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Relative contributions of MCM1 and STE12 to transcriptional activation of a- and α-specific genes from *Saccharomyces cerevisiae*

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Summary. We have examined the relative contributions of MCM1 and STE12 to the transcription of the **a**-specific *STE2* gene by using a 367 bp fragment from the *STE2* 5′-noncoding region to drive expression of a reporter *lacZ* gene. Mutation of the MCM1 binding site destroyed MCM1·α2-mediated repression in α cells and dramatically reduced expression in **a** cells. The residual expression was highly stimulated by exposure of cells to pheromone. Likewise, the loss of STE12 function reduced *lacZ* expression driven by the wild-type *STE2* fragment. In the absence of both MCM1 and STE12 functions, no residual expression was observed. Thus, the *STE2* fragment appears to contain two distinct upstream activation sequences (UASs), one that is responsible for the majority of expression in cells not stimulated by pheromone, and one that is responsible for increased expression upon pheromone stimulation. In further support of this idea, a chemically synthesized version of the *STE2* MCM1 binding site had UAS activity, but the activity was neither stimulated by pheromone nor reduced in *ste12* mutants. Although transcription of α-specific genes also requires both MCM1 and STE12, these genes differ from **a**-specific genes in that they have a single, MCM1-dependent UAS system. The activity of the minimal 26 bp UAS from the α-specific *STE3* gene was both stimulated by pheromone and reduced in *ste12* mutants. These data suggest that at α-specific genes STE12 and MCM1 exert their effects through a single UAS.

Key words: Yeast – Transcription – **a**- and α-specific genes – MCM1 – STE12

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Introduction

The yeast *Saccharomyces cerevisiae* exhibits either of two mating types, **a** or α. Each mating type synthesizes a characteristic set of proteins, most notably unique pheromones and receptors for those pheromones, which enable cell-cell communication and mating to proceed efficiently. The differential synthesis of these proteins is controlled by the mating-type locus (*MAT*), in particular the *MAT*α allele. This allele encodes two regulatory proteins that govern transcription of the α- and **a**-specific gene sets; α1 is required to activate transcription of α-specific genes, whereas α2 is required to repress transcription of **a**-specific genes (for review see Herskowitz 1988, 1989; Sprague 1990).

Even though these two gene sets are transcribed in different cell types, their expression exhibits common features.

(a) The same general transcription factor, MCM1 (also called PRTF and GRM), in cooperation with α1 and α2, allows regulated transcription of both gene sets (Hayes et al. 1988; Jarvis et al. 1989; Passmore et al. 1989; Keleher et al. 1989; Ammerer 1990).

(b) Transcription of both gene sets increases in response to pheromone stimulation (Strazdis and MacKay 1983; Hagen and Sprague 1984; Hartig et al. 1986; Kronstad et al. 1987; Achstetter 1989).

(c) Both gene sets require the STE12 product for maximal transcription in the absence of pheromone (Fields and Herskowitz 1985; Hartig et al. 1986; Fields et al. 1988). STE12, which is part of the pheromone response pathway (Hartwell 1980), therefore contributes to both basal and pheromone-stimulated transcription (Dolan et al. 1989; Errede and Ammerer 1989). Although qualitatively the transcription of **a**- or α-specific genes involves several common components, the quantitative contribution of these components to transcriptional activation of the two gene sets has not been established. In this study we determine the contribution of MCM1

and STE12 at two genes: the **a**-specific *STE2* gene and the α-specific *STE3* gene.

MCM1 appears to have two roles in expression of **a**-specific genes. First, it serves as a co-repressor with α2 to repress this gene set in α and **a**/α cells (Johnson and Herskowitz 1985; Keleher et al. 1988; Sauer et al. 1988). MCM1 and α2 bind cooperatively to a 33 bp operator in vitro, MCM1 to the P box sequence in the center of the operator and α2 to sequences on both sides of the P box. Both the MCM1 and α2 sites must be intact for repression to occur in vivo (Keleher et al. 1988). Second, MCM1 is believed to contribute to activation of the gene set in **a** cells. The upstream control regions of **a**-specific genes contain single copies of good, but not perfect, versions of the symmetric, 16 bp P box (Johnson and Herskowitz 1985; Bender and Sprague 1987; Jarvis et al. 1988). The functional importance of these P boxes is suggested by two findings. (i) Deletion of sequences including the P box from the *BAR1* gene reduced transcription 10- to 20-fold (Kronstad et al. 1987). (ii) DNA segments containing P boxes function as upstream activation sequences (UAS) in reporter gene constructs (Keleher et al. 1988; Jarvis et al. 1988; Errede and Ammerer 1989; Passmore et al. 1989). The interpretation of these experiments is not straightforward, however. For no **a**-specific gene have both the UAS activity and protein binding properties of P box mutations lying in the context of extensive upstream sequence been examined, nor has the UAS activity of just the P box been examined.

STE12 has been shown to be part of protein-DNA complexes that form on three **a**-specific genes, *STE2*, *MFA1*, and *MFA2* (Dolan et al. 1989; Errede and Ammerer 1989). STE12 apparently binds alone to these DNAs, albeit very poorly, but binds strongly and cooperatively with MCM1. These STE12-containing complexes form on the pheromone response element (PRE), a DNA sequence that mediates pheromone stimulation of transcription (Kronstad et al. 1987; Van Arsdell and Thorner 1987). Since the upstream regions from **a**-specific genes typically contain two or more PRE sequences, these genes potentially contain two or more elements capable of serving as a UAS, the PREs and the P box.

In contrast to the potential complexity of the upstream control regions from **a**-specific genes, the analysis of α-specific genes has thus far revealed a single UAS system, which is MCM1-dependent. The MCM1 binding sites present in α-specific UAS elements poorly match the symmetric P box – half of the dyad is degenerate. In compensation, these UASs contain a second sequence element, the Q box, immediately adjacent to the degenerate side of the P box. MCM1 can bind and activate transcription only in conjunction with the co-activator α1 (Bender and Sprague 1987; Inokuchi et al. 1987; Jarvis et al. 1988; Tan et al. 1988; Flessel et al. 1989). Moreover, MCM1 is essential for UAS activity as deletion of its binding site abolishes both basal and pheromone-stimulated transcription.

Given the finding that, at least for *STE3*, all transcription is dependent on the MCM1/P box system, how is transcription of α-specific genes influenced by STE12 and by pheromone stimulation? STE12-dependent protein-DNA complexes have not been detected on DNA fragments from this gene set (Bender and Sprague 1987; G. Ammerer, personal communication). The upstream regions of α-specific genes contain good matches to PRE, but as already pointed out these sequences do not have detectable UAS activity in the absence of the MCM1 system. Moreover, DNA segments from the *STE3* upstream control region, which do not contain good matches to the PRE – no better than 5 out of 7 nucleotides identical to the PRE – nonetheless have UAS activity that is stimulated by **a**-factor pheromone (Jarvis et al. 1988). We therefore considered the possibility that the STE12 and pheromone regulatory inputs to *STE3* transcription may be organized somewhat differently than proposed for **a**-specific genes. In particular, we asked whether the 26 bp QP sequence from *STE3* is sufficient to confer α-specific expression, as expected, and whether that transcription is stimulated by pheromone and reduced in *ste12* mutants.

Materials and methods

Yeast strains and media. Three sets of isogenic yeast strains were used. HR125-5d, SY816, and SY817 are **a**, α, and **a**/α *leu2-3, -112 his3 his4 trp1 ura3-52 gal2* strains. SY1599 is a *ste12::URA3* derivative of HR125-5d. SY1202 is an **a** *leu2 ura3 trp1 met14 lys2oc pep4::LEU2* strain, and SY1565 is an *ste12::URA3* derivative of SY1202. The third set of strains was 246-1-1 (α *trp1 leu2 ura3 his4-519 can1-101*), SF167-1c (246-1-1 made *ste12::LEU2*), EG123 (246-1-1 except **a**), and SF167-5a (EG123 made *ste12::LEU2*) (Fields et al. 1988). The media used were the following: YEPD (same as YPD; Sherman et al. 1986) and SD-Leu or SD-Ura (SD supplemented with adenine, L-tryptophan, L-histidine, L-arginine, L-methionine, L-tyrosine, L-lysine, L-phenylalanine, L-threonine, and uracil or L-leucine; Sherman et al. 1986).

Plasmids and DNA manipulations. Two plasmids carrying a reporter gene were used to assay the UAS activity of wild-type and mutant DNA fragments and of synthetic oligonucleotides. Plasmid pSL709 harbors *CYC1-lacZ* on pJDB207, a *LEU2* 2 μm-circle-based plasmid (Beggs 1981; Jarvis et al. 1989). Plasmid pΔSS harbors *CYC1-lacIlacZ* on YEp24, a *URA3* 2 μm-circle-based plasmid (Botstein et al. 1979; Johnson and Herskowitz 1985). The *CYC1* segments of these plasmids provide a TATA element and a translation initiation codon but lack a UAS element.

A 367 bp *Hin*dIII fragment from *STE2* (coordinates −172 to −538 with respect to the translation initiation codon) was inserted into each reporter plasmid, creating pSL1163 (pSL709) and pSL1169 (pΔSS). Similarly, the equivalent *Hin*dIII fragment in which the P box sequence had been mutated was inserted into each plasmid, creating pSL1161 (pSL709) and pSL1190 (pΔSS). To facilitate subcloning and mutagenesis of the *STE2 Hin*dIII fragment, it was first inserted into the single strand-gen-

erating plasmid Bluescript SK$^+$ (Stratagene), creating plasmid pSL1098. Single-stranded DNA was prepared after transformation of *Escherichia coli* strain CJ236 (*dut1*$^-$ *ung1*$^-$ *thi1 relA1*/pCJ105 (Cm^r); Kunkel et al. 1987) and infection with M13 helper phage KO7 (Vieira and Messing 1987). The single-stranded DNA was hybridized with a mutant oligonucleotide 5′ – CAACCATGTAAA**TGTATCTCGAGTTTAA**GTACATGATG (bold face residues indicating the mutant P box). Following hybridization, the DNA was converted to double-stranded circles in vitro and then introduced into *E. coli* strain SB115 by transformation (Sambrook et al. 1989). Plasmids harboring the mutant P box were identified initially by virtue of a *Xho*I site contained within the altered sequence. The structures of the putative mutant was verified by DNA sequencing (Sanger et al. 1977). The mutant *Hin*dIII fragment from one such plasmid, pSL1101, and the wild-type *Hin*dIII fragment from pSL1098 were excised as *Sal*I-*Bam*H1 fragments (using restriction sites in the parent Bluescript SK+ vector) and cloned into pSL709 and pΔSS.

The DNA oligonucleotide P (*STE2*) was synthesized as two complementary fragments: 5′-tcgacTTTCCTAATTGGGTAAg, and 5′-tcgacTTACCCAATTAGGAAAg. Uppercase letters represent the P box and lower case letters represent residues added to facilitate cloning manipulations. Likewise, the sequence QP (*STE3*) was synthesized as two complementary fragments: 5′-tcgaCTGTCATTGTGACACTAATTAGGAAAg and 5′-tcgacTTTCCTAATTAGTGTCACAATGACAG. In addition, a perfectly symmetric dyad, P(PAL-16), was synthesized as the self-complementary fragment 5′-tcgacTTTCCTAATTAGGAAAg. All oligonucleotides were synthesized by the University of Oregon Biotechnology Laboratory with an Applied Biosystems DNA Synthesizer Model 380B (phosphoramidite chemistry). The duplex fragments were inserted into the *Xho*I sites of pSL709 and pΔSS, and the structures of the resulting plasmids were verified by DNA sequencing. Plasmid pSL1195 contains P (PAL-16) in pSL709, and pSL1196 contains P (PAL-16) in pΔSS. For P (*STE2*), plasmids were identified in which the P sequence had the natural orientation found in the *STE2* upstream region or the reverse orientation. Plasmids pSL1199 and pSL1203 contain the natural orientation of P (*STE2*) in pSL709 and pΔSS, whereas plasmids pSL1201 and pSL1204 contain the reverse orientation, P (*STE2*-R), in pSL709 and pΔSS. Fortuitously, a P (*STE2*) fragment which contained a 2 bp deletion was identified (5′-TTTC()TA()TTGGGTAA, parentheses indicating the positions of the deleted nucleotides). This fragment was inserted in the natural orientation into pΔSS, generating pSL1205. Plasmids pSL904 and pSL1117 contain QP (*STE3*) in pSL709 and pΔSS in the natural orientation found at *STE3*. Plasmid pSL906 contains the reverse orientation of QP (*STE3*) in pSL709.

Standard methods (Sambrook et al. 1989) were used for DNA preparations, bacterial transformations, and plasmid constructions. *E. coli* strains SB69 (Hagen and Sprague 1984) and SB115 were used for plasmid propagation. SB115 is SB69 made *pyrF74*::Tn*5*(Km^r) by transduction using phage P1 grown on MC1066 (Casadaban et al. 1983). Yeast transformations were by the spheroplast method of Beggs (1978) or by electroporation (Becker and Guarente 1990).

β-Galactosidase and DNA binding assays. β-Galactosidase activities of plasmid-bearing strains were measured as described previously (Jarvis et al. 1988). In cases when expression in response to pheromone was to be assayed, cultures were incubated with pheromone for 2.5 h. **a**-Factor preparations were the culture filtrates of strain DC5 (*MAT***a** *leu2-3, 112 his3 can1 gal2*) grown to saturation in YEPD medium at 30° C. Cells were removed by filtration through a membrane of pore size 0.2 μm (Nalgene filter unit). The filtrate was used at a final concentration of $\times^1/_2$. α-Factor was purchased from Sigma Chemical Corporation and used at a final concentration of 4×10^{-7} M.

Binding of protein to DNA was assayed by electrophoretic mobility-shift of radioactive DNA fragments (Fried and Crothers 1981; Garner and Revzin 1981). DNA fragments to be tested were excised from plasmids by restriction endonuclease digestion and purified by agarose gel electrophoresis and electroelution. The fragments were labeled with ^{32}P at their 3′ ends using the Klenow fragment of DNA polymerase I and then repurified by electrophoresis and electroelution.

Protein extracts of yeast cells were prepared as described previously (Bender and Sprague 1987; Jarvis et al. 1989) except that precipitation was carried out with ammonium sulfate at 75% saturation. The ammonium sulfate pellet was resuspended in 2× the original extract volume, dialysed against buffer A100 (20 mM $NaPO_4$, pH 7.3, 5% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl), and diluted 1:1 with 100% glycerol. To carry out DNA binding assays, 18 μg of crude yeast extract in a volume of 8 μl (50% A100, 50% glycerol) was added to 10 μl of A100M (A100 containing 15 mM $MgCl_2$). When present, 1 μl affinity-purified antibodies raised to an MCM1 peptide were first added to the 18 μl of A100M, and the mix was incubated at room temperature for 15 min. Approximately 8×10^{-5} μg of ^{32}P-labelled DNA fragment and 1.0 μg of sonicated salmon sperm DNA were added simultaneously in a volume of 2 μl. Reactions were incubated for an additional 20 min at room temperature and were stopped by loading onto a nondenaturing 4% polyacrylamide gel. Electrophoresis was performed as described by Bender and Sprague (1987), except that gels were dried without prior fixing.

Results and discussion

Activity of wild type and mutant UAS_{STE2}

To investigate the role of P box DNA in transcriptional activation of **a**-specific genes we used a 367 bp *Hin*dIII fragment derived from the upstream region of the *STE2* gene (coordinates −172 to −538 with respect to the translation initiation codon) (Hartig et al. 1986). This segment contains one copy of the P box and four copies

Table 1. Upstream activation sequence (UAS) activities of *STE2* fragments and synthetic oligonucleotides

Plasmid	Fragment	Orientation	Recipient	β-galactosidase activity[a]						
				a/α	α	α + **a**F[b]	**a**	**a** + αF[b]	**a** *ste12*	**a** *ste12* + αF
pSL709	None			18	17	17	17	17	15	15
pSL1163	$UAS_{STE2-Pwt}$	Natural	pSL709	15	13	20	1390	1740	460	470
pSL1161	$UAS_{STE2-Pmt}$	Natural	pSL709	5	10	310	7	350	4	5
pΔSS	None			0.1	0.6	0.2	0.3	<0.1	0.6[c]	ND[d]
pSL1169	$UAS_{STE2-Pwt}$	Natural	pΔSS	0.8	0.6	0.8	340	470	36[c]	ND
pSL1190	$UAS_{STE2-Pmt}$	Natural	pΔSS	0.4	1.1	4.5	0.7	6.3	0.7[c]	ND
pSL709	None			15	14	15	15	14	15	15
pSL1195	P (PAL-16)		pSL709	1060	1150	1280	1050	1085	1000	1010
pSL1199	P (*STE2*)	Natural	pSL709	580	1040	1110	890	990	600	660
pSL1201	P (*STE2*)	Reversed	pSL709	740	960	1060	980	940	ND	ND
pΔSS	None			0.4	0.4	0.4	0.3	0.3	ND	ND
pSL1196	P (PAL-16)		pΔSS	8	22	22	23	26	ND	ND
pSL1203	P (*STE2*)	Natural	pΔSS	5	12	13	12	12	ND	ND
pSL1204	P (*STE2*)	Reversed	pΔSS	4	9	9	10	10	ND	ND
pSL1205	P (*STE2*−Δ2 bp)	Natural	pΔSS	0.5	0.6	0.9	0.8	0.9	ND	ND

[a] Plasmids were transformed into the isogenic series of yeast strains derived from HR125-5d, and β-galactosidase activity determined as described in the Materials and methods. The values reported are the average of assays of three separate transformants

[b] αF, α-factor; **a**F, **a**-factor

[c] These values were from plasmids transformed into SF167-5a, an **a** *ste12* derivative of 246-1-1; in the isogenic **a** *STE12* strain, EG123, these plasmids conferred activities virtually identical to those listed for the **a** *STE12* strain, HR125-5d

[d] ND, not done

of the pheromone response element (PRE), which confers increased transcription in response to pheromone treatment (Kronstad et al. 1987; Van Arsdell and Thorner 1987). Two of the PREs are perfect matches to the consensus (5′-TGAAACA), one has a C to A substitution at the sixth position, and one has an A to C substitution at the third position. Two versions of the *Hin*dIII fragment were used: one with a wild-type P box ($UAS_{STE2-Pwt}$), the other with a substitution mutation in which the 16 bp P box (5′-TTTCCTAATTGGGTAA) was replaced by an unrelated sequence of identical base composition (5′-TGTATCTCGAGTTTAA) ($UAS_{STE2-Pmt}$). The wild-type and mutant fragments were inserted into two different *lacZ* reporter plasmids, pSL709, a *LEU2* 2 μm-based plasmid, and pΔSS, a *URA3* 2 μm-based plasmid. In the absence of an inserted UAS fragment the two plasmids give very different levels of basal β-galactosidase activity (Jarvis et al. 1988 and Table 1), but each serves as a sensitive test for the UAS activity of inserted DNA fragments.

The $UAS_{STE2-Pwt}$ fragment directed the synthesis of β-galactosidase levels that were 100- to 600-fold greater in **a** cells than in α or **a**/α cells (Table 1). Moreover, β-galactosidase activity in **a** cells was inducible by incubating the cells in the presence of α-factor, although the magnitude of induction was modest. We have never observed β-galactosidase activities greater than 1800–2000 units for pSL709 or greater than 500 units for pΔSS. Therefore the modest pheromone induction conferred by $UAS_{STE2-Pwt}$ is probably the result of a limitation on gene expression other than inherent UAS activity. In any event, the **a**-specific, pheromone inducible expression is in accord with the pattern of transcription of the *STE2* chromosomal locus (Hartig et al. 1986).

The expression conferred by $UAS_{STE2-Pmt}$ differed in three ways from that conferred by $UAS_{STE2-Pwt}$ (Table 1). First, β-galactosidase levels in **a** cells were reduced to essentially the levels seen for plasmids pSL709 and pΔSS lacking UAS elements. Second, β-galactosidase activities increased greatly (10- to 50-fold) when **a** cells were treated with α-factor. Thus, in the absence of the P box, other sequences function as a UAS whose activity can be stimulated by pheromone. Third, expression was no longer limited to **a** cells: the residual UAS activity observed in **a** cells was also apparent in α cells, in particular when they were treated with **a**-factor. The loss of **a** cell specificity that results from the P box mutation reflects a loss of α2-mediated repression. This observation is in agreement with results of Keleher et al. (1988) who found that mutation of the P box within the 33 bp α2 operator from *STE6* destroyed the ability of the operator to confer α2-mediated repression.

UAS activity in ste12 *mutants*

STE12 gene product is required for maximal basal expression of **a**- and α-specific genes (and of some genes transcribed in both **a** and α cells), as well as for induced expression following stimulation by pheromone (Hartig et al. 1986; McCaffrey et al. 1987; Fields et al. 1988). In *ste12* mutants, transcript levels are reduced 5- to 50-fold, depending on the gene. To investigate the contribution of STE12 to UAS activity, the plasmids discussed above were introduced into an **a** *ste12* mutant strain and assayed for β-galactosidase activity. The activity of $UAS_{STE2-Pwt}$ was diminished 3- to 10-fold, even in cells not stimulated by pheromone (Table 1). As expected giv-

en that STE12 is required for response to pheromone, treatment with pheromone did not result in elevated β-galactosidase activity. $UAS_{STE2-Pmt}$ had no activity in the *ste12* strain under any condition. Thus, STE12 makes a contribution to the basal activity of UAS_{STE2} and is required for the pheromone-stimulated activity of the UAS. There are no sequences in this *STE2* DNA fragment that can function as a UAS in the absence of the P box and STE12 function.

Protein-DNA complex formation on UAS_{STE2}

To complement the results of the in vivo assays of UAS_{STE2} activity we have investigated the binding of proteins to wild-type and mutant DNA fragments in vitro. MCM1 has been shown to bind to UAS_{STE2} (Bender and Sprague 1987; Tan et al. 1988). In addition, STE12 binds cooperatively with MCM1 to a 97 bp fragment which contains the P box and a single PRE (Errede and Ammerer 1989) and binds alone, albeit poorly, to DNA fragments from the **a**-specific *MFA1* and *MFA2* genes which contain two copies of PRE (Dolan et al. 1989). We used electrophoretic mobility shift (bandshift) assays to determine whether there is a correlation between the DNA binding by MCM1 and STE12 in vitro and the UAS activity in vivo of the wild-type and mutant *STE2* fragments. In particular, to account for the inducible activity of the $UAS_{STE2-Pmt}$ we wanted to learn whether STE12 could bind to this fragment even though the opportunity for cooperation with MCM1 is presumably precluded. Conversely, perhaps MCM1 could be recruited to bind to the UAS by STE12 or other proteins even in the absence of its own binding site. Under the conditions of our assay, neither of these possibilities was realized.

When an extract of a wild-type **a** strain was incubated with $UAS_{STE2-Pwt}$, three protein-DNA complexes were revealed by bandshift assay as one major band plus two minor, more slowly migrating bands (Fig. 1, lane 7). The slowest complex is STE12-dependent, as it is absent when complexes are formed with a *ste12* mutant extract (Fig. 1, lane 9). To determine whether any of the complexes contained MCM1, we incubated the extracts with affinity-purified antibodies raised against an MCM1 peptide (MIP; Jarvis et al. 1989) and then examined the mobility of protein-DNA complexes formed on the UAS fragment. The inclusion of antibodies reduced the mobility of the major (fastest) complex and the slowest complex (Fig. 1, lanes 8 and 10; compare with 7 and 9, respectively) presumably as the result of formation of a ternary MCM1 (and STE12)/DNA/antibody complex. Whether the very minor, middle complex (Fig. 1, lane 7) contains MCM1 cannot be determined from these data. The specificity of the antibody reaction was demonstrated by preincubation of the antibodies with peptides. The peptide MIP, to which the antibodies were raised, prevented the additional shift presumably by blocking recognition sites on the antibodies, whereas a second peptide, derived from a different segment of MCM1, did not have this effect (data not shown).

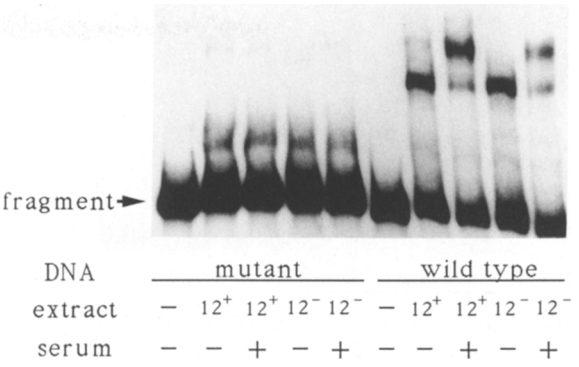

Fig. 1. MCM1- and STE12-dependent complexes form on wild-type *STE2* DNA. Bandshift assays were performed using two different *STE2* DNA fragments, a 367 *Hin*dIII fragment wild-type for the P box ($UAS_{STE2-Pwt}$) and the same fragment mutant at the P box ($UAS_{STE2-Pmt}$). Extracts were prepared from **a** *STE12* (SY1202) and **a** *ste12::URA3* (SY1565) strains and incubated with the DNAs as described in the Materials and methods. In some cases the extract was preincubated with affinity-purified antibodies raised against the MCM1 peptide, MIP (Jarvis et al. 1989). Decreased mobility of a complex when antibodies are included in the incubation (compare lanes 7 and 8 and lanes 9 and 10) implies that MCM1 is part of the complex. The position of the free labelled DNA is indicated by the arrow

When wild-type or *ste12* mutant extracts were incubated with $UAS_{STE2-Pmt}$, no MCM1- or STE12-dependent complexes were detected (Fig. 1, lanes 1–5). Two novel complexes were observed however. Whether these complexes result from sequence-specific interaction of proteins with the mutant *STE2* fragment has not been explored.

*UAS activity of P (*STE2*)*

The experiments described above demonstrate that the P box segment of UAS_{STE2} makes a major contribution to overall UAS activity, especially in cells not stimulated by pheromone. We expected that the P box alone would have significant UAS activity, given the previously demonstrated activity of a perfectly symmetric version of the P box and of P box-containing DNA fragments from *STE6* and *STE2* (Jarvis et al. 1988; Keleher et al. 1988; Errede and Ammerer 1989; Ammerer 1990). To test this possibility directly and to determine whether P (*STE2*) conferred expression that was sensitive to *STE12* genotype or to pheromone, we inserted either P (*STE2*) or P (PAL-16), a symmetric P box, into pSL709 and pΔSS. In addition, a version of P (*STE2*) harbouring a 2 bp deletion was inserted into pΔSS. P (*STE2*) displayed UAS activity both in the orientation found naturally at the *STE2* locus and in the reversed orientation, and the activity was nearly equal to that conferred by P (PAL-16). The 2 bp deletion abolished nearly all UAS activity. None of these synthetic P box oligonucleotides conferred UAS activity that was influenced by the *STE12* genotype or by pheromone stimulation (Table 1). The ability of protein-DNA complexes to form on P (*STE2*) was assessed by bandshift assay. As shown in

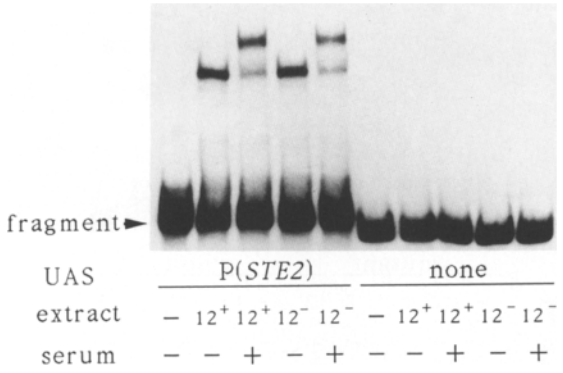

Fig. 2. MCM1 binds to DNA containing P (*STE2*). Bandshift assays were performed using two different DNA fragments. One fragment is 141 bp *Hin*dIII-*Bam*HI fragment from plasmid pSL709, which contains no UAS (lanes 6–10). The second fragment, from pSL1199, is identical except that the P (*STE2*) synthetic oligonucleotide (22 bp) was inserted at the *Xho*I site of the *Hin*dIII-*Bam*HI fragment (lanes 1–5). The experiment was performed exactly as described for Fig. 1

Fig. 2, MCM1 bound to DNA containing P (*STE2*), but no STE12-dependent complexes were detected.

Together the results described above demonstrate that MCM1 and its binding site make a major contribution to the expression of the **a**-specific *STE2* gene, particularly in cells not stimulated by pheromone. The expression that remains in the absence of the MCM1 binding site is highly inducible by pheromone treatment and is STE12-dependent. In vitro, we detect three protein-DNA complexes that form on wild-type *STE2* upstream regulatory sequences, at least two of which contain MCM1. In addition, formation of one complex is STE12-dependent, and this complex presumably contains STE12 as well as MCM1 (Errede and Ammerer 1989). Removed from the context of the *STE2* upstream region, the MCM1 binding site (P box) has UAS activity, but transcription directed by the *STE2* P box alone is not regulated by pheromone or by STE12. Thus, these two proteins appear to account for the pattern of *STE2* transcription observed in vivo: MCM1 directs the constitutive basal transcription and STE12 is responsible for increased transcription upon stimulation by pheromone. Moreover, MCM1 and STE12 act synergistically, because the transcription activity when both functions are present is substantially greater than the sum of the transcription activities observed when only one function is operating. In the absence of MCM1 function (following mutation of the P box) and the absence of *STE12* function (by chromosomal deletion), the *STE2* upstream fragment that we used had no residual UAS activity, indicating that there are no other proteins that alone are capable of binding to this fragment and activating transcription.

UAS activity of QP (STE3)

Although *STE2* appears to contain two distinct UAS elements, one that is MCM1-dependent and one that is both stimulated by pheromone and STE12-dependent, deletion of just the MCM1 binding site from the α-specific *STE3* gene abolished transcription under all conditions tested (Jarvis et al. 1988). To determine whether the MCM1/QP box system of *STE3* confers sensitivity to pheromone and STE12, we inserted the 26 bp QP (*STE3*) sequence into pSL709 and pΔSS. In both the natural and reversed orientation the oligonucleotide conferred α-specific expression to the reporter *lacZ* gene (Table 2). In addition, expression of β-galactosidase was sensitive to pheromone and STE12 regulatory inputs: the activity level increased modestly when wild-type α cells were treated with **a**-factor and was reduced in *ste12* mutants. Thus, the QP (*STE3*) sequence is responsible not only for cell-type-specific transcription but also for at least part of the pheromone stimulation and STE12 dependence that characterizes transcription of *STE3*.

Two equally plausible explanations for this finding can be offered. First, unlike the organization seen for

Table 2. UAS activity of QP (*STE3*)

Plasmid	Fragment	Orientation	Recipient	β-Galactosidase activity[a]				
				a	α	α + **a**F[b]	α *ste12*	α *ste12* + **a**F
pSL709	None			17[c]	17[c]	17[c]	16	16
pSL1195	P (PAL-16)		pSL709	1050[c]	1150[c]	1280[c]	1190	1470
pSL1199	P (*STE2*)	Natural	pSL709	890[c]	1040[c]	1110[c]	1170	1330
pSL904	QP (*STE3*)	Natural	pSL709	29	500	1130	82	98
pSL906	QP (*STE3*)	Reversed	pSL709	23	600	1170	110	140
pΔSS	None			ND[d]	0.6	0.8	0.6	0.8
pSL1196	P (PAL-16)		pΔSS	ND	22	24	24	28
pSL1203	P (*STE2*)	Natural	pΔSS	ND	11	13	14	14
pSL1117	QP (*STE3*)	Natural	pΔSS	ND	4	12	1.7	1.5

[a] pSL709-based plasmids were transformed into the isogenic series of yeast strains derived from HR125-5d, and pΔSS-based plasmids were transformed into the isogenic strains derived from 246-1-1. β-Galactosidase activity was determined as described in the Materials and methods. The values reported are the average of assays of three separate transformants

[b] **a**F, **a**-factor

[c] For clarity, these values are repeated from Table 1

[d] ND, not done

a-specific genes, the MCM1 and STE12 sites may overlap in the *STE3* UAS. The QP (*STE3*) sequence includes no good matches to the consensus PRE; there are, however, three 5 out of 7 matches to the consensus PRE located within the segment. Perhaps these poor PRE matches enable STE12 to bind to the UAS in vivo or perhaps STE12 can join the protein-DNA complex by protein-protein interaction with MCM1 or α1. To date, however, STE12 has not been detected as part of protein-DNA complexes that form on α-specific UAS elements in vitro. Second, STE12 may influence the activity of the QP (*STE3*) UAS not by being part of complexes that form on the UAS, but by acting indirectly, through another gene product, to influence the activity of α1 or the α1·MCM1 complex.

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