MAT α 1 can mediate gene activation by a-mating factor

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In the yeast *Saccharomyces cerevisiae*, expression of α -specific genes is governed by the MAT α 1 and MCM1 gene products. MAT α 1 and MCM1 bind cooperatively to PQ elements upstream of α -specific genes. The PQ element not only directs α -specific expression but can also direct gene induction in response to treatment with a-mating pheromone. We have used gene fusions to investigate whether induction conferred by the PQ box is mediated through either MAT α 1 or MCM1, or a combination of both. When MCM1 is fused to the DNA-binding domain of the bacterial repressor LexA, this fusion protein is capable of *trans*-activating a *lacZ* reporter gene driven by a LexA operator. However, the transcriptional activity of the MCM1–LexA fusion is not further enhanced by treatment of cells with a-factor. A MAT α 1–LexA fusion protein is also capable of *trans*-activation through a LexA operator. Moreover, the activity of the MAT α 1–LexA fusion protein can be further induced by treatment with a-factor. When progressive deletions are made from the amino terminus of MAT α 1 in the fusion protein, the basal level of *trans*-activation progressively decreases, but the inducibility of the fusion protein increases. MAT α 1–LexA fusion proteins, which have ≥ 57 amino acids deleted from the amino terminus of MAT α 1 are not capable of *trans*-activation. In addition, the activity of the MAT α 1–LexA fusion protein is dependent on the functions of the *STE7*, *STE11*, and *STE12* genes that encode components of the pheromone response pathway.

[Key Words: MAT α 1; MCM1; mating pheromone; transcription; SRF; PQ box]

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The yeast *Saccharomyces cerevisiae* has three cell types designated **a**, α , and **a**/ α . The **a**/ α diploid cell type is formed by conjugation of the two haploid cells **a** and α (for review, see Herskowitz 1988). The mating of two haploid cells is mediated by two extracellular mating pheromones referred to as **a**- and α -mating factor. These mating pheromones bind to receptors on the surface of haploid yeast cells and induce cellular responses that include increased transcription of specific genes, increased agglutinability and, finally, growth arrest. The failure of yeast to grow in the presence of extracellular mating pheromones has been used to isolate resistant mutants, which are consequently unable to mate. Thus, the genes in this pathway have been designated sterile genes (*STE*). Many of the sterile genes encode proteins that are part of the signal transduction pathway in response to mating pheromones (Herskowitz 1989). The *STE2* and *STE3* genes encode receptors for the α - and **a**-type mating pheromones, respectively (Burkholder and Hartwell 1985; Nakayama et al. 1985; Hagen et al. 1986; Blumer et al. 1988). The *STE4* and *STE18* genes encode proteins homologous to the β - and γ -type subunits of G proteins (Whiteway et al. 1989). The *STE7* and *STE11* genes encode protein kinases (Teague et al. 1986; Rhodes et al. 1990), and the *STE12* gene encodes a transcription

factor (Dolan et al. 1989; Errede and Ammerer 1989). Deletions of many of these genes reduce expression of **a** and α cell type-specific genes, as well as certain haploid-specific genes required for cell fusion such as *FUS1* (McCaffrey et al. 1987; Trueheart et al. 1987; Fields et al. 1988).

Each of the three yeast cell types expresses a unique set of gene products. The expression of cell type-specific genes in yeast is determined by the gene products of the mating-type locus. In α cells, the MAT α 1 gene product activates the expression of α -specific genes, whereas the MAT α 2 gene product represses expression of **a**-specific genes (Sprague et al. 1983; Johnson and Herskowitz 1985). In **a** cells, the lack of expression of these two gene products leads to the opposite result. However, MAT α gene products do not function alone but in cooperation with the product of the essential MCM1 locus that has also been referred to as PRTF (pheromone/receptor transcription factor) or GRM (general regulator of mating type) (Keleher et al. 1988; Jarvis et al. 1989; Passmore et al. 1989; Ammerer 1990). Both MAT α 1 and MAT α 2 bind cooperatively with MCM1 upstream of the promoters of cell type-specific genes to exert their effects (Bender and Sprague 1987; Keleher et al. 1988, 1989; Sauer et al. 1988; Tan et al. 1988).

MCM1 and MAT α 1 bind to a common sequence motif found upstream of α -specific genes known as the PQ box

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(Jarvis et al. 1988). This 26-bp sequence includes the binding sites for both MCM1 and MAT α 1. MCM1 can bind by itself to the P box. MAT α 1 probably contacts the Q site but only when MCM1 is bound to the P box (Tan et al. 1988; Jarvis et al. 1989; Passmore et al. 1989). Previously, we have shown that the PQ box is sufficient to mediate in addition to α cell-specific gene expression, a pheromone-induced transcription in α cells (Sengupta and Cochran 1990). Mutations in either the *MCM1* or *MAT α 1* genes or in the P or Q sites abolish this response (Bender and Sprague 1987; Jarvis et al. 1988; Passmore et al. 1988; Sengupta and Cochran 1990). In this study we have investigated how the signal generated by *a*-mating factor binding to its receptor is able to up-regulate the expression of α -specific genes. We find that the MAT α 1 gene product is sufficient to mediate this response and that the *trans*-activation activity of the MAT α 1 gene product is dependent on the expression of other sterile genes.

Results

A MAT α 1-LexA fusion protein activates gene expression and is inducible by a-factor

It is well established that MAT α 1 and MCM1 bind cooperatively to PQ boxes upstream of α -specific genes to

stimulate gene expression (Bender and Sprague 1987; Tan et al. 1988). However, it is not known whether the subsequent gene activation and responsiveness to *a*-mating factor is mediated by one or both of these proteins. We have investigated whether MAT α 1 alone is capable of *trans*-activation or is responsive to *a*-factor, or both. Because MAT α 1 is not known to bind DNA by itself, a hybrid gene was constructed by fusion of the entire *MAT α 1* gene to sequences encoding the DNA-binding domain of the bacterial repressor protein LexA. The fusion protein was expressed from the *ADH1* promoter on a 2- μ m-based plasmid. To test whether this MAT α 1-LexA fusion is capable of *trans*-activation, yeast cells were cotransformed with the plasmid expressing the fusion protein and a reporter plasmid carrying either the LexA operator or a PQ box upstream of the transcription start site of an inactive *GAL1* promoter fused to *lacZ* (Hanes and Brent 1989). *GAL1-lacZ* gene expression was measured by assaying the β -galactosidase activity of transformed strains.

Figure 1 shows the results of this experiment in an α strain. The data show that the MAT α 1-LexA fusion does activate gene expression. Target genes lacking upstream binding sites were not stimulated by MAT α 1-LexA fusion proteins, whereas target genes carrying one copy of the LexA operator were activated $\sim$ 20-fold. This activation was enhanced an additional three- to fourfold when

PLASMIDS				
producer	target	-aF	+aF	+aF/-aF
--	0.6	0.8	
	1 x LexA	1.3	1.4	
	4 x LexA	1.6	2.2	
	1 x PQ	7.0	30	4.2X
--	1.0	0.7	
	1 x LexA	2300	2700	
	4 x LexA	nd	nd	
	1 x PQ	7.5	26	3.5X
--	1.0	1.0	
	1 x LexA	2.7	3.5	
	4 x LexA	27	32	1.2X
	1 x PQ	47	135	2.9X
--	0.9	0.9	
	1 x LexA	26	50	2.0X
	4 x LexA	87	210	2.4X
	1 x PQ	110	170	1.5X

Figure 1. A MAT α 1-LexA fusion protein activates gene expression and is inducible by *a*-factor. Producer fusion plasmids and target reporter plasmids containing the indicated binding sites upstream of a *GAL1-lacZ* fusion gene were cotransformed into the MAT α strain 246-1-1. The *MCM1-lexA* and the *MAT α 1-lexA* fusion plasmids contain fusions of the entire coding regions of the *MCM1* gene and the *MAT α 1* gene, respectively, to the DNA-binding domain of LexA (for details, see Materials and methods). The control plasmids containing the LexA DNA-binding domain alone or a *GAL4-lexA* fusion gene have been described previously (Hanes and Brent 1989). The binding sites are the LexA operator sequence in single or multiple copies and a single copy of the 26-bp PQ box from the *STE3* upstream activating sequence (UAS). β -Galactosidase activity was assayed after growth with or without *a*-factor [aF]. Assays were done in triplicate for each plasmid, and the data shown represent the average of three separate experiments. Values are reported in Miller units. (nd) Not determined.

four copies of the LexA operator were used as the target. When one copy of the PQ box was used as a binding site on the reporter plasmid, the basal level expression driven by the PQ box also increased ~15-fold in the presence of the MAT α 1–LexA fusion protein. This increase is probably due to the MAT α 1–LexA fusion protein retaining the ability to interact with MCM1 at the PQ box. The same increase in activity at the PQ box is seen when wild-type MAT α 1 is overexpressed (data not shown). Overproduction of MAT α 1 could drive formation of a MAT α 1–MCM1 complex at the PQ box by mass action to increase occupancy and activation. In contrast, the strong activator GAL4–LexA stimulated gene expression ~90-fold better than the MAT α 1 fusion at the LexA operator but had no effect at the PQ box. The activity of the MAT α 1–LexA fusion protein is not caused by a fortuitous activation by fusion to LexA, as both overexpressed MAT α 1 and the MAT α 1–LexA fusion protein increase expression of the endogenous *STE3* mRNA (an α -specific gene) to comparable extents (data not shown). The MAT α 1 fusion protein is also active to similar levels in a congenic *a* strain (data not shown).

With the addition of *a*-factor, the β -galactosidase activity stimulated by the MAT α 1 fusion at the LexA operator was further increased 2.0- to 2.4-fold over basal levels. This level of induction is somewhat lower than the induction of the endogenous *STE3* gene or that driven by a PQ box in the absence of the fusion protein (4.2 $\times$). The lower level of induction could be due to the overproduction of MAT α 1 itself, which may reduce the level of induction. This is supported by the fact that in the presence of the MAT α 1–LexA fusion an even smaller induction (1.5 $\times$ vs. 4.2 $\times$) is seen upon addition of *a*-factor when the PQ box is used as the target-binding site, although the basal level of expression is higher in this case. Neither the LexA domain by itself nor the GAL4–LexA fusion confers *a*-factor inducibility. Therefore, the MAT α 1–LexA fusion is capable of both constitutively activating transcription and conferring *a*-factor responsiveness onto a heterologous promoter when bound to DNA.

An MCM1–LexA fusion is not inducible by a-factor

To test whether an MCM1–LexA fusion was also capable of responding to *a*-factor, a hybrid gene consisting of the entire MCM1-coding region and the LexA DNA-binding domain was constructed and assayed in experiments similar to those described above. The MCM1–LexA fusion was found to be capable of *trans*-activating transcription, albeit at lower levels than the MAT α 1 fusion (Fig. 1). This result is in accordance with previous observations where it has been shown that MCM1 can stimulate transcription from a P box upstream of a heterologous promoter. In general, it has been found that the higher the affinity of the MCM1-binding site or the greater the level of MCM1 expression, the higher the level of transcription mediated by the P box becomes (Jarvis et al. 1988, 1989; Passmore et al. 1989). The lower level of activity seen here could be accounted for par-

tially by lower levels of expression of the MCM1 fusion protein relative to the MAT α 1 fusion protein (data not shown). However, the MCM1–LexA fusion protein increased the basal level activity driven by the PQ box about sevenfold, implying that MCM1–LexA is overproduced compared with wild-type MCM1. Alternatively, it is possible that the MCM1–LexA fusion protein assumes a different conformation than a wild-type protein bound to the PQ box. Previously, it has been shown that the tertiary structure of MCM1 can differ when bound to different DNA sequences (Tan and Richmond 1990). Because the fusion protein is bound to DNA by the LexA domain, it may not be in the same conformation as when bound to the PQ box.

With the addition of *a*-factor, the β -galactosidase activity driven by the MCM1 fusion at the LexA operator was not further enhanced. This is also consistent with previous observations where it has been shown that expression driven by a high-affinity MCM1 site is not further inducible by *a*-factor (Jarvis et al. 1988; Sengupta and Cochran 1990). Because expression driven by the PQ box is inducible in strains expressing MCM1–LexA fusion, the fusion protein itself must not interfere with signal transduction. The MCM1 fusion protein was also active in a congenic *a* strain and was not inducible by α -factor (data not shown). Therefore, although the MCM1–LexA fusion protein has *trans*-activation properties, it is not inducible by *a*-factor.

Sequences in the amino-terminal region of MAT α 1 are required for both basal and induced levels of expression

To delineate sequences of the MAT α 1 protein that are required for *trans*-activation, progressive deletions from the amino-terminal end of the gene were made and then fused in-frame to the LexA DNA-binding domain. These plasmids were introduced into yeast, and extracts of the transformed cells were analyzed by Western blotting with anti-LexA antisera. This analysis demonstrated that fusion proteins with predicted molecular weights were produced at approximate equal levels (data not shown). These deletion constructs were then assayed for their ability to *trans*-activate a reporter gene bearing four copies of the LexA operator.

The results are shown in Figure 2. As deletions were made from the amino-terminal end of MAT α 1 through the first 39 amino acid residues, the basal level of *trans*-activation activity of the MAT α 1–LexA fusion was found to decrease progressively. However, the level of activity measured after treatment with *a*-factor remained relatively constant. As a consequence, the inducibility of these fusion proteins increased from 3-fold for the MAT α 1 full-length fusion to 14-fold when the amino-terminal 39 amino acids were deleted. Thus, a domain of the protein encoded by the first 39 amino acids is required for basal level expression. Surprisingly, deletion of an additional 6 amino acids increased the basal level expression as well as the level of induced activity. Deletion of a further 12 amino acids essentially

PLASMIDS		target	-aF	+aF	+aF/-aF
producer
4 x LexA	80	250	3.0
4 x LexA	67	240	3.5
4 x LexA	38	250	6.6
4 x LexA	16	210	14
4 x LexA	12	170	14
4 x LexA	120	820	6.8
4 x LexA	1.2	1.4	1.2
4 x LexA	0.9	0.7	0.8
4 x LexA	1.0	3.5	3.5
4 x LexA	1.0	1.1	1.1

Figure 2. Sequences in the amino-terminal region of MAT α 1 are required for both basal and induced levels of expression. Producer plasmids express MAT α 1-LexA fusion proteins. The indicated number of residues have been sequentially deleted from the amino terminus of the MAT α 1-LexA fusion proteins as described in Materials and methods. Target reporter plasmids carry four copies of the LexA operator upstream of the *GAL1-lacZ* fusion gene. The producer and target plasmids were cotransformed into the MAT α strain 246-1-1, and β -galactosidase activity was assayed. +aF/-aF represents the ratio of the β -galactosidase activity after growth with a-factor to activity after growth without a-factor. Values reported are in Miller units. Assays were done in triplicate and represent the average of three separate experiments.

abolished the remaining level of basal activity and most, if not all, of the inducibility conferred by the fusion protein. Therefore, it appears that the basal level *trans*-activation activity of the MAT α 1 fusion protein is partially separable from the inducible level of activity. Deletion of the first 39 amino acids reduces the basal level without diminishing the overall inducible level of expression. The exception to this was the Δ 1-45 truncation that regained a high level of basal activity and a high level of inducibility. Deletion of amino acids 1-57 resulted in loss of both basal level expression and overall inducibility.

To investigate whether the domain of MAT α 1 coding for the amino acids 45-57 is sufficient for *trans*-activation, a fusion protein was constructed between the LexA DNA-binding domain and the sequences encoding amino acids 45-57 of MAT α 1. This fusion protein was cotransformed with a reporter plasmid, and β -galactosidase activity was measured. It was found that this domain of MAT α 1 was not sufficient to confer *trans*-activation onto a LexA operator. Expression of this fusion protein did not further increase activity driven by the PQ box. Western blotting showed that a protein with the expected molecular weight was being produced. Thus, the carboxyl terminus of MAT α 1 is needed along with amino acid residues 45-57 for activity.

Deletions made progressively from the carboxyl terminus of the MAT α 1 protein were also made and fused to LexA. These deletions showed no ability to *trans*-activate but appeared to produce very unstable fusion proteins, as little or no fusion products were detected by Western blotting with anti-LexA antisera (data not shown). It is likely that sequences in the carboxy-terminal region of MAT α 1 are required for protein stability.

The activity of the MAT α 1-LexA fusion protein is dependent on STE12

STE12 is a DNA-binding factor that is required both for the basal and induced levels of expression of many cell type-specific genes in yeast (Fields and Herskowitz 1985; Fields et al. 1988). STE12 has been shown to bind to multimers of a repeated sequence known as the pheromone response element (PRE), which is found in multiple copies upstream of pheromone-responsive genes (Kronstad et al. 1987; Dolan et al. 1989). Although the PQ box contains no consensus PRE sequences, expression driven by the PQ box is dependent on STE12 (Sengupta and Cochran 1990). Therefore, we investigated the activities of the MCM1 and MAT α 1 fusions in a strain carrying a disruption in the STE12 gene. Isogenic strains

carrying either a wild-type *STE12* or a *ste12::LEU2* allele were cotransformed with the plasmids expressing the fusion genes and the reporter plasmids carrying either the LexA operator or the PQ box as binding sites.

Figure 3 shows the results of this experiment. Disruption of *STE12* has no effect on the activity of the GAL4–LexA fusion protein. The *trans*-activation of the LexA operator by the MCM1–LexA fusion was enhanced in the *ste12* mutant strain. This effect is not caused by increased amounts of the fusion protein as detected by Western blots (data not shown). In contrast, the activity driven by the PQ box was decreased by the *ste12* mutation in these strains as is consistent with the *STE12* dependence of this element.

The activity of the MAT α 1–LexA fusion protein, however, was found to be dramatically lower in the *ste12* mutant strain. Expression of target genes with one copy or four copies of the LexA operator was reduced by ~8-fold and 30-fold, respectively, in the *ste12* mutant strain producing the MAT α 1 fusion. Interestingly, when expression driven by the PQ box was assayed, overproduction of the MAT α 1 fusion protein suppressed the effect of the *ste12* deletion at the PQ box. Thus, although the activity of the MAT α 1–LexA fusion is largely dependent on *STE12* at the LexA operator, overproduction of MAT α 1 suppresses the *STE12* dependence of the PQ box. This may be due to overexpressed MAT α 1 stimulating the binding of MCM1 to the PQ box by mass action.

The activity of the MAT α 1–LexA fusion but not the MCM1–LexA fusion is dependent on STE7 and STE11

The products of the *STE7* and *STE11* genes are known to be necessary for both cell type-specific transcription and induction of genes involved in mating (Fields et al. 1988). *STE7* and *STE11* encode protein kinases and appear to act before *STE12* in the pheromone response pathway (Teague et al. 1986; Rhodes et al. 1990). It has been shown previously that overproduction of *STE12* can suppress the mating defect of the *ste7* and *ste11* mutations (Dolan and Fields 1990). We investigated the effect of deletions in the *STE7* and *STE11* genes on the activity of the MCM1 and MAT α 1 fusions.

Fusion and reporter plasmids were cotransformed into congenic *ste7* and *ste11* mutant strains, and β -galactosidase activity was measured. Figure 4 shows the results of this experiment. The activity of the GAL4–LexA fusion protein was not affected by the mutations. β -Galactosidase activity of LexA operator-bearing target genes was enhanced in both the *ste7* and *ste11* mutant strains producing the MCM1 fusions. Overexpression of MCM1–LexA also caused the basal level expression driven by the PQ box to increase, but this activity was slightly reduced in *ste11* and *ste7* strains. Thus, MCM1 has a *trans*-activation activity that is not dependent on *STE7* and *STE11*. Instead, *STE7* and *STE11* function to decrease MCM1 activity slightly.

PLASMIDS			
producer	target	WT	$\Delta ste12$
LexA	--	0.7	1.0
	1 x LexA	1.3	1.7
	4 x LexA	1.4	2.6
	1 x PQ	8.2	3.0
LexA GAL4	--	0.7	1.5
	1 x LexA	2400	2900
	4 x LexA	nd	nd
	1 x PQ	9.6	2.8
LexA MCM1	--	0.8	1.7
	1 x LexA	2.7	4.5
	4 x LexA	13	57
	1 x PQ	35	10
LexA MAT α 1	--	0.9	1.9
	1 x LexA	28	3.3
	4 x LexA	110	3.7
	1 x PQ	110	101

Figure 3. The activity of the MAT α 1–LexA fusion protein is dependent on *STE12*. The producer fusion plasmids and target reporter plasmids described in Fig. 1 were cotransformed into the MAT α strain 246-1-1 (WT) and a congenic *ste12* mutant strain SF167-1c (*ste12::LEU2*), and β -galactosidase activity was assayed. Assays were done in triplicate for each plasmid, and the data shown represent the average of three separate experiments. Values are reported in Miller units. (nd) Not determined.

PLASMIDS				
producer	target	WT	<i>ste7</i>	<i>ste11</i>
--	0.8	0.9	0.9
	1 x LexA	1.4	3.0	1.6
	4 x LexA	1.6	1.6	1.8
	1 x PQ	11	0.9	1.9
--	0.8	1.0	0.6
	1 x LexA	2500	1400	2500
	4 x LexA	nd	nd	nd
	1 x PQ	10	1.5	1.5
--	0.7	1.1	0.9
	1 x LexA	1.8	2.2	3.9
	4 x LexA	7.4	20	36
	1 x PQ	15	6.1	8.5
--	0.8	1.1	0.8
	1 x LexA	31	2.9	2.5
	4 x LexA	130	1.9	2.7
	1 x PQ	120	30	81

Figure 4. The activity of the MAT α 1–LexA fusion protein is dependent on *STE7* and *STE11*. The producer fusion plasmids and target reporter plasmids described in Fig. 1 were cotransformed into a set of congenic strains. (WT) MAT α strain 246-1-1; (*ste7*) *ste7* mutant strain DC130; (*ste11*) *ste11* mutant strain DC39 (Fields et al. 1988). The β -galactosidase activity of at least three independent transformants was assayed. Values reported are in Miller units and represent the average of three separate experiments. (nd) Not determined.

In contrast, activity of LexA operator-bearing target genes was reduced or completely abolished in the *ste7* and *ste11* mutant strains carrying the MAT α 1–LexA fusion. However, as in the case of the *ste12* mutant strain, overproduction of MAT α 1 appeared to suppress partially the *ste7* and *ste11* effects on expression driven by the PQ box. Therefore, although the activity of the MCM1–LexA fusion appears to be independent of the components of the pheromone response pathway, the activity of the MAT α 1 fusion requires the pathway to be intact even for basal levels of expression.

Discussion

Function of MAT α 1

α -Cell-specific promoters in yeast are complex regulatory units and require the function of MAT α 1, MCM1, and several sterile (*STE*) genes (Fields and Herkowitz 1985; Bender and Sprague 1987; Fields et al. 1988; Passmore et al. 1988). Although both PQ boxes and PRE-like sequences are found upstream of α -specific genes, the PQ box by itself is sufficient to confer α -cell specificity and a-factor inducibility onto heterologous promoters (Jarvis et al. 1988; Sengupta and Cochran 1990). In this study we have investigated the mechanisms by which α -specific

gene activation is regulated by a complex of the MAT α 1–MCM1 proteins.

It has been demonstrated previously that MAT α 1 and MCM1 bind cooperatively to the PQ sequence (Bender and Sprague 1987; Tan et al. 1988). MCM1 binds to the P site, and MAT α 1 likely contacts the Q site. MCM1 can bind to P sites by itself, but MAT α 1 binds only in conjunction with MCM1. Previous experiments have suggested that MCM1 is capable of *trans*-activating gene expression when bound to a P box (Jarvis et al. 1988; Passmore et al. 1989). P boxes with greater in vitro affinity for MCM1 have greater *trans*-activation activity, and overexpression of MCM1 can increase expression of a heterologous promoter driven by an upstream P-box element (Jarvis et al. 1988, 1989; Passmore et al. 1989). Because the P-box site in the context of the PQ element is a relatively weak binding site for MCM1 on its own, it might be anticipated that in the absence of MAT α 1, a PQ box would have relatively little UAS activity because the P site would have a relatively low occupancy rate.

One question addressed by our studies is whether MAT α 1 functions only to increase the occupancy rate of MCM1 at the PQ box or whether it has a separate and distinct activity on its own. The fact that a MAT α 1–LexA fusion protein can *trans*-activate through a LexA operator indicates that MAT α 1 has a *trans*-activation ac-

tivity that is independent of MCM1 binding. In these experiments the MAT α 1–LexA fusion protein is a stronger *trans*-activator than the MCM1–LexA fusion protein, suggesting that most of the UAS activity may be due to MAT α 1. However, this may not be a general conclusion as this inference is based on the activities of a fusion protein that is overexpressed and not on those of the wild-type gene product. Moreover, it is possible that the interaction of MAT α 1 and MCM1 induces conformational changes that cause the complex to have greater activity than either protein alone. Consistent with this possibility is the observation of Tan and Richmond (1990) that MCM1 can assume differing conformations depending on the sequence to which it is bound.

Furthermore, several lines of evidence lead to the conclusion that the ability of *a*-mating pheromone to stimulate activity of the MAT α 1–MCM1 complex appears to be largely dependent on MAT α 1. A P box by itself confers no *a*-factor inducibility onto a heterologous promoter (Jarvis et al. 1988; Sengupta and Cochran 1990). A MCM1–LexA fusion, although capable of weakly *trans*-activating through a LexA operator, is not further inducible by treatment of α cells by *a*-factor. However, when MAT α 1 is brought in proximity to DNA by fusion to the LexA DNA-binding domain, it can *trans*-activate and its activity is further induced by *a*-factor. These results suggest that MCM1 is not responsive to *a*-factor in this complex but, instead, serves primarily to bring MAT α 1 to the PQ box. It may be that a MAT α 1–MCM1 complex is more responsive to *a*-factor than is the chimeric fusion protein, but it is clear that MAT α 1 is the principal target of this signal. Furthermore, when deletions are made from the amino terminus of MAT α 1 in the context of a MAT α 1–LexA fusion protein, inducibility of this fusion protein becomes even more pronounced. The amino-terminal 39 residues of MAT α 1 may be responsible for the basal level of transcription that is then reduced when they are removed. Other residues may be more directly involved in responding to signals generated by *a*-factor. Complicating this interpretation, however, is the increased basal level activity as well as increased inducibility of one of the deletion mutants (Δ 45). It is possible that amino acids 39–45 reduce the activity of a protein that may interact with adjacent amino acid residues to confer inducibility. Deletion of the first 57 amino acid residues eliminates all of the activity of the fusion protein.

MAT α 1–lexA activity requires STE12

Although MAT α 1 can *trans*-activate when fused to LexA, the MAT α 1 protein does not appear to possess an intrinsic *trans*-activation domain such as the acid blob motif that has been described for GAL4 (Gill and Ptashne 1987). This is demonstrated by the fact that the activity of the MAT α 1–LexA fusion protein is almost entirely dependent on the activity of the STE7, STE11, and STE12 gene products. Because overexpression of STE12 can suppress the effects of STE7 and STE11 deletions partially, STE12 is believed to be downstream of STE7 and STE11

in the pheromone response pathway (Dolan and Fields 1990). STE12 is a DNA-binding protein that has been shown to bind multimers of a 7-bp repetitive sequence known as PRE, which is found upstream of a number of genes that are inducible by both *a*- and α -mating factors (Dolan et al. 1989; Hagen et al. 1991). Because the consensus PQ box has only a single nonfunctional PRE-like element (Sengupta and Cochran 1990) and the LexA operator contains no PRE sequences, it is unlikely that the STE12 dependence of the α 1–LexA fusion protein is due to STE12 binding to its cognate recognition sequence alongside MAT α 1. However, it cannot be ruled out that an interaction between STE12 and MAT α 1 allows STE12 to bind DNA at a nonconsensus site.

Despite the fact that the MAT α 1–LexA fusion protein is dependent on STE12 function, overexpression of MAT α 1 will partially suppress the STE12 effect on the PQ box. One explanation of this phenomenon would be that overexpression of MAT α 1 is driving formation of MAT α 1–MCM1 complex at the PQ box. *Trans*-activation would occur by MCM1, which is independent of STE12. This hypothesis could be tested by *in vivo* footprinting the PQ box.

How, then, might STE12 regulate MAT α 1 function? Two possible models are diagrammed in Figure 5. In the first model, STE12 forms a complex with MAT α 1 and MCM1 directly. Because Song et al. (1991) have shown that STE12 can respond to α -pheromone when fused to a GAL4 DNA-binding domain, in this model the *trans*-activation function of MAT α 1 would be supplied by STE12. This complex would not necessarily require STE12 binding to a PRE site or even binding to DNA at all. Instead, binding would be mediated principally by protein–protein interactions between STE12 and MAT α 1. Although STE12 is a DNA-binding protein, there is evidence from Errede and Ammerer (1989) that STE12 may complex with other transcription factors at the Ty1 activator sequence in a manner that is independent of its own binding to DNA. However, our attempts to detect STE12-dependent complexes at the PQ box, similar to those found for the Ty1 promoter, have yet to prove successful (data not shown). There are other difficulties with the direct model as well. PRE boxes and the STE12–GAL4 fusion protein have very low levels of basal activity in the absence of mating factor. In contrast, PQ elements and the MAT α 1–LexA fusion protein show significant basal levels of activity, almost all of which are dependent on the presence of STE12. In the STE2 UAS that both binds STE12 and has a high basal level of activity, the presence of a nearby MCM1-binding site may account for the basal level activity (Errede and Ammerer 1989).

An alternative view of the STE12 dependence of MAT α 1 would be that STE12 functions in an indirect manner to influence the activity of MAT α 1. It is known that STE12 is a sequence-specific DNA-binding protein and that it is involved in the regulation of a number of genes, some of which are themselves components of the pheromone response pathway (Fields et al. 1988). The indirect model would hold that deletion of STE12 would

Figure 5. Models for the role of STE12 at the PQ box. (A) Direct model: STE12 is involved directly at the PQ box by a protein-protein interaction with MAT α 1 and provides *trans*-activation activity. Upon addition of a-factor, modifications of the complex, possibly by phosphorylation of STE12 and/or MAT α 1-MCM1, result in increased activity. (B) Indirect model: STE12 regulates the expression of an unknown gene X by binding to PRE sequences. X interacts with the MAT α 1-MCM1 complex at the PQ box by MAT α 1. Pheromone treatment then leads to enhanced activity mediated by modifications of X and/or MAT α 1-MCM1 (for details, see text).

eliminate or reduce the expression of some other unknown gene that influences MAT α 1 function either by directly complexing with MAT α 1 or modifying MAT α 1 in some manner. Current work in the laboratory is directed toward testing these two models.

In either case, the mechanism by which a-factor would enhance activation through the MCM1-MAT α 1 complex remains an open question. Given the presence of the STE7 and STE11 kinases just upstream of STE12 in the pheromone response pathway, it is likely that the phosphorylation of MAT α 1 or STE12 is involved in the pheromone response. If STE12 is part of the complex at the PQ box, then pheromone induction could simply be mediated by the pheromone-dependent phosphorylation of STE12 (Song et al. 1991). Another model of pheromone

activation would be that modification of MAT α 1 or a MAT α 1-STE12 complex would enhance the efficiency of MCM1-MAT α 1 binding to the PQ box. This would be consistent with the observation that overproduction of MAT α 1 or MCM1 on multicopy plasmids can enhance the activity of a PQ box-driven promoter. However, the fact that a MAT α 1-LexA fusion protein is itself inducible would indicate that induced activity is due to more than an enhanced occupancy of the PQ box by the MCM1-MAT α 1 complex.

Relationship to mammalian transcription factors

MCM1 is a member of a family of transcription factors that includes the mammalian serum response factor (SRF) and several homeotic genes in plants (Hayes et al. 1988; Norman et al. 1988; Sommer et al. 1990; Yanofsky et al. 1990). The best characterized of these other family members is SRF. SRF is involved in the regulation of transcription of a variety of mammalian early response genes including the *c-fos* proto-oncogene (Gilman et al. 1986; Treisman 1986; Greenberg et al. 1987). It is also implicated in the regulation of tissue-specific transcription in muscle (Phan-Dinh-Tuy et al. 1988; Walsh 1989; Sartorelli et al. 1990; Webster and Kedes 1990). As with its counterpart in yeast, SRF appears to be capable of complexing with other proteins that may modulate its activity. One of these proteins is p62^{TCF}, which forms a ternary complex between SRF and the serum response element upstream of the *c-fos* gene (Shaw et al. 1989; Schroter et al. 1990). p62^{TCF} is in many ways analogous to MAT α 1 in yeast. This protein does not appear to bind DNA on its own, but does so only in conjunction with SRF. Furthermore, p62^{TCF} contacts DNA on only one side of SRF as MAT α 1 likely does with MCM1. Moreover, as with the MAT α 1 requirement for the Q box, mutation of the contact points for p62^{TCF} alters the response of the serum response element to various extracellular agonists, including phorbol esters (Shaw et al. 1989; Graham and Gilman 1991). Although *c-fos* has a characteristic time course of induction that involves a rapid burst of transcription followed by a rapid shutoff, *in vivo* footprinting experiments suggest that both SRF and p62^{TCF} remain bound to DNA before, during, and after this rapid burst of *c-fos* transcription (Herrera et al. 1989). To date, there are no biochemical modifications of either p62^{TCF} or SRF known that correlate with *c-fos* induction. It is possible that p62^{TCF} functions in a manner analogous to MAT α 1 in yeast. Likewise, it is possible that there may be a mammalian protein that is functionally analogous to STE12. A similar case of conservation of a multicomponent transcription complex has been found recently for the heterotrimeric CCAAT box-binding factors (Chodosh et al. 1988; Oleson and Guarente 1990). Given the similarity between p62^{TCF}-SRF and MAT α 1-MCM1, it is possible that insights gained from yeast will apply to functions of the mammalian factors and that yeast may be used profitably as a tool to identify proteins that are involved in regulation of the mammalian *c-fos* gene.

Materials and methods

Strains and media

Bacteriological work was done with standard techniques (Ausubel et al. 1989). Yeast strains were grown in YPD or SD (Sherman et al. 1986), supplemented with appropriate amino acids to maintain selection for plasmids. Yeast transformation was done with a modified LiOAc procedure (Schiestl and Gietz 1989).

All *S. cerevisiae* strains used were congenic to 246-1-1 (*MAT α* , *trp1*, *leu2*, *ura3*, *his4*, *can1*). The mating type of 246-1-1 was switched by transformation with a plasmid carrying the HO gene to produce the congenic a strain 195-a. SF167-1c carries a gene disruption *ste12::LEU2⁺*, DC39 carries a *ste11- Δ 1*, and DC130 carries a *ste7- Δ 1* gene deletion. All of these strains, with the exception of 195-a, were the kind gift of Dr. Stan Fields (Fields et al. 1988).

β -Galactosidase assays

Assays were performed essentially as described previously (Sengupta and Cochran 1990), except cultures were allowed to grow to an OD₆₀₀ of 1–2. In the case of the *ste* strains, a-factor was not added and assays were performed at the end of the 3.5-hr incubation period in YPD.

Plasmid constructions

DNA manipulations were carried out by standard methods. Restriction enzymes, Klenow, T4 DNA ligase, T4 DNA polymerase, and linkers were obtained from New England Biolabs (Beverly, MA). Exonuclease III and S1 were obtained from Promega (Madison, WI). Polymerase chain reaction (PCR) was performed with reagents from Perkin-Elmer Cetus (Emeryville, CA). Sequencing was carried out with the Sequenase kit from U.S. Biochemical (Cleveland, OH). Oligonucleotides were synthesized at the Biopolymer Laboratory (MIT).

The LexA-containing plasmid pSH2-1 and the LexA-GAL4 fusion-carrying plasmid pSH17-4 are described in Hanes and Brent (1989). The *TRP1* gene was inserted into the *SacI* site of pSH2-1 by adding *SacI* linkers to an *EcoRI* fragment containing the *TRP1* gene [obtained from the plasmid YEP427 (Ma et al. 1987)]. All fusions were constructed in pSH2-1 carrying the *TRP1* gene.

The *MCM1-lexA* fusion was constructed by first digesting the plasmid pED629 (Dubois et al. 1987) with *SspI* and *ClaI*. *SspI* digests 22 bp upstream of the initiator ATG of *MCM1*, and *ClaI* digests 6 bp downstream of the stop codon. This 890-bp fragment was then fused in-frame to the LexA DNA-binding domain at the *BamHI* site of pSH2-1 by using *BamHI* linkers.

The *MAT α 1-lexA* fusion was constructed in two steps. First, the *MAT α 1* gene was obtained as an *EcoRI-BamHI* fragment by using PCR on the plasmid pSL599 (Bender and Sprague 1987). The PCR was done with 50 ng of template DNA and 1.0 μ M each of the primers 5'-CCCGAATTCATGTTTACTTCGAAGCCT-3' and 5'-CCCGATCCCAACCTCCGCCACGA-3'. The reaction was carried out for 30 cycles at the annealing, extension, and denaturation temperatures of 60, 72, and 94°C, respectively, in a Perkin-Elmer Cetus thermocycler. This fragment was then cloned in-frame into the *EcoRI* and *BamHI* sites of the polylinker in pSH2-1. The sequence of the PCR product was confirmed by dideoxy sequencing.

The plasmid LexA/ α 1 20 carries a domain of MAT α 1 consisting of the amino acids 45–57 fused to the LexA DNA-binding domain. This plasmid was constructed by cloning an oligonucleotide encoding the MAT α 1 domain in-frame into the *BamHI* site of pSH2-1. The top strand sequence of the LexA/MAT α 1

45–57 fusion and corresponding amino acid sequence is as follows: -ATC_(pSH2-1)^(α 1)CGACCACTCAAGAAAGATATCCAG-ATTCCTGTTCCCTCCTAAGGATCC; I-RPLKKDIQIPVPS.

The reporter plasmids containing one LexA operator (p1840) and four LexA operators (pSH18-8) upstream of a *GAL1-lacZ* fusion gene in the plasmid LR1 Δ 1 were as described in Hanes and Brent (1989). The sequence of the operators in pSH18-8 differs slightly from that in p1840 (Ebina 1983). LR-PQ was constructed by inserting the *STE3* UAS oligonucleotide (Sengupta and Cochran 1990) into the *XhoI* site at –167 from the *GAL1-lacZ* transcription start site of LR1 Δ 1.

Generation of deletions in MAT α 1

The *MAT α 1* gene (obtained by PCR, as described) was cloned into the *EcoRI* and *BamHI* sites of pBluescript SKII (Stratagene, La Jolla, CA) to obtain SK- α 1. To generate 5' deletions, SK- α 1 was digested with *ApaI* and *HindIII*. Deletions were made by using exonuclease III and S1, as recommended by the manufacturer. The exonuclease III reaction was carried out at 22°C, and samples were removed at intervals of 30 sec for a total of 10 min. The samples from each time point were then filled in with Klenow, ligated, and transformed into competent HB101. Plasmid DNA was prepared from transformants and digested to obtain an estimate of the extent of deletions. Appropriate plasmids were then sequenced by the dideoxy method to determine the deletion end points. The selected deletions in SK- α 1 were then fused in-frame to the LexA DNA-binding domain in pSH2-1 by using the following strategies: LexA/ α 1 Δ 1-8–pSH2-1 was digested with *BamHI* and filled in with Klenow. SK- α 1 carrying the deletion was digested with *Asp718* and *BamHI*, filled in with Klenow, and ligated to pSH2-1. LexA/ α 1 Δ 1-17, Δ 1-26, Δ 1-57, Δ 1-64, Δ 1-77–pSH2-1 was digested with *EcoRI* and filled in with Klenow, followed by digestion with *BamHI*. SK- α 1 carrying the deletions was digested with *Asp718*, filled in with Klenow, digested with *BamHI*, and ligated to pSH2-1. LexA/ α 1 Δ 1-39, Δ 1-45–pSH2-1 was digested with *BamHI*. SK- α 1 carrying the deletions was digested with *Asp718* and *BamHI*, and filled in with Klenow, and *BamHI* linkers were added before ligation with pSH2-1.

The top strand sequences across the junctions of the LexA/ α 1 deletions and the corresponding amino acid sequences are as follows: LexA/ α 1 Δ 1-8–ATC_(pSH2-1)GTACCG_(α 1)AAAAATT; I-VP-KI; LexA/ α 1 Δ 1-17–GAATT_(pSH2-1)GTACCG_(α 1)ATACAGA; E-LYR-YR; LexA/ α 1 Δ 1-26–GAATT_(pSH2-1)GTACCG_(α 1)GCTGAAA; E-LYR-LK; LexA/ α 1 Δ 1-39–ATCC_(pSH2-1)GCGGTA-CC_(α 1)TGCTTC; I-RGT-CF; LexA/ α 1 Δ 1-45–ATCC_(pSH2-1)GCGGTACCG_(α 1)GACCACTC; I-RGTG-PL; LexA/ α 1 Δ 1-57–GAATT_(pSH2-1)GTACCG_(α 1)CTCTCGA; E-LYR-SR; LexA/ α 1 Δ 1-64–GAATT_(pSH2-1)GTACC_(α 1)TCCAAATT; E-LYL-QI; LexA/ α 1 Δ 1-77–GAATT_(pSH2-1)GTACCG_(α 1)GTTTCGA; E-LYR-FR.

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MAT alpha 1 can mediate gene activation by a-mating factor.

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