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Functional Domains of the Yeast STE12 Protein, a Pheromone-Responsive Transcriptional Activator

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The pheromone response pathway of the yeast *Saccharomyces cerevisiae* is necessary for the basal level of transcription of cell-type-specific genes, as well as the induced level observed after pheromone treatment. The STE12 protein binds to the DNA sequence designated the pheromone response element and is a target of the pheromone-induced signal. We generated 6-nucleotide linker insertion mutants, internal-deletion mutants, and carboxy-terminal truncation mutants of STE12 and assayed them for their ability to restore mating and transcriptional activity to a *ste12Δ* strain. Two of these mutant proteins retain the capacity to mediate basal transcription but show little or no induced transcription upon pheromone treatment. Cells producing these proteins cannot mate, formally demonstrating that the ability to respond to pheromone by increasing gene expression is essential for the mating process. Since distinct domains of STE12 appear to be required for basal versus induced transcription, we suggest that the pheromone-induced signal is likely to target residues of the protein different from those targeted by the basal signal because of the constitutive activity of the response pathway. Our analysis of mutant STE12 proteins also indicates that only the DNA-binding domain is sensitive to the small changes caused by the linker insertions. In addition, we show that, while the carboxy-terminal sequences necessary for STE12 to form a complex with the transcription factor MCM1 are not essential for mating, these sequences are required for optimal transcriptional activity.

Haploid cells of *Saccharomyces cerevisiae* are of mating type **a** or α, which differ principally in the kind of pheromone and pheromone receptor produced; **a** cells synthesize **a**-factor and the receptor for α-factor, and α cells synthesize α-factor and the receptor for **a**-factor. Exchange of the pheromones activates a signaling pathway that leads to changes in transcription, cell cycle arrest, and ultimately, mating (for reviews, see references 3, 21, and 38). The ability of **a** and α cells to display these phenotypic differences is the result of cell-type-specific transcription (for reviews, see references 5 and 16). This transcription is regulated by DNA-binding proteins that are either synthesized in a cell-type-specific manner (the *MAT* products), expressed in both **a** and α cells with activity regulated by the pheromone signal from the opposite mating type (*STE12* product), or ubiquitously expressed and not regulated by signaling (the *MCM1* product). This combinatorial control, thus, provides a model for understanding cell-type-specific and hormonally induced transcription in more-complex systems.

STE12, a protein of 688 amino acids, binds to the pheromone response element found in the upstream region of many genes inducible by α-factor or **a**-factor (6, 9). The minimal DNA-binding domain of the protein lies between residues 40 and 204 and shows a low level of homology to the homeodomain (42). STE12 becomes rapidly phosphorylated after treatment of cells with pheromone, a phenomenon consistent with hyperphosphorylated forms of the protein being the active species for transcriptional induction (34). In addition to promoting this induced transcription in cells treated with pheromone, STE12 as well as other components of the pheromone response pathway is required for basal levels of cell-type-specific transcription (10, 11, 14, 15, 22, 41). This requirement suggests that the pathway is constitutively active at a low level, maintaining STE12 in a partially modified state for basal transcription (see reference 4 for a discussion).

We present here a mutational analysis of STE12, including linker insertion mutants, internal-deletion mutants, and carboxy-terminal truncation mutants. These mutants delineate regions in this protein capable of mediating basal transcriptional activation and other regions required for mediating transcriptional induction in response to pheromone treatment. The fact that distinct domains appear to be required for these two functions has implications for how this response pathway operates to modulate transcription.

MATERIALS AND METHODS

Yeast strains and methods. Yeast strains used were SF167-5a (*MAT***a** *ste12::LEU2 leu2 ura3 his4 trp1*) (12), 469 (*MAT***a** *ste12::LEU2 MFA2-lacZ leu2 ura3 his4 trp1*), which is derived from a cross of an **a** strain carrying the *MFA2-lacZ* allele (provided by Susan Michaelis) with an α *ste12::LEU2* strain, IS-4D (*MAT***a** *ste12::LEU2 FUS1-lacZ::LEU2 ura3 leu2 trp1 his3 lys2 can1*), and 271 (*MAT*α *lys1*). Media for growing *S. cerevisiae* were either yeast extract-peptone-dextrose, synthetic complete medium lacking uracil (SD-ura), or synthetic minimal media (SD) (33). Transformation into *S. cerevisiae* was performed by the lithium chloride method (19). Mating was tested by assaying the ability of patches to form diploids in a procedure in which transformants were grown on an SD-ura plate and replica plated onto a lawn of tester α cells on an SD minimal plate which was incubated for 2 to 3 days. Only diploids are capable of growth on the minimal plate because of the presence of different auxotrophies in each of the haploid stains. Quantitative mating assays were performed by growing transfor-

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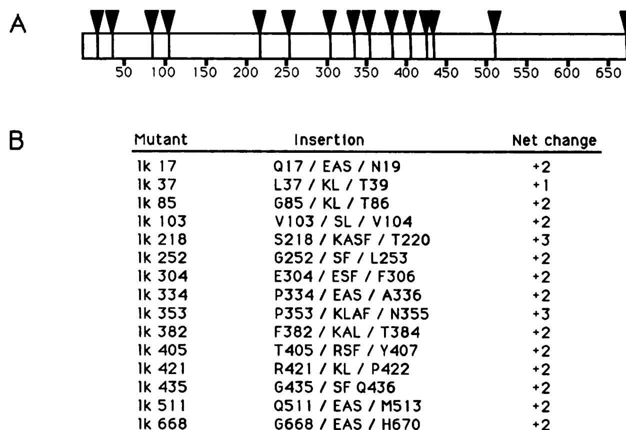
mants in SD-ura medium to mid-logarithmic phase, mixing them with an excess of the α STE^{+} strain, and plating the mixtures on SD minimal plates to select diploids. Total numbers of cells tested in the assay were determined by plating diluted aliquots on yeast extract-peptone-dextrose plates. The mating efficiency is the number of diploids divided by the number of haploid **a** cells in the mating reaction.

Plasmids. A 5-kb *Sph*I-*Cla*I fragment containing the *STE12* gene (12) was cloned into the vector YCp50 (30), which had been cut with *Sal*I and *Eco*RI. The *Hin*dIII site was destroyed by filling in the 5′ overhang with DNA polymerase I (Klenow fragment) (31) to generate plasmid p650. For insertion of linkers, this plasmid was linearized by one of two methods, either treatment with dilute DNase I in 50 mM Tris-Cl (pH 7.5)–1 mM $MnCl_2$ or partial digestion with *Alu*I, *Hae*III, *Xmn*I, *Rsa*I, or *Mbo*II. The linear DNAs were separated from uncut or multiply cut forms by electrophoresis on a 0.7% agarose gel and purified by adsorption onto glass (Geneclean; Bio 101, Inc.). The phosphorylated *Hin*dIII linker AAGCTT was ligated to the linear plasmid, and the ligation mix was used to transform *Escherichia coli*. A pool of transformants was used to obtain plasmid DNA, which was cut with *Hin*dIII, and then linear DNA was purified, ligated, and transformed. This two-step procedure resulted in a significant enrichment of plasmids that contained an inserted linker. Individual plasmids were screened by *Hin*dIII and *Sac*I double digestion to identify those with a linker within the *STE12* coding sequence. The exact site of the insertion was identified by DNA sequencing using the dideoxy method (32).

Carboxy-terminal truncations were generated by cutting plasmids containing a linker insertion with *Hin*dIII and ligating them to the self-complementary oligonucleotide AGCTTAGCTAGCTA, which ensures a stop codon in all three reading frames. Internal deletions were constructed by using two plasmids containing linker insertions. The plasmid with the linker at the 5′ border of the deletion provided all of the vector sequences. This plasmid was cut with *Hin*dIII and with *Eco*RI (which lies 3′ to the end of the coding sequence) and treated with phosphatase. The plasmid with the linker at the 3′ border was cut with *Hin*dIII and *Eco*RI to generate the fragment of the *STE12* gene and with *Hinc*II and *Pst*I to prevent religation of this plasmid. The two *Hin*dIII-*Eco*RI fragments were ligated together, and the resultant plasmids were sequenced across the deletion site. If necessary, the reading frame was restored by cutting with *Hin*dIII and ligation to either of the self-complementary oligonucleotides AGCTCCAAGCTTGG and AGCTCCCAAGCTTGGG.

Immunodetection of STE12. Yeast extracts were prepared as described previously (27) from 500 ml of culture grown in SD-ura. A 40% ammonium sulfate precipitation was used to concentrate the extracts. Samples were resolved on a 6% sodium dodecyl sulfate-polyacrylamide gel and transferred to nitrocellulose. Blots were blocked with 3% gelatin and probed with 150 μl of rabbit polyclonal antiserum (34). The filters were then treated with biotinylated goat anti-rabbit immunoglobulin G, streptavidin-alkaline phosphatase, nitroblue tetrazolium, and 5-bromo-4-chloro-3-indolylphosphate according to the instructions of GIBCO-BRL. The rabbit antiserum also recognizes several proteins that migrate close to the position of the full-length STE12, such that only smaller derivatives could be unambiguously identified.

β-Galactosidase assay. Cultures were grown in SD-ura media and split in half, with one half treated with 1 μM α-factor (Sigma) for 2 h (for MFA2-lacZ expression) or 2 μM α-factor for 3 h (for FUS1-lacZ expression). β-Galactosidase activity was determined as previously described (4), with activity calculated in Miller units (23). The pattern of activities for the MFA2-lacZ fusion obtained for different mutants remained consistent, although the absolute values varied from experiment to experiment. Therefore, each experiment was normalized to the wild-type value of that experiment, and Table 3 presents the mean percentages of the wild-type level for up to 10 separate experiments each. The absolute activity of the strain with the wild-type *STE12* gene was approximately 100 Miller units, and with the vector it was 18 Miller units. For FUS1-lacZ activity, the values in Table 4 are the absolute activities and are the means for four separate experiments. The standard errors for these assays were typically 30% or less of the mean values.



Mutant	Insertion	Net change
lk 17	Q17 / EAS / N19	+2
lk 37	L37 / KL / T39	+1
lk 85	G85 / KL / T86	+2
lk 103	V103 / SL / V104	+2
lk 218	S218 / KASF / T220	+3
lk 252	G252 / SF / L253	+2
lk 304	E304 / ESF / F306	+2
lk 334	P334 / EAS / A336	+2
lk 353	P353 / KLAF / N355	+3
lk 382	F382 / KAL / T384	+2
lk 405	T405 / RSF / Y407	+2
lk 421	R421 / KL / P422	+2
lk 435	G435 / SF Q436	+2
lk 511	Q511 / EAS / M513	+2
lk 668	G668 / EAS / H670	+2

FIG. 1. Six-base oligonucleotide linker mutagenesis of STE12. (A) Summary of the positions of the inserted linkers. Positions (numbers) of the amino acid residues of the 688-residue STE12 protein and the points of linker insertions (triangles) are indicated. (B) Descriptions of the linker mutations. Each mutant is designated "lk" followed by the last intact amino acid residue. The last intact residue and its codon number, the new amino acids inserted at that site, and the residue, with its codon number, at which the STE12 protein sequence resumes are indicated (in that order). Net change, net number of amino acids inserted.

RESULTS

Linker insertions affect only the DNA-binding domain. The *STE12* gene was cloned into a derivative of the centromere-containing plasmid YCp50 (30) in which the unique *Hin*dIII site had been eliminated. The resultant plasmid, designated p650, allowed *STE12* expression from its own promoter in a vector present at only one or two copies per cell. A 6-base oligonucleotide linker, corresponding to a *Hin*dIII site, was inserted throughout the plasmid sequence (1), and restriction analysis was used to detect 15 linkers present in the *STE12* coding sequence (Fig. 1A). In cases in which DNA sequence analysis indicated that the insertion led to the loss of nucleotides, thereby changing the STE12 reading frame, nucleotides were inserted at the *Hin*dIII site to restore the frame. Thus, the net gains of the linker mutations, designated "lk" followed by the number of the last intact STE12 amino acid residue, varied from one to three amino acids (Fig. 1B).

Plasmids containing the mutations were transformed into strain SF167-5a (12), whose relevant genotype is *MAT***a** *ste12::LEU2*. A patch of each transformant was replica

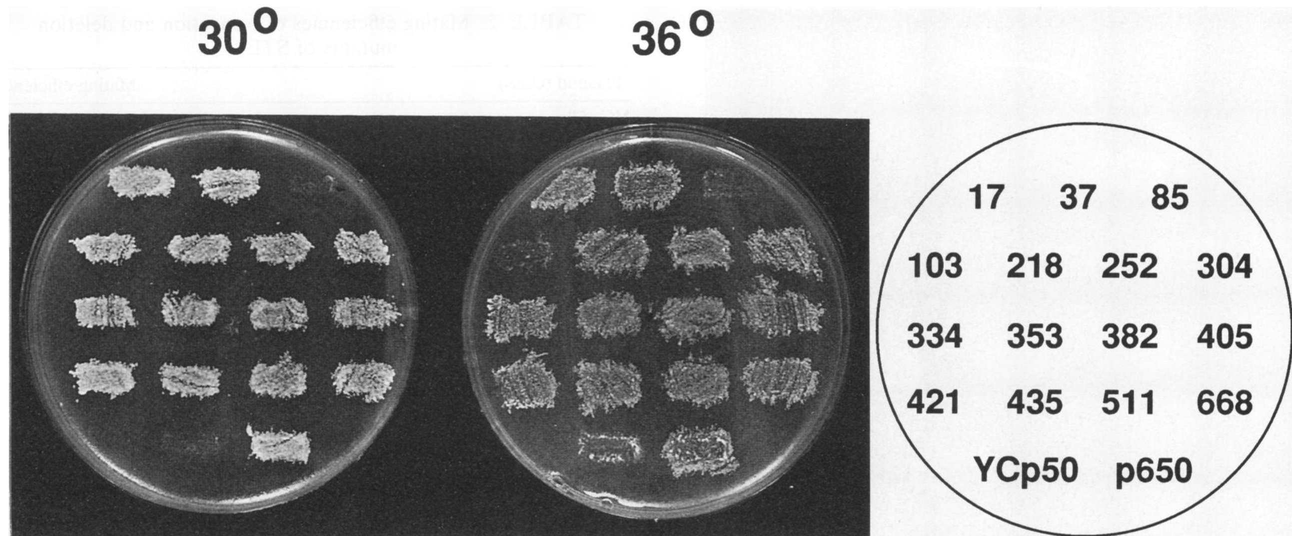


FIG. 2. Mating assay of the linker mutants. The numbers on the right represent the linker mutants shown in the plate assays; YCp50 is the vector and p650 is the plasmid carrying the wild-type *STE12* gene. Transformants of an **a** *ste12* strain expressing the indicated linker mutants were grown at 30 or 36°C and replica plated onto a lawn of α *STE*$^+$ cells spread on a minimal-medium plate. These minimal-medium plates were incubated at 30 or 36°C, respectively, and photographed after 3 days. Growth of diploids indicates complementation of the *ste12* mutation by a plasmid expressing the STE12 protein with its linker insertion.

plated onto a minimal-medium plate spread with a lawn of *MAT*α *STE*$^+$ cells (strain 271); each of the strains also contains other complementary auxotrophic markers. Mating between the transformant and cells in the lawn leads to formation of a prototrophic diploid, whose growth can be detected on the minimal-medium plate. This assay is a qualitative measure of mating ability and provided an initial classification of mutant phenotypes. At 30°C, all of the linker mutants except lk85 mated similarly to the wild type (Fig. 2). By a quantitative mating assay, lk85 was as defective as the strain transformed with the vector alone (Table 1). However at 36°C, lk103 was also defective in mating (Fig. 2), with its mating efficiency reduced 24-fold at the higher temperature (Table 1). These two linker insertions fall within the minimal domain (residues 40 to 204) sufficient for DNA binding by the STE12 protein (42). The remainder of the protein, required for transcriptional activation and induction as well as for interaction with the MCM1 protein (9), is insensitive to the small changes introduced by these linker mutations. These mutations were also transformed into an α *ste12* strain, to determine whether there were any cell-type-specific phenotypes, but these and all other mutations constructed gave identical results in the α strain (data not shown).

Carboxy-terminal truncation mutants define a minimal functional STE12 fragment. Using the sites generated by the linker insertions, we generated a series of truncation mutants

TABLE 1. Mating efficiencies of linker mutants of STE12 that have decreased activity

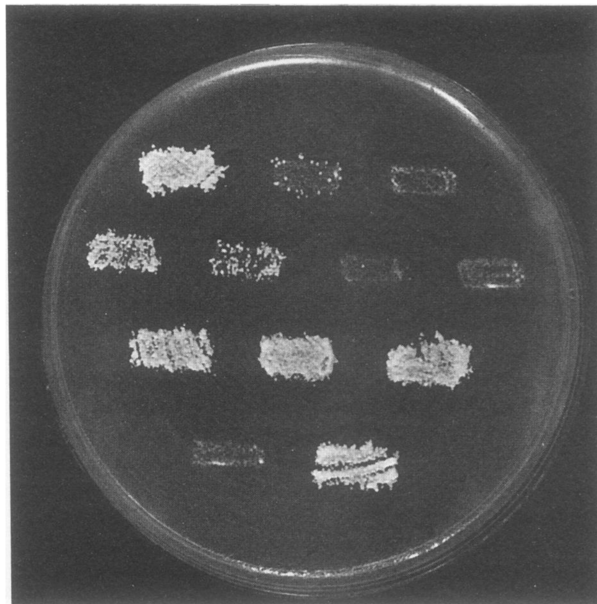
Plasmid	Mating efficiency	
	30°C	35°C
YCp50	$<10^{-6}$	$<10^{-6}$
p650	1.1×10^{-1}	2.8×10^{-1}
lk85	$<10^{-6}$	ND[a]
lk103	7.3×10^{-2}	3.0×10^{-3}

[a] ND, not determined.

by cutting plasmids at their unique *Hin*dIII site and ligating them to an oligonucleotide that encoded a stop codon in all three reading frames. The resulting mutants were assayed by patch mating tests (Fig. 3) and quantitative matings (Table 2). Truncation to residue 435, removing the carboxy-terminal third of the protein, could still promote mating, albeit at only 0.2% of the wild-type level. Further truncation to residue 421 led to even more reduced function, and truncation to residue 405 or 382 essentially eliminated STE12 activity. These results indicate that, as with other transcriptional activators such as GAL4 and GCN4 (18, 20), a substantial fraction of the STE12 protein can be eliminated while much of its function is retained.

It is notable that truncation to residue 587 or 511 caused a severe reduction in mating (0.02 and 0.006% of the wild-type level, respectively) even though smaller fragments of STE12 allowed mating at more than 10-fold higher efficiencies than these two truncations. The failure of the 587 and 511 truncations to function is apparently due to their low levels in *S. cerevisiae* (Fig. 4), which may be caused by protein instability. Only a small amount of the 587 truncation and none of the 511 truncation were detectable on a Western blot (immunoblot), whereas some of the smaller fragments were detectable. By contrast, deletion mutants Δ588-669 and Δ512-669 (described more fully below), which delete residues similar to those deleted by the 587 and 511 truncations but retain the carboxy-terminal 19 residues of STE12, were present in *S. cerevisiae* at readily detectable levels. These two deletions were also capable of promoting mating similar to that of the wild type (Fig. 3 and Table 2).

Internal deletions identify functionally redundant regions of STE12. Combinations of two linker mutants were used to create internal deletions of the *STE12* gene (see Materials and Methods), which are designated by the first and last amino acid residues deleted from the protein. When necessary, additional nucleotides were inserted at the *Hin*dIII site of the resultant plasmid to restore the reading frame. The deletion mutants were introduced into the **a** *ste12* strain and



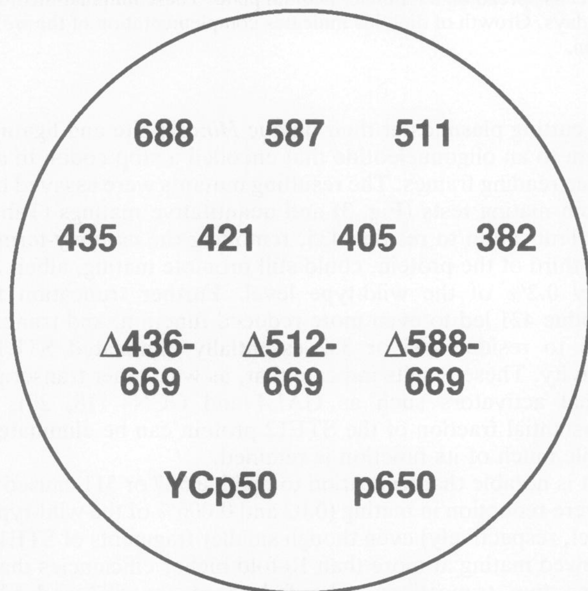


FIG. 3. Mating assay of truncation mutants and comparable internal-deletion mutants. The last intact amino acid residues of the carboxy-terminal truncation mutants (first two rows), the residues deleted in three deletion mutants that are similar to three of the truncation mutants except for the presence of the carboxy-terminal 19 residues of STE12 (third row), and the negative (YCp50) and positive (p650) controls (fourth row) are indicated at the bottom. The results of the mating assay at 30°C, described in the legend to Fig. 2, are shown at the top.

assayed for mating ability (Fig. 5). On the basis of this assay and transcription assays described below, the mutants could be grouped into four general classes (Fig. 6). Class I consists of deletion mutants that were capable of restoring a mating ability to the *ste12* strain that was comparable to that for the wild-type plasmid. Nine deletion mutants, each removing approximately 100 to 200 amino acid residues, showed this phenotype. Since these deletions are overlapping (Fig. 6), all of the residues between positions 253 and 669 are removed by this set of mutations. However, the truncation data

TABLE 2. Mating efficiencies of truncation and deletion mutants of STE12

Plasmid (class)	Mating efficiency
YCp50	$<10^{-6}$
p650	1.1×10^{-1}
587 truncation	1.9×10^{-5}
511 truncation	6.7×10^{-6}
435 truncation	2.2×10^{-4}
421 truncation	8.5×10^{-5}
405 truncation	3.5×10^{-6}
382 truncation	$<10^{-6}$
Δ253-305 (I)	1.5×10^{-1}
Δ305-406 (I)	1.1×10^{-1}
Δ436-669 (I)	2.0×10^{-3}
Δ512-669 (I)	4.7×10^{-2}
Δ588-669 (I)	4.3×10^{-2}
Δ104-219 (II)	$<10^{-6}$
Δ172-252 (II)	$<10^{-6}$
Δ253-335 (III)	$<10^{-6}$
Δ255-354 (III)	5.8×10^{-6}
Δ305-587 (IV)	$<10^{-6}$
Δ383-669 (IV)	$<10^{-6}$

indicate that the minimal fragment of STE12 that can restore any degree of mating is the amino-terminal 421 residues. Thus, these deletion results suggest that, while no specific residues carboxy terminal to amino acid 253 are absolutely required for mating activity, one or more essential functions may be redundantly encoded.

Class II consists of two deletion mutants, Δ104-219 and Δ172-252, that remove substantial portions of the DNA-binding domain. These mutants were completely defective in restoring mating ability (Table 2), suggesting that these deletions destroy DNA-binding activity or that the loss of this critical region destabilizes the mutant proteins. Class III comprises two mutants with deletions of residues in the domain carboxy terminal to the DNA-binding domain. These two mutants, Δ253-335 and Δ255-354, which remove 83 and 100 residues, respectively, from this domain, were expressed well in *S. cerevisiae*, to a level comparable to that of other mutant proteins that promoted mating (data not shown). These mutants showed virtually no ability to promote mating, indicating the likelihood that the region encoding another essential function, which we show below to be

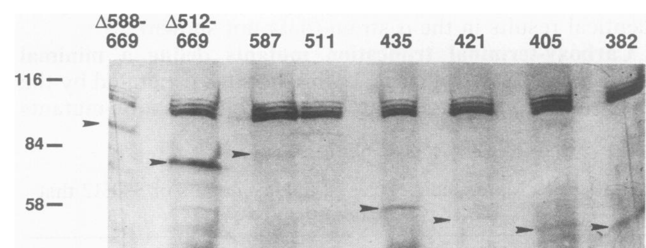
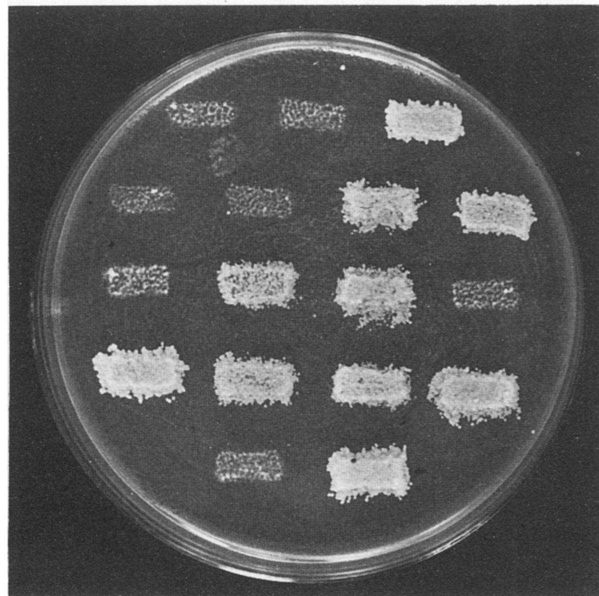


FIG. 4. Protein levels of the truncation mutants and comparable internal-deletion mutants. An immunoblot using anti-STE12 antibody is shown for transformants expressing either of two internal-deletion mutants or one of six carboxy-terminal truncation mutants (the last intact amino acid residue is indicated). Positions of STE12 proteins with a truncation or a deletion are indicated (arrowheads). The sizes (in kilodaltons) of molecular size markers are shown on the left.



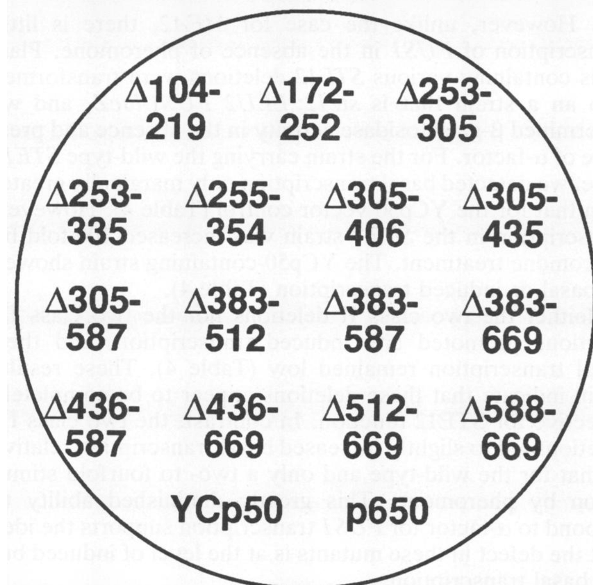


FIG. 5. Mating assay of internal-deletion mutants. The amino acid residues deleted and the negative (YCp50) and positive (p650) controls are indicated at the bottom. The results of the mating assay at 30°C, described in the legend to Fig. 2, are shown at the top.

transcriptional induction, has been deleted. However, these mutants are in contrast to those with mutations in the DNA-binding domain, in which even 2-amino-acid insertions could abolish function. Two mutants, Δ253-305 and Δ305-406, remove the amino-terminal and carboxy-terminal halves, respectively, of the region between residues 253 and 354, yet they could mate at an efficiency comparable to that of the wild type (Table 2). Thus, the comparison of the two class III mutants with Δ253-305 and Δ305-406 suggests that this essential function is redundantly provided in the STE12 protein.

Class IV consists of two mutants with large deletions, Δ305-587 and Δ383-669, each of which removes nearly 300 residues; only the Δ305-587 deletion mutant was present in

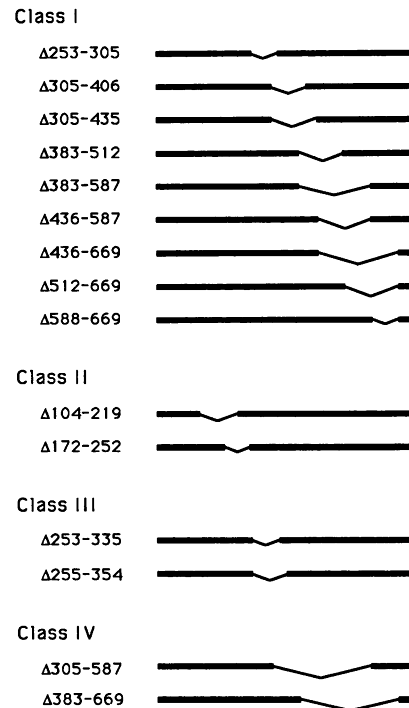


FIG. 6. Summary of the four classes of internal-deletion mutants. The thick lines represent STE12 residues present, and the thin lines represent the residues deleted. Class I mutants are capable of restoring mating to a *ste12* strain, class II mutants are mating defective and remove portions of the DNA-binding domain, class III mutants are mating defective and promote basal transcription but fail to induce transcription in response to pheromone, and class IV mutants are mating defective and fail to promote basal or induced transcription.

S. cerevisiae at a level comparable to those of mutant proteins that promoted mating (data not shown). Both of these mutants failed to restore mating ability (Table 2). This result, combined with the analysis of the truncations, suggests that there is an additional essential function, carried out by sequences carboxy terminal to residue 382. This function, which data below suggest to be transcriptional activation, may also be redundantly encoded, in that the overlapping deletion mutants Δ383-587 and Δ436-669 were capable of promoting mating whereas Δ383-669 was defective.

Distinct domains appear to mediate basal transcription and pheromone-induced transcription. To assess the ability of the mutant STE12 proteins to promote basal and induced levels of transcription, we transformed the appropriate plasmids into an **a** strain that carries the *ste12::LEU2* allele as well as an *MFA2-lacZ* gene. We chose to assay transcription of the *MFA2* gene, which encodes the pheromone **a**-factor, because expression of this gene is dependent on STE12 function and occurs at an easily detectable level in the absence of α-factor treatment (10). Thus, we could measure the activity of STE12 mutants over a wide range. Comparison of the *ste12::LEU2 MFA2-lacZ* strain carrying either the wild-type *STE12* gene or the vector indicates that fivefold more β-galactosidase was produced by the strain with the *STE12* gene (Table 3). Additionally, treatment of the STE12-containing cells with α-factor led to a 1.7-fold induction in β-galactosidase activity. The *ste12* strain carrying the vector showed no

TABLE 3. Transcriptional activation of an *MFA2-lacZ* reporter gene mediated by deletion mutants of STE12

Plasmid (class)	β-Galactosidase activity[a]		Fold induction[b]
	Without α-factor	With α-factor	
p650	100	174	1.7
YCp50	18	16	0.9
Δ253-305 (I)	149	272	1.8
Δ305-406 (I)	125	190	1.5
Δ436-669 (I)	53	60	1.1
Δ512-669 (I)	144	196	1.4
Δ588-669 (I)	85	152	1.8
Δ104-219 (II)	27	26	0.9
Δ172-252 (II)	21	23	1.1
Δ253-335 (III)	89	98	1.1
Δ255-354 (III)	98	92	0.9
Δ305-587 (IV)	27	28	1.0
Δ383-669 (IV)	22	23	1.0

[a] Calculated in Miller units (23). The data are percentages of the activity for the strain carrying p650 (wild-type *STE12* gene) and not treated with α-factor.
[b] Ratio of the normalized β-galactosidase activity in the presence of α-factor to the activity in the absence of α-factor.

TABLE 4. Transcriptional activation of a *FUS1-lacZ* reporter gene mediated by deletion mutants of STE12

Plasmid (class)	β-Galactosidase activity[a]		Fold induction[b]
	Without α-factor	With α-factor	
YCp50	0.1	0.1	1.0
p650	0.2	20	100
Δ253-305 (I)	0.6	12	20
Δ305-406 (I)	4.0	20	5.0
Δ436-669 (I)	0.3	4.8	16
Δ512-669 (I)	2.7	40	15
Δ588-669 (I)	1.2	42	35
Δ104-219 (II)	0.1	0.1	1.0
Δ172-252 (II)	0.1	0.2	2.0
Δ253-335 (III)	0.5	2.0	4.0
Δ255-354 (III)	0.6	1.1	1.8
Δ305-587 (IV)	0.2	0.1	0.5
Δ383-669 (IV)	0.1	0.1	1.0

[a] In Miller units (23).
[b] Ratio of β-galactosidase activity in the presence of α-factor to the activity in the absence of α-factor.

induction, as expected, in that *ste12* mutants do not respond to α-factor (15).

Internal-deletion mutants that were incapable of promoting mating showed one of two transcriptional patterns (Table 3). Both class II deletion mutants, which remove parts of the DNA-binding domain, and class IV deletion mutants, which remove large carboxy-terminal regions, showed little basal transcription and no induction in response to α-factor. These results suggest that the inability to promote mating could be due to the failure of the class II deletion mutants to bind DNA and the failure of the class IV deletion mutants, such as Δ305-587, to carry out transcriptional activation. Class III deletion mutants, however, showed near wild-type levels of basal transcription but little or no transcriptional induction. The results for these deletion mutants suggest that the residues between positions 253 and 335 are essential only for the ability of the STE12 protein to respond to the signal generated by α-factor treatment, thereby separating the induction function from the basal transcriptional activation function. The phenotype of these two deletions also indicates that induction is absolutely essential for mating.

The class I deletions, which promoted mating, were generally proficient at both basal transcription and induced transcription (Table 3), a result consistent with the abilities of these mutant STE12 proteins to bind DNA, activate transcription, and respond to the pheromone signal. However, Δ436-669 showed a twofold reduction in basal transcription, which was not substantially increased by treatment of cells with α-factor. While the *MFA2* promoter showed no induction, another STE12-dependent promoter was capable of induced transcription with this mutant (see below). Thus, the strain presumably regulates some, but not all, cell-type-specific genes, and this overall pattern would allow mating. The *ste12 MFA2-lacZ* strain, which contains only a single functional **a**-factor gene, *MFA1*, produces detectable **a**-factor when carrying the Δ436-669 deletion but not the vector (data not shown); however, we have not assayed the inducibility of the *MFA1* gene upon α-factor treatment.

We also assayed expression of the *FUS1* gene, which is highly inducible by pheromone and dependent on STE12 (22, 40). However, unlike the case for *MFA2*, there is little transcription of *FUS1* in the absence of pheromone. Plasmids containing various *STE12* deletions were transformed into an **a** strain that is *ste12::LEU2 FUS1-lacZ*, and we determined β-galactosidase activity in the absence and presence of α-factor. For the strain carrying the wild-type *STE12* gene, we detected basal transcription only marginally greater than that for the YCp50 vector control (Table 4). However, transcription in the *STE*$^+$ strain was increased 100-fold by pheromone treatment. The YCp50-containing strain showed no basal or induced transcription (Table 4).

Neither the two class II deletions nor the two class IV deletions promoted any induced transcription, and their basal transcription remained low (Table 4). These results again indicate that these deletions appear to be completely defective for STE12 function. In contrast, the two class III deletions led to slightly increased basal transcription relative to that for the wild-type and only a two- to fourfold stimulation by pheromone. This greatly diminished ability to respond to α-factor for *FUS1* transcription supports the idea that the defect in these mutants is at the level of induced but not basal transcription.

Three of the class I deletion mutants showed a higher basal transcription than the wild type, and these deletions could mediate induced transcription to 50 to 200% of the wild-type level (Table 4). The high basal transcription suggests that STE12-dependent gene expression may normally be negatively regulated in the absence of pheromone. The Δ436-669 mutant showed a 16-fold induction by pheromone (Table 4), suggesting that the mating promoted by this deletion mutant is due to its ability to induce transcription of at least some cell-type-specific genes.

DISCUSSION

The STE12 protein binds to the pheromone response element, the DNA sequence that both mediates increased transcription of genes in cells treated with pheromone and contributes to the levels of basal transcription from these genes. This protein must therefore be capable of three functions: site-specific DNA binding, transcriptional activation, and transcriptional induction in response to pheromone. We have assayed a series of mutants with mutations

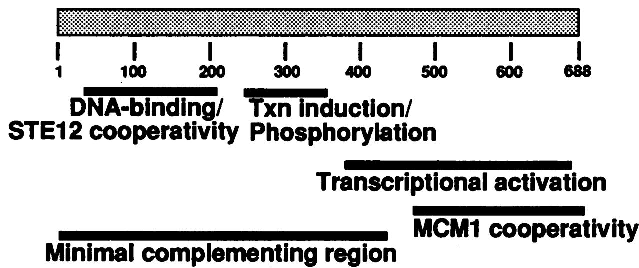


FIG. 7. Summary of the functional domains of STE12. Positions (numbers) of amino acid residues of STE12 and regions (thick lines) necessary for STE12 activities are indicated. Txn induction, the region required for transcriptional induction in response to pheromone; Phosphorylation, at least one domain implicated in pheromone-dependent phosphorylation; Transcriptional activation, the region containing redundant domains for basal transcriptional activation.

in the *STE12* gene, including linker insertions, truncations, and internal deletions. These assays, in conjunction with other studies (9, 34, 42), delineate functional domains of STE12 (Fig. 7).

The residues between positions 253 and 354 appear to be necessary for transcriptional induction in response to pheromone but not for basal transcription. Mutants incapable of carrying out transcriptional induction do not mate. This result formally establishes that a dynamic transcriptional response to pheromone is an absolute prerequisite for successful mating. This requirement is not unexpected, given the many genes involved in mating whose expression is inducible by pheromone. However, a weakness in previous studies in which transcriptional induction was abolished by *ste* mutations (e.g., references 10, 11, 14, 15, 22, and 41) is that the mutant strains were also severely deficient in basal transcription. Therefore, it was not possible to observe the effects of activating the response pathway upstream of STE12 solely in the absence of induced transcription.

Either of the regions between 253 and 305 and between 305 and 354 can mediate this induction, suggesting that there may be at least two independent targets for pheromone-dependent modification. This result is consistent with the results of assays that indicated multiple phosphorylations of the STE12 protein in cells treated with pheromone (34), and thus, these modifications might be responsible for changes in STE12 activity. The ability to obtain mutants defective only in this induction has implications for an understanding of the pheromone response pathway's role in transcription. STE12-mediated basal transcription is severely reduced in cells with mutations in the response pathway. This result may reflect the loss of some STE12 modification catalyzed by the low-level constitutive activity of the pathway. The characterization of the induction-defective mutants suggests the possibility that new STE12 residues become modified in the presence of pheromone rather than that the same residues become modified in a larger fraction of the STE12 population. Four proteins, STE7, STE11, FUS3, and KSS1 (2, 7, 8, 29, 39), with homology to protein kinases are required for pheromone response, and these are candidates for the activities that modify STE12 in the absence or presence of pheromone.

Sequences required for transcriptional activation in the absence of pheromone lie in the carboxy-terminal half of the STE12 protein, and the ability to mediate basal transcription appears to be essential for the mating process. The fact that the truncation to residue 421 is capable of promoting some degree of mating whereas truncation to residue 383 is not suggests that amino acids between positions 383 and 421 are important in carrying out this function. Sequences carboxy terminal to residue 435 may also be capable of mediating this transcriptional activation, as Δ305-435 can promote mating comparable to that for the wild-type protein. The mutants with large deletions that fail to activate transcription also show no induced transcription in response to pheromone. This result suggests that inducible transcription may require sequences mediating basal transcription as well as the separate domain functioning only in induction.

The DNA-binding domain lies in the amino-terminal 204 residues of the protein (42). Of 15 linker mutations throughout the coding sequence, only those inserted immediately following residues 85 and 103 eliminated the capacity to promote mating. On the basis of a proposed homology to the homeodomain (see reference 42), lk85 falls in the loop between the match with helix 1 and helix 2 and lk103 falls in the middle of the match with helix 2. The lk103 mutation causes a less severe effect, resulting only in a temperature-sensitive phenotype. Although it is surprising that the protein can partially tolerate a two-amino-acid insertion at this position, this result is consistent with the presence of a proposed loop of 18 residues within helix 2 of the liver factor LF-B1, which can be deleted without significant loss of DNA-binding ability (25).

STE12 can bind to a single pheromone response element, such as one present in the *STE2* gene, in cooperation with MCM1 bound to an adjacent binding site (9, 24, 28). This cooperativity requires the carboxy-terminal domain (residues 470 to 688) of STE12 (9). The mating ability promoted by a STE12 protein truncated to residue 435, as well as the mating promoted by the Δ436-669 deletion mutant, indicates that this MCM1 cooperativity is not absolutely essential for STE12 function. This result was observed previously with a STE12 protein truncated at residue 469 (9). STE12 may bind to multiple pheromone response elements present in genes such as *FUS1* by virtue of STE12-STE12 cooperativity, which requires the amino-terminal DNA-binding domain of the protein (42). Thus, STE12 may be transcriptionally active in the absence of the STE12-MCM1 interaction. However, we note that the Δ436-669 deletion shows poor transcriptional activity for the *MFA2-lacZ* gene, suggesting that MCM1 cooperativity may be necessary for optimal STE12 activity on at least some genes.

With its functional domains apparently present on separate regions of the polypeptide, STE12 resembles other inducible transcriptional activators. For example, the glucocorticoid receptor contains a hormone-binding domain, whose deletion leads to constitutive transcriptional activation (13, 17). The yeast heat shock factor plays a role in both constitutive transcription and heat-inducible transcription at heat shock promoters, and analyses of truncations and deletions indicated that separable regions of the protein mediate these activities (26, 35). Like STE12, this heat shock factor also is phosphorylated upon activation (35–37). It remains to be determined how such changes in phosphorylation affect the activities of these factors.

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