either any specific DNA sequence or any other yeast protein.

Discussion

Transcription of the yeast cell type-specific genes is dependent on the combinatorial controls exerted by the MAT-encoded proteins, by proteins that compose the pheromone response pathway, and by the MCM1 protein. In the case of *a*-specific genes, transcriptional activation is not dependent on any proteins encoded by MAT. Instead, MCM1 and STE12 are necessary for activation, with STE12 being the pheromone-responsive component (Fig. 10). In the case of α -specific genes, the binding site for $\alpha 1$ and MCM1 confers both cell type specificity as well as transcriptional induction by the *a*-factor pheromone; both of these transcriptional activities are also dependent on the STE12 protein. How do the proteins bound at the α -specific UAS respond to the *a*-factor signal? Our genetic and biochemical results indicate a direct interaction between the STE12 and $\alpha 1$ proteins, suggesting that STE12 may mediate the signal for induction of α -specific gene transcription (Fig. 10). A similar model was proposed by Sengupta and Cochran (1991) based on their data indicating that $\alpha 1$ is the STE12-responsive component functioning at the α -specific UAS.

We have isolated the *STE12* and *MAT $\alpha 1$* homologs from the yeast *K. lactis* and analyzed their ability to promote transcription and mating in *S. cerevisiae*. Our genetic evidence for a STE12– $\alpha 1$ interaction derives from several findings. First, the *Kl STE12* gene on a low-copy plasmid is capable of restoring *STE12* function to an *S. cerevisiae a ste12* mutant considerably better than to an $\alpha ste12$ mutant. By quantitative mating assays, this difference is at least 10-fold. By transcriptional assays of

Figure 10. Model for the activation of *a*-specific and α -specific transcription. (A) In *a* cells, MCM1 and STE12 are necessary for the transcription of *a*-specific genes, with STE12 binding to DNA and acting as the target of the activity of the pheromone response pathway (wavy arrow). (B) In α cells, $\alpha 1$, MCM1, and STE12 are necessary for the transcription of α -specific genes, with STE12 also responding to the pheromone response pathway but held in the complex by interaction with $\alpha 1$. STE12 could also be interacting with DNA and/or MCM1.

cell type-specific genes, an α cell carrying the *Kl STE12* gene expresses an α -specific gene at a level two- to sixfold lower than if it carries the *S. cerevisiae* gene. In contrast, the difference in an α cell expressing an α -specific gene is 30- to 40-fold. The *Kl STE12* protein has a similar affinity for the PRE binding site of α -specific genes as has the *S. cerevisiae* protein and a similar capacity to induce transcription in response to pheromone. Therefore, its differential activity in the two haploid cell types appears to be primarily the result of its poor ability to act through the $\alpha 1$ /MCM1-binding site.

Second, a hybrid protein consisting of the DNA-binding domain of the *Kl STE12* protein and the activation domain of the *Sc STE12* protein functions efficiently and promotes mating equally in α and α cells. This result supports the idea that binding to PREs through the STE12 DNA-binding domain is similar for the two homologs. In contrast, a hybrid protein consisting of the *Sc STE12* DNA-binding domain and the *Kl STE12* activation domain promotes mating at low levels and is much less efficient in α cells than in α cells. Thus, it is likely that the carboxy-terminal STE12 sequences involved in transcriptional activation and induction are defective in interaction with some α -specific protein.

Third, and most significant, the poor mating mediated by the *Kl STE12* gene in α cells can be enhanced 50-fold more by the presence of the *Kl MAT $\alpha 1$* gene than by that of the *Sc MAT $\alpha 1$* gene. This synergistic interaction of the two *K. lactis* homologs is suggestive of a physical interaction between the two proteins, which appears to involve at least the carboxy-terminal domain of STE12. Formally, this specificity could be thought of as an evolutionary allele-specific suppression, which is a genetic result consistent with direct protein-protein interaction.

Our genetic findings are supported by the ability of the GST-STE12 fusion protein to bind $\alpha 1$. This binding indicates that the STE12- $\alpha 1$ interaction can occur in solution and does not require DNA binding by either protein. However, we cannot exclude the possibility that in vivo STE12 does contact DNA at the α -specific UAS. In addition, the ability of bacterially derived STE12 and $\alpha 1$ to bind indicates that other yeast proteins or yeast-dependent modifications are not essential for this interaction. However, such potential modifications, which might be catalyzed by activities of the pheromone response pathway in the absence or presence of pheromone stimulation, could change the affinity or the conformation of the STE12- $\alpha 1$ complex.

The STE12 homolog

The *Sc STE12* protein has been shown to possess several activities, including DNA binding, differential transcriptional activation in the absence and presence of pheromone treatment, and interaction with the MCM1 protein (Dolan et al. 1989; Errede and Ammerer 1989; Song et al. 1991; Yuan and Fields 1991; Kirkman-Correia et al. 1993). The DNA-binding domain has been postulated to consist of a highly divergent homeo domain, with loops between helices 1 and 2 and between helices 2 and 3

(Yuan and Fields 1991). A comparison of the *Sc STE12* with the *Kl STE12* shows that this DNA-binding domain is the only large region of high homology. The two proposed loops are as conserved as the proposed helical domains, implying that they may carry out an essential function, perhaps STE12-STE12 cooperativity.

In addition to the DNA-binding domain, three short sequences are highly conserved. The first two of these are located in the region of STE12 required for transcriptional induction in response to pheromone (Song et al. 1991; Kirkman-Correia et al. 1993). Although the absolute levels of α - and haploid-specific transcription mediated by *Kl STE12* were lower than that by *Sc STE12*, a similar pheromone induction ratio was observed. This result argues that the *Kl STE12* protein is capable of responding to the pheromone-generated signal, which may occur through STE12 phosphorylation (Song et al. 1991). The third conserved sequence lies in the domain of STE12 required for cooperative interaction with MCM1 (Errede and Ammerer 1989) and required for negative regulation of activity (Song et al. 1991). These conserved sequences might be recognized by proteins of the pheromone response pathway, such as protein kinases. Determination of the role of these sequences awaits their specific mutagenesis, as well as the cloning of the *K. lactis* MCM1 homolog.

As in the *Sc STE12* gene, the upstream regulatory sequence of the *Kl STE12* gene contains sequences that may function as PREs. These include one copy of a consensus PRE (TGAAACA) (Kronstad et al. 1987; Van Arsdell and Thorner 1987) 356 nucleotides upstream of the first codon and two sequences that contain a 6/7 match 304 and 413 nucleotides upstream of the first codon. On the basis of *lacZ* fusions, transcription of the *Sc STE12* gene is severalfold inducible by pheromone treatment (J.W. Dolan and S. Fields, unpubl.). The presence of these PREs in the *Kl STE12* gene suggests that transcription of this gene may also be pheromone inducible, providing a mechanism for amplification of the pheromone signal.

The MAT $\alpha 1$ homolog

The $\alpha 1$ protein has several functions. It may contact DNA, both double-stranded and single-stranded, it interacts with MCM1, and it responds to the signal generated by α -factor treatment (Bender and Sprague 1987; Jarvis et al. 1988; Sengupta and Cochran 1991; Grayhack 1992). The *Kl* $\alpha 1$ protein shows an overall lower similarity to its counterpart than does the *Kl STE12* homolog. However, the efficient mating mediated by the *Kl* $\alpha 1$ protein in the *S. cerevisiae mata1* mutant suggests that the sites for contacting the *S. cerevisiae* MCM1 and STE12 proteins are conserved in *Kl* $\alpha 1$. Comparison of the $\alpha 1$ sequences indicates that the most highly conserved region corresponds to the residues in *Sc* $\alpha 1$ that have the only significant homology to the *Neurospora crassa mtA-1* product (Glass et al. 1990) (Fig. 11). Strikingly, this same region also shows some similarity to sequences present in the SAP-1 protein (homology region B) and the elk-1 protein (Fig. 11), two Ets-related proteins that interact

K. lactis $\alpha 1$	K K Q I N D F I A F R S Y Y S R L L N G I L
S. cerevisiae $\alpha 1$	K K Y L N S F M A F R A Y Y S Q F G S G V K
N. crassa mtA-1	K K K V N G F M G F R S Y Y S P L F S Q L P
SAP-1	T S S R N D Y I H S G L Y S S F T L N S L N
ELK-1	R S S R N E Y M R S G L Y S T F T I Q S L Q

Figure 11. Similarity between $\alpha 1$ proteins, *N. crassa* mtA-1, and ets-like proteins that interact with SRF. This region of similarity may indicate the residues of the $\alpha 1$ proteins that interact with MCM1.

with serum response factor (SRF) at the *c-fos* serum response element (Hipskind et al. 1991; Dalton and Treisman 1992). SRF is structurally related to MCM1, and the homology region B of SAP-1 appears to be involved in the interaction of this protein with SRF (Dalton and Treisman 1992). The presence of this similarity in the five proteins allows us to speculate that these residues in $\alpha 1$ may be involved in the interaction with MCM1.

Although the $\alpha 1$ protein may make sequence-specific DNA contacts, it has not been placed in any of the large families of sequence-specific DNA-binding proteins, such as homeo domain, zinc finger, or helix-loop-helix. We note that the two $\alpha 1$ homologs fail to display any large region of extensive homology, as do the STE12 homologs, that might reflect residues involved in DNA binding. Near the carboxyl terminus of the $\alpha 1$ proteins, however, are two conserved tryptophan residues spaced 19 residues apart, and such residues and spacing are characteristic of the DNA-binding domain of Myb proteins (Frampton et al. 1991) and Ets proteins (Macleod et al. 1992). In Kl $\alpha 1$, one of these tryptophans is part of the sequence NWEIIA, which is highly similar to NWEIIG of *Drosophila* Myb and DWTLIA of human A Myb (Katzen et al. 1985; Nomura et al. 1988). Other conserved features of Myb or Ets proteins, though, do not appear to be present in the $\alpha 1$ proteins.

Implications for control of cell type-specific transcription

The interaction between a protein involved in cell identity ($\alpha 1$) with one involved in signal response (STE12) suggests an additional mechanism to couple these two processes. α -Factor induction of α -specific transcription could occur by modification of STE12 to a more active form, which collaborates with $\alpha 1$ and MCM1 at the α -specific UAS to increase the frequency of transcription initiation. On the basis of studies of a GAL4-STE12 hybrid in α cells responding to α -factor (Song et al. 1991), a likely possibility is that the activated STE12 is hyperphosphorylated. Such modification could enhance STE12- $\alpha 1$ interaction, or it could result in conversion of STE12 to a more potent transcriptional activator.

The idea of cross coupling of distinct pathways in the control of eukaryotic transcription is also emerging from studies of mammalian systems. For example, the Jun-Fos proteins and steroid hormone receptors appear to interact with each other to mediate transcriptional repres-

sion (for review, see Ponta et al. 1992). Similarly, the MyoD and Jun proteins interact physically, and each protein can suppress the transcription mediated by the other (Bengal et al. 1992). The DNA-binding activity of SRF can be enhanced by certain human homeo domain proteins, a phenomenon that also couples a protein involved in signal transduction with proteins that play a role in cell identity (Grueneberg et al. 1992). Our results with yeast α -specific transcription suggest the likelihood of additional cases of cross coupling leading to transcriptional activation. Furthermore, such activation might include other examples of the collaboration among proteins from the homeo domain, SRF, and Myb- or Ets-like families.

Materials and methods

Yeast strains

S. cerevisiae strain CK3-1B1 (*MAT α ste12::LEU2 mfa2::lacZ trp1 ura3 his4*) (Kirkman-Correia et al. 1993) was used for the cloning of the STE12 homolog, and strain 23 α 189 (*mata1 trp1 leu2 ura3 his4*) (Siliciano and Tatchell 1984) was used for the cloning of the MAT α 1 homolog. A strain carrying both *ste12::LEU2* and *mata1* mutations was constructed by crossing strain 23 α 189 (*mata1 trp1 leu2 ura3 his4*) (Siliciano and Tatchell 1984) carrying a wild-type MAT α 1 gene on a plasmid and SF208 (*MAT α ste12::LEU2 trp1 ura3 his4*) carrying a wild-type *Sc* STE12 gene on a plasmid. The diploid cells were allowed to lose both of the plasmids and were then sporulated. Tetrads were tested for mating ability, pheromone production, and nutritional markers to identify a strain with the *ste12::LEU2* and *mata1* mutations. Mating tester strains were 227a (*MAT α cry1 lys1*) or AM227 α (*MAT α lys1*). Reporter gene expression was assayed in strain CK3-1B1, strain CK2-6b (*MAT α mfa1::lacZ ste12::LEU2 his4 his3⁺ trp1 ura3*) (Kirkman-Correia et al. 1993), and strain SF458 (*MAT α fus1::lacZ ste12::LEU2 trp1 his4*).

Plasmid constructions

All of the STE12 constructions in Figures 5 and 8 are based on YCp50, which is a centromere-based plasmid carrying the URA3 gene. *Sc* STE12 is the intact *Sc* STE12 gene inserted into YCp50 (Kirkman-Correia et al. 1993). *Kl* STE12 is the intact *Kl* STE12 gene inserted as a *Bam*HI-*Hind*III fragment into YCp50, in which both *Bam*HI and *Hind*III sites are obtained from the polylinker region of the Bluescript vector. Hybrid 2, which contains the amino-terminal 215 residues of *Sc* STE12 and the carboxy-terminal 451 residues of *Kl* STE12, was made from *Sc* STE12. A PCR fragment containing the *Kl* STE12 sequence with a 5' *Xba*I site and 3' *Eco*RI site was used to replace the *Xba*I-*Eco*RI fragment of *Sc* STE12. Hybrid 1, which contains the amino-terminal 215 residues of *Kl* STE12 and the carboxy-terminal 475 residues of *Sc* STE12, and *Kl* STE12(*Psc*), which contains the intact coding region of *Kl* STE12 under the control of the *Sc* STE12 promoter, were made from a modified *Sc* STE12 clone in which an *Mlu*I site was created just before the first amino acid codon. Hybrid 1 was made from a PCR fragment containing the first 215 residues of *Kl* STE12 plus an *Mlu*I site at its 5' end and an *Xba*I site at its 3' end, which was cloned into the modified *Sc* STE12 clone to replace the *Mlu*I-*Xba*I fragment of *Sc* STE12. *Kl* STE12(*Psc*) was made by a similar strategy: A PCR fragment containing the intact coding region of *Kl* STE12 plus an *Mlu*I site at its 5' end and a *Bam*HI site at its 3' end was

cloned into the modified *Sc STE12* clone to replace the *MluI*–*BamHI* fragment. The *Kl ADH1*–*MAT α 1* gene was constructed using a PCR fragment that contained the coding region with flanking *HindIII* sites. This fragment was cloned into *HindIII*-digested pAAH5 (Ammerer 1983), and the resultant *BamHI* fragment containing the *MAT α 1* gene with *ADH1* promoter and terminator was moved into YCplac22 (Gietz and Sugino 1988). For expression of the *Kl STE12* DNA-binding domain in *E. coli*, a PCR fragment corresponding to amino acid residues 1–217 was synthesized with flanking *BamHI* sites. This *BamHI* fragment was ligated into the T7 expression vector pET-3c (Studier et al. 1990).

Cloning and mapping of the *Kl STE12* and *MAT α 1* genes

The *K. lactis* genomic library, which was generously provided by Chiron, is present on a 2 μ plasmid vector with the *URA3* gene as a selectable marker. Transformation of strain CK3-1B1 or 23 α 189 was done by the high-efficiency method of Schiestl and Gietz (1989). After 2–3 days, growth on SD–ura plates (Sherman et al. 1986), the Ura⁺ transformants containing the *K. lactis* library were transferred to a lawn of the opposite type of cells spread on a minimal plate. The plasmids were isolated from all of the mating-positive colonies (Hoffman and Winston 1987), amplified in *E. coli*, and retransformed back into the proper strain to confirm their ability to confer mating.

Subclones generated by restriction enzyme digestions were used to map both the *Kl STE12* and *Kl MAT α 1* genes. The original clones, which were ~12–15 kb, were digested with *XbaI*, *HindIII*, or *PstI*, and the pools of DNA were subcloned into the vector YEpl352 (Hill et al. 1986). Pools of subclones were then transformed back into the appropriate yeast strain, and the transformants were assayed for mating ability. Plasmids were purified from the mating-positive clones and analyzed for the size of their inserts. The *Kl STE12* gene was localized to a 5-kb *XbaI* fragment and the *Kl MAT α 1* gene to a 1.4-kb *HindIII* fragment. Furthermore, because *EcoRI* digestion of the original *Kl STE12* clone did not result in any mating-positive transformants, it was likely that there was at least one *EcoRI* site in the coding region of the *Kl STE12* gene. Therefore, we sequenced the *Kl STE12* gene beginning from this *EcoRI* site in both directions.

Sequencing and sequence alignment

DNA sequencing was done by the dideoxy chain-termination method (Sanger et al. 1977) modified for double-stranded DNA. Sequence alignment was performed by the BestFit command in the GCG programs.

Footprinting assay

The amino-terminal 215-residue fragment of *Sc STE12* and the 217-residue fragment of *Kl STE12* were synthesized in *E. coli* and used in a footprinting assay as described by Yuan and Fields (1991). Bacterial extracts were fractionated on an SDS–polyacrylamide gel and stained with Coomassie blue to normalize the amounts of the two different *STE12* fragments used in the footprinting reaction. For Figure 3, lanes 2–4 contained 1.5, 0.15, and 0.015 μ g, respectively, of total protein, and lanes 5–7 contained 1.0, 0.1, and 0.01 μ g, respectively, of total protein. Lane 8 contained 1 μ g of total protein from the control extract.

Mating assay

The patch mating assay was used as a qualitative measurement of mating efficiency. Cells were inoculated onto an SD plate

with the appropriate amino acid supplements. After 2 days growth, the well-grown patches were replica plated to the mating plates, which are SD-minimal plates spread with the opposite type of cells, either 227 α or AM227 α . The diploid cells formed colonies after 3 days of incubation.

For the quantitative mating assay, cells were grown in SD medium with the appropriate amino acid supplements to ~10⁷ cells/ml. Aliquots of serial dilutions were plated onto SD-minimal plates spread with the tester cells to determine the number of mating-competent cells and onto YEPD plates (Sherman et al. 1986) to determine the total number of the cells. The mating efficiency is the number of cells that mated divided by the total number of cells.

β -Galactosidase assay

For an α -specific gene, the transcription of *MFA2* was measured in strain CK3-1B1; for an α -specific gene, the transcription of *MFA1* was measured in strain CK2-6b; for a haploid-specific gene, the transcription of *FUS1* was measured in strain SF458. A plasmid carrying the *STE12* gene was transformed into the appropriate strain. The transformants were grown in selective SD medium to an OD₆₀₀ between 0.5 and 1, and half of each culture was incubated for 2 hr with 1 μ M synthetic α -factor from Sigma or α -factor obtained as the supernatant of a saturated culture of α cells. β -Galactosidase assays were carried out as described (Dolan and Fields 1990).

In vitro binding

The GST–*STE12* fusion was constructed by ligating the *EcoRI* fragment containing the *STE12* gene from OF22 (Song et al. 1991) into pGEX1 (Smith and Johnson 1988). *E. coli* containing the GST–*STE12* (or control plasmids) were grown to an OD₆₀₀ of ~1. Expression was induced for 1.5 hr by the addition of IPTG to 0.4 mM. Cells were pelleted, washed in 100 mM NaCl, 10 mM Tris-HCl at pH 8.0, 1 mM EDTA, and lysed in GEX-C buffer [20 mM HEPES at pH 7.6, 100 mM NaCl, 1.2 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 1% Triton X-100, 0.1 μ M PMSF, 5 μ g/ml of leupeptin, 5 μ g/ml of pepstatin] by two passages in a French press (14,000 psi). The lysate was clarified by centrifugation at 10,000 rpm for 10 min (SS34 rotor) and 100,000 rpm for 15 min (TL100 ultracentrifuge), and the supernatant was frozen in aliquots at –70°C. The $\alpha 1$ protein was produced using plasmid pTAC $\alpha 1$ (Grayhack 1992). As a control, the Tac promoter and $\alpha 1$ -coding sequences were deleted by digestion with *EcoRI* and *HindIII*, followed by treatment with DNA polymerase I (Klenow fragment) and religation.

The binding reaction was carried out for 2.5 hr at 4°C with 1 mg of GST extract, 100 μ l of $\alpha 1$ extract, and 200 μ l of a 1 : 1 slurry of glutathione–agarose (Sigma) in 1 ml of GEX-C buffer. The agarose was pelleted by a 5-sec spin in a microcentrifuge and washed 4 $\times$ with 1 ml of GEX-C buffer. Pelleted material was analyzed by electrophoresis in a 15% SDS–polyacrylamide gel, and the fractionated proteins were transferred to nitrocellulose (Hybond-ECL, Amersham). The blot was blocked for 1 hr in 5% nonfat dry milk, 10 mM Tris-HCl (pH 8.0), and 150 mM NaCl, and then incubated for 1 hr in a 1 : 200 dilution of polyclonal antiserum 191 against the carboxy-terminal 9 residues of *Sc* $\alpha 1$ (Bender and Sprague 1987) in TBST (10 mM Tris-HCl at pH 8.0, 150 mM NaCl, 0.05% Tween 20). The blot was washed 4 $\times$ (15 min each) in TBST and incubated for 1 hr in TBST containing a 1 : 4000 dilution of horseradish peroxidase-conjugated goat antirabbit antibody (U.S. Biochemical). The blot was washed again as described above and developed using the ECL detection kit (Amersham). For binding in the presence of ethid-

ium bromide, the GST fusion protein was incubated with ethidium bromide for 0.5 hr at 4°C, the $\alpha 1$ protein extract was then added, and incubation continued for another 2 hr.

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Note added in proof

The Kl STE12 and Kl MAT $\alpha 1$ sequence data have been submitted to the GenBank data library under accession numbers L21156 and L21155, respectively.

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