The N-Terminal 96 Residues of MCM1, a Regulator of Cell Type-Specific Genes in *Saccharomyces cerevisiae*, Are Sufficient for DNA Binding, Transcription Activation, and Interaction with $\alpha 1$

LAURAKAY BRUHN, JEN-JEN HWANG-SHUM, AND GEORGE F. SPRAGUE, JR.*

*Institute of Molecular Biology and Department of Biology,
University of Oregon, Eugene, Oregon 97403*

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MCM1 performs several functions necessary for its role in regulating cell type-specific gene expression in the yeast *Saccharomyces cerevisiae*: DNA binding, transcription activation, and interaction with coregulatory proteins such as $\alpha 1$. We analyzed a set of MCM1 deletion derivatives using in vivo reporter gene assays and in vitro DNA-binding studies to determine which regions of MCM1 are important for its various activities. We also analyzed a set of LexA-MCM1 hybrids to examine the ability of different segments of MCM1 to activate transcription independent of MCM1's DNA-binding function. The first third of MCM1 [MCM1(1–96)], which includes an 80-residue segment homologous to the mammalian serum response factor, was sufficient for high-affinity DNA binding, for activation of reporter gene expression, and for interaction with $\alpha 1$ in vitro and in vivo. However, the ability of MCM1(1–96) to activate transcription and to interact with $\alpha 1$ was somewhat reduced compared with wild-type MCM1 [MCM1(1–286)]. Optimal interaction with $\alpha 1$ required residues 99 to 117, in which 18 of 19 amino acids are acidic in character. Optimal transcription activation required a segment from residues 188 to 286, in which 50% of the amino acids are glutamine. Deletion of this segment from MCM1 reduced expression of reporter genes by about twofold. Moreover, LexA-MCM1 hybrids containing this segment were able to activate expression of reporter genes that rely on LexA binding sites as potential upstream activation sequences. Thus, glutamine-rich regions may contribute to the activation function of yeast transcription activators, as has been suggested for glutamine-rich mammalian proteins such as Sp1.

The MCM1 protein from the yeast *Saccharomyces cerevisiae* is an intriguing and versatile regulatory protein that binds DNA, interacts with several coregulatory proteins, and participates in both transcription activation and repression. As a result of these activities, MCM1 plays a central role in governing the transcription of cell type-specific genes in *S. cerevisiae*. The two haploid cell types, a and α , must each express a unique set of genes in order to communicate and mate efficiently with cells of the other type. MCM1 mediates proper regulation of the a - and α -specific gene sets by operating in conjunction with two coregulators, $\alpha 1$ and $\alpha 2$, which are encoded by the α mating type locus and are therefore present only in α cells. Three regulatory complexes—MCM1, MCM1- $\alpha 1$, and MCM1- $\alpha 2$ —account for activation and repression of the two gene sets (for review, see references 11 and 34).

The molecular mechanisms that underlie this transcription regulation hinge on the nature and sequence context of MCM1 binding sites within the upstream control regions of a - and α -specific genes. In the case of a -specific genes, the control regions contain nearly perfect versions of the palindromic MCM1 binding site, the P box. MCM1 binds to these P boxes in vitro (1, 2, 15, 16, 36), and they are crucial for transcription of the gene set in a cells (12, 17). The MCM1 binding sites are also essential for repression of a -specific genes in α cells. The P boxes of a -specific genes are flanked by binding sites for $\alpha 2$ protein. MCM1 and $\alpha 2$ bind cooper-

atively to the combined $\alpha 2$ /P box/ $\alpha 2$ site in vitro, and the ternary complex is required for repression in vivo (12, 15, 29). Thus, MCM1 is required for transcription of a -specific genes in a cells and for their repression in α cells.

MCM1 is also required for transcription of α -specific genes. The control regions of these genes contain versions of the P box in which one-half of the palindrome is degenerate. However, a conserved 10-bp sequence, the Q box, is present immediately adjacent to the degenerate side. $\alpha 1$ and MCM1 together bind to the QP sequence, but neither protein binds well alone (2, 36). In addition to its role in recruiting MCM1 to bind at QP box sequences, $\alpha 1$ is thought to have two additional roles that contribute to transcription of α -specific genes. First, $\alpha 1$ may induce a conformational change that converts MCM1 to a form which is more competent to activate transcription (37). Second, $\alpha 1$ itself may have the ability to activate transcription (30). In any event, because $\alpha 1$ is present only in α cells, a transcriptionally active QP box-MCM1- $\alpha 1$ complex is formed at α -specific genes only in α cells.

Despite the diversity of functions carried out by MCM1, it is a relatively small protein, 286 amino acids in length. The sequence of MCM1 (22) reveals several striking features that might be important for the execution of these functions (see Fig. 1). The amino-terminal one-third of MCM1 is homologous (68% identity) to a region of serum response factor (SRF), a mammalian protein that binds to the serum response element and mediates the transient transcriptional activation of genes in response to growth factors (20). Given that this segment of SRF has been shown to be necessary

* Corresponding author.

and sufficient for DNA binding and dimerization (20), it is likely that the N-terminal third of MCM1 is responsible for binding to P boxes. Immediately following the SRF homology is an acidic stretch of amino acids in which 19 of 20 residues are aspartate or glutamate. Acidic regions have been shown to play an important role in the transcription activation function of several proteins (e.g., GAL4 and GCN4), although these activating regions tend to be much less acidic (~30%) than that of MCM1 (reviewed in reference 24). The latter two-thirds of MCM1 is highly enriched for glutamine residues (42%), some of which are present in polyglutamine tracts of up to 10 residues. Glutamine-rich regions were first shown to be important for the activation function of a mammalian transcription factor, Sp1, and have been identified in a variety of eukaryotic regulatory proteins (reviewed in reference 19). However, in vivo and in vitro experiments indicate that Sp1 does not efficiently activate transcription in yeast cells (25), and a glutamine-rich segment has not been shown to be important for the activity of any yeast transcription regulator.

To begin to understand the specific mechanisms by which MCM1 acts, we sought to delineate, by deletion analysis, regions of the primary amino acid sequence of MCM1 responsible for DNA binding, transcription activation, and interaction with $\alpha 1$ protein. While these studies were in progress, two other groups have also analyzed the properties of MCM1 deletion mutants (5, 23). Christ and Tye relied solely on in vivo reporter gene assays to conclude that the first third of MCM1 was sufficient to activate transcription from palindromic P boxes but was markedly deficient in activating transcription from α -specific QP boxes (5). Primig et al. complemented this analysis with qualitative biochemical experiments that showed that the N-terminal third of MCM1 binds to DNA and has some capacity to interact with $\alpha 1$ (23). In general, our findings are in agreement, but we have extended the analysis in three important ways: we have quantitated the binding of MCM1 deletion derivatives to DNA, we have assessed the ability of each of these deletion derivatives to interact with $\alpha 1$ in vitro, and we have examined the transcription activation ability of segments of MCM1 independent of its DNA-binding function by constructing a set of LexA-MCM1 fusions. We find that the first 96 residues of MCM1 are sufficient to bind DNA, activate transcription, and interact with $\alpha 1$. The acidic region is not important for transcription activation but may enhance MCM1's ability to interact with $\alpha 1$. Finally, a glutamine-rich region appears to contribute to the transcription activation function of MCM1.

MATERIALS AND METHODS

Strains and media. The yeast strains used in this research were derived from an a/α diploid strain, C842La, in which one copy of the entire MCM1 coding sequence is replaced by the LEU2 gene. C842La, generously provided by Gustav Ammerer, contains the following genetic markers, each homozygous: *leu2-3,112 trp1-1 his3-11,15 ura3 ade2-1 can1-100*. Plasmid pSL1483 (see below), which contains MCM1 in a CEN/ARS-TRP1 vector, was introduced into strain C842La to create YY1887. YY1888 is a MAT α *mcm1::LEU2* pSL1483-containing segregant from YY1887 and was used to analyze the MCM1 deletion series. SY2158, used to analyze the LexA-MCM1 fusions, is a MAT α segregant from YY1887 that contains a wild-type chromosomal MCM1 locus.

The UAS-*lacZ* reporter gene constructs carried on plasmids pSL1477, pSL1972, and pSL1973 (see below) were

introduced into YY1888 by a two-step gene transplacement (26). The resulting strains contain a single copy of P(PAL-16)-*lacZ* (YY2047), QP(STE3)-*lacZ* (YY2049), or P(STE3)-*lacZ* (YY2050) at the FUS1 locus, as confirmed by Southern analysis (33). Isogenic MAT α derivatives (YY2052, YY2054, and YY2055, respectively) of these strains were made by HO-promoted mating type interconversion (11a). Plasmid pSL1574, which contains MCM1 on a CEN/ARS-URA3 vector, was then introduced into the α and a versions of the reporter gene-containing strains, and plasmid pSL1483 was lost by passage on nonselective medium. Subsequently, plasmids expressing the MCM1 deletion series were introduced into these strains, and pSL1574 was lost by using the plasmid shuffle technique (3).

The *Escherichia coli* strains used have been described previously: SB115 (12), DH5F' (Bethesda Research Laboratories), and CJ236 (18).

Standard media and methods were used for propagating yeast and bacterial strains (27, 31). YEPD and SD media have been described elsewhere (14, 31). S-Raf medium contains 2% raffinose in place of the glucose present in SD medium. Plasmid-containing yeast strains were grown in synthetic, selective medium (e.g., SD-Trp or S-Raf-His-Ura).

Plasmids. The plasmids used in this research were constructed by standard methods (27) and include UAS-*lacZ* plasmids used to place reporter genes at the FUS1 locus, MCM1 plasmids used to express the MCM1 deletion series, LexA-MCM1 fusion plasmids, and plasmids used to purify fragments for band shift assays.

Plasmid pSL1476 contains a version of a *CYC1-lacZ* reporter gene which lacks the *CYC1* upstream activation sequence (UAS), flanked by FUS1 sequences to allow insertion of the reporter at the FUS1 locus. Plasmid pSL1476 served as the recipient for test UAS fragments, specifically P(PAL-16), QP(STE3), and P(STE3), and was created as follows. A HindIII-BamHI fragment containing FUS1 upstream sequences but lacking its UAS (−1668 to −268 relative to the ATG) was ligated into HindIII- and BamHI-cut pSL87 (a derivative of YIp5 [35] lacking the EcoRI site) to create pSL1473. A BglII linker was placed at the EcoRI (filled-in) site within the FUS1 upstream sequences to create pSL1474, and then a filled-in EcoRI-HindIII fragment containing FUS1 sequences from +410 to ca. +2630 was ligated into the NruI site of the vector to create pSL1475. A three-part ligation combined a HindIII (filled-in)-BssH2 fragment (−1.8 kb) from pSL709 (13) and a BssH2-BamHI fragment (−1.6 kb) from pMC1871 (4) with XbaI (filled-in)- and BamHI-cut pSL1475 to create pSL1476. The different UAS sequences were put into pSL1476 by replacing the XbaI (filled-in)-BssH2 fragment (containing a polylinker, *CYC1* TATA element, and first part of the *lacZ* gene) with HindIII (filled-in)-BssH2 fragments from pSL1195 (12), pSL905 (13), and pSL719 (13). These three plasmids contain the *CYC1-lacZ* fusion with P(PAL-16), QP(STE3), and P(STE3) UAS sequences placed at the XhoI site of the polylinker. The resulting plasmids were designated pSL1477, pSL1972, and pSL1973, respectively.

The MCM1 deletion series was expressed under control of the MCM1 upstream promoter region in vector pRS314, a CEN/ARS TRP1 yeast shuttle vector (32). Blunt-ended fragments containing upstream sequences (from the SphI site ~860 bp upstream of the MCM1 ATG) and the coding sequences described below were introduced into the SmaI site of the polylinker in pRS314 so that both orientations of MCM1 within the vector were obtained. Constructs in which MCM1 is transcribed in the same and opposite directions as

the *bla* gene of the vector are designated R and L, respectively. Except for the constructs expressing *MCM1*(1–286) and *MCM1*(Δ 99–117), which contain ~10 bp of sequence downstream of the stop codon, the other plasmids in the deletion series do not include any *MCM1* sequence downstream of the stop codons. The C-terminal deletions were constructed by using naturally occurring restriction sites or ones created by standard site-directed mutagenesis techniques. *MCM1*(1–187), *MCM1*(1–117), *MCM1*(1–98), and *MCM1*(1–96) were created so that a stop codon immediately follows the codon for the last amino acid designated. *MCM1*(1–157) contains additional sequences coding for amino acids glycine, arginine, and serine between residue 157 and the stop codon, and *MCM1*(1–91) contains sequences coding for amino acids valine and leucine between residue 91 and the stop codon. *MCM1*(Δ 99–117) contains a deletion of the sequences coding for residues 99 to 117 and was created by site-directed mutagenesis. The configuration of the deletion endpoints in the series was confirmed by sequencing by the dideoxy method (28).

Two additional plasmids, pSL1483 and pSL1574, were used during construction of the yeast strains described above. pSL1483 contains the same *MCM1* upstream and coding sequences described for *MCM1*(1–187) above, inserted at the *Hind*III and *Spe*I sites in pRS314. pSL1574 contains the same *MCM1* upstream and coding sequences described for *MCM1*(1–286) above, inserted at the *Hind*III site (filled in) of pRS316 (32). The *MCM1* gene in both these plasmids is transcribed in the same direction as the *bla* gene.

The *lexA-MCM1* fusion series were expressed under control of the yeast *ADH1* promoter and transcription termination sequences in plasmid pSH2-1, generously provided by Roger Brent's laboratory (10). The fusions were made by using naturally occurring restriction sites and sites created by site-directed mutagenesis, as for the *MCM1* deletion series. All *MCM1* sequences were ligated into pSH2-1 so that they were in frame with *lexA* and any additional, artificial codons present downstream of the *lexA* coding sequences. The additional codons that connect the *lexA* and *MCM1* moieties in *LexA-MCM1*(1–286), -(Δ 99–117), -(1–187), -(1–98), -(1–96), and -(1–91) specify the sequence RPEFPGIPHYSHPAK. The connecting codons in *LexA-MCM1*(32–286), -(32–187), -(118–286), -(158–286), and -(187–286) specify the sequence RPEFPGI, and those in *LexA-MCM1*(92–286) and -(92–187) specify the sequence RP. Except for *lexA-MCM1*(1–91), which contains additional C-terminal sequences coding for amino acids VDLQPS, all the *lexA* fusions have stop codons immediately following the last codon designated.

Plasmid pSH17-4 (10) contains a *lexA-GAL4* fusion in pSH2-1 and was donated by Roger Brent's laboratory along with reporter gene constructions to test the activities of the *LexA* hybrids. Plasmid LR1 Δ 1 contains a fusion of the *GAL1* promoter region, lacking the *GAL4* binding sites, to the *lacZ* gene (38). pSH18-8 and pSH18-34 contain four and eight *LexA*-binding sites, respectively, inserted upstream of the *GAL1-lacZ* fusion.

The UAS-containing fragments used in the band shift assays were obtained from plasmids pSL1874 [P(PAL-16)] and pSL1121 [QP(*STE3*)]. pSL1874 contains a *Hind*III-*Bam*HI fragment from pSL1195 (12) ligated into *Hind*III- and *Bam*HI-cut vector pBluescript KS⁺ (Stratagene). pSL1121 was constructed by ligation of a *Hind*III-*Hpa*I fragment from pSL905 (13) into *Hind*III- and *Pvu*II-cut vector pSP64 (Promega).

β -Galactosidase assays. Plasmid-bearing yeast strains were

grown at 30°C overnight to late-log phase in selective medium as indicated in the figure legends. The cells were diluted to a density of ~10⁷ cells per ml in fresh selective medium and grown for two doublings (~5 h). The cells were prepared and assayed, and the units of β -galactosidase activity were calculated as described previously (14).

Protein extracts. Crude protein preparations were made from plasmid-bearing yeast strains as follows. Cultures were grown in 100 ml of selective medium to mid-log phase, and the cells were harvested and washed with water. The pellets were suspended in a volume (in milliliters) of A100G (20 mM NaPO₄ [pH 7.4], 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, 10% glycerol) equal to the pellet weight (in grams) times 2.5. Phenylmethylsulfonyl fluoride was added at 10 μ g/ml, and 2.5 g of glass beads (Sigma; ~300 μ m in diameter, acid washed) was added per ml of A100G used. Cells were broken by vortexing the suspension at top speed for five 1-min intervals. Between agitations, the suspensions were held on ice for 1 min. Liquid was drawn out and centrifuged in a microcentrifuge for 30 min to remove cellular debris. Protein concentrations were determined with the Bio-Rad Protein Assay Kit. Recombinant α 1 protein, expressed and purified from *E. coli*, was generously donated by the F. W. Dahlquist laboratory.

Band shift assays. Radioactively labeled UAS-containing DNA fragments were prepared for band shift assays as follows. *Hind*III-*Sst*I fragments from pSL1874 and pSL1121 were purified by agarose gel electrophoresis and electroelution and subsequently quantitated spectrophotometrically (27). The P(PAL-16)-containing fragment from pSL1874 is 148 bp long, and the QP(*STE3*)-containing fragment is 158 bp long. Fragments were labeled by filling in the *Hind*III end with the Klenow fragment of DNA polymerase and [α -³²P] dATP (6,000 Ci/mmol; New England Nuclear).

Band shift reaction mixes were incubated for 20 min at room temperature in a final volume of 15 to 20 μ l, containing 20 mM NaPO₄ (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 120 mM NaCl (for the experiments presented in Fig. 6, it contained 20 mM NaCl plus 100 mM KCl), 5 μ g (or the amounts indicated in the figure legends) of crude yeast extract, 60 ng of double-stranded poly(dI-dC) (Pharmacia) per μ g of extract, and 100 pM labeled DNA fragment. Additional unlabeled P(PAL-16) fragment was added with the other DNA to achieve a range of fragment concentrations from 100 to 400 pM for the quantitative band shift analysis. Where indicated, α 1 protein was mixed with the yeast extract on ice before addition of DNA.

Gels for resolving protein-DNA complexes were 4% polyacrylamide:bisacrylamide (38:1.25) containing 10% glycerol in 1 $\times$ TGE (24.8 mM Tris, 192 mM glycine, 1 mM EDTA) or 0.5 $\times$ TBE (1 $\times$ TBE is 89 mM Tris, 89 mM borate, 2.4 mM EDTA), as indicated in the figure legends. Gels were prerun for 30 min at 150 V, 15 to 20 μ l of each binding reaction mix was loaded as the gel was running, and electrophoresis was continued for 2 h. After electrophoresis, the gels were dried and autoradiographed with an intensifying screen at -70°C. For quantitation, regions of the gel containing free and bound DNA were identified from the autoradiograph and excised from the gel. The radioactivity present at each region was measured by scintillation counting. The concentrations of free and bound DNA in each reaction were calculated from the known amount of DNA present in each reaction mix and the percentage of total radioactivity per lane present in the bound and free bands.

RESULTS

MCM1 deletions. The MCM1 amino acid sequence contains several striking features (see the introduction). To investigate the role of these features, we first created a set of deletion derivatives of MCM1 (Fig. 1). In addition to the wild type, MCM1(1–286), the different versions analyzed include partial deletions of the Gln-rich sequences [MCM1(1–187) and MCM1(1–157)] and a complete deletion of the Gln-rich region [MCM1(1–117)]. We also made a deletion lacking both the Gln-rich region and the acidic domain [MCM1(1–98)] and two further deletions [MCM1(1–96) and MCM1(1–91)] which lack small parts of the region homologous to SRF. Additionally, we constructed an internally deleted variant, MCM1(Δ 99–117), which specifically lacks the acidic region.

From preliminary experiments, we anticipated that deletion derivatives might produce different amounts of protein product owing to differences in transcript or protein stability. In the hope of being able to analyze mutants expressing similar levels of protein, we inserted the constructs in two orientations (R and L) into the plasmid vector. Constructions with the L orientation yielded ca. twofold-lower levels of protein than constructions with the R orientation (see below and data not shown). Additionally, in a given orientation, wild-type MCM1(1–286) and MCM1(Δ 99–117) routinely yielded about twofold-lower levels of protein than did the C-terminally deleted constructs. Consequently, we analyzed strains carrying constructs with MCM1(1–286) and MCM1(Δ 99–117) in the R orientation and strains carrying the other constructs in the L orientation so that we were comparing the different versions of MCM1 at approximately equivalent levels of expression.

Expression of palindromic P box-controlled genes. The different versions of MCM1 were first analyzed for expression of the P(PAL-16)-*lacZ* reporter gene, which is under control of a synthetic 16-bp perfectly palindromic P box. All the reporter genes used in this study were integrated at the *FUS1* locus of a yeast strain that carries a disruption of the chromosomal copy of *MCM1*. Because *MCM1* is an essential gene, the mutant derivatives were introduced into α and α versions of these strains on single-copy plasmids by the plasmid shuffle technique (3) in order to determine whether the MCM1 deletions retained the function(s) necessary for viability. With the exception of MCM1(1–91), all the MCM1 derivatives tested were able to maintain viability upon loss of the original wild-type copy of *MCM1* and, furthermore, did not confer an observable growth rate deficiency upon the strain (data not shown).

Figure 1 shows the results of β -galactosidase assays from α cells containing an integrated copy of P(PAL-16)-*lacZ* and the different versions of MCM1. MCM1(1–187)_L gave ~40% of the expression mediated by MCM1(1–286)_R, while other C-terminal deletions of the codons for amino acids 187 to 96 caused no further reduction in activity. Strains carrying MCM1(Δ 99–117)_R showed a small decrease in expression of P(PAL-16)-*lacZ*, but since there was no reduction in activity between MCM1(1–117)_L and MCM1(1–98)_L, it is unlikely that the acidic region contributes directly to expression of P(PAL-16)-*lacZ*. As shown below, the reduced expression mediated by the C-terminal deletion derivatives did not seem to be due to differences in protein concentration or DNA-binding affinity compared with that of wild-type MCM1(1–286). Moreover, the R and L orientations of the different versions of MCM1 showed only modest differences in P(PAL-16)-*lacZ* expression, even though the two orientations gave MCM1 concentrations that differed by about

FIG. 1. MCM1 deletion derivatives and expression of P(PAL-16)-*lacZ*. The diagram of wild-type MCM1 shows the region homologous to SRF (hatched box), the acidic region (dashes), and the glutamine-rich region, where each black bar represents a glutamine residue. The endpoints of the C-terminal deletions are indicated below the diagram. The MCM1 deletion constructs (see Materials and Methods) were introduced into strain YY2047 by the plasmid shuffle technique (3). The strains were grown in SD-Trp medium and assayed for β -galactosidase activity as described in Materials and Methods. Each value reported is the mean for at least three independent transformants, and the standard deviation was typically less than 10% of the mean value. nd, assays not done.

twofold (Fig. 1; see Fig. 4; data not shown). In accord with previous results (5, 23), these data indicate that the N-terminal third of MCM1 is sufficient for viability and for transcription activation, although the transcription activation function of this truncated MCM1 is somewhat reduced compared with that of wild-type MCM1.

Protein stability and DNA-binding affinity. To determine whether the deleted versions of MCM1 were altered with respect to protein stability or DNA binding, we quantitated the concentrations and binding affinities of the mutant proteins in comparison to wild-type MCM1. Two methods were used to assess the relative amounts of MCM1 present in crude extracts. Initially, we used Western immunoblot analysis with MCM1 N-terminal peptide-specific antibodies. Although we were able to detect the longer versions of MCM1, MCM1(1–117), MCM1(1–98), and MCM1(1–96) were difficult to detect by this method. Since all the deletions should contain the epitope to which the antibodies were generated, our inability to detect the smaller versions of MCM1 was probably due to a difference in ability to transfer to nitrocellulose in the Western blot analysis.

As an alternative method, we used the DNA-binding property of MCM1 to quantitate the relative amounts of the mutant proteins. As shown in Fig. 2, extracts prepared from each of the viable MCM1 mutant strains contain MCM1 protein, as judged by the ability to bind and retard the

FIG. 2. MCM1 deletion derivatives bind to P(PAL-16). Band shift assays were performed with 100 pM radiolabeled P(PAL-16) fragment as described in the text. Lane 1, no extract; lanes 2 through 8, 5 μ g of extract from strains expressing the version of MCM1 shown above each lane. MCM1-P(PAL-16) complexes were separated from free DNA, indicated by the arrowhead, in a polyacrylamide gel in 0.5 $\times$ TBE.

electrophoretic mobility of a radiolabeled DNA fragment containing P(PAL-16) in a band shift assay. To quantitate the concentration and binding affinities of the MCM1 protein present in these crude extracts, we used Scatchard-type analysis of band shift experiments in which a constant amount of extract was added to a series of known P(PAL-16) fragment concentrations (6, 8, 9). The amount of bound and free fragment was calculated for each DNA concentration as described in Materials and Methods, and the data were plotted graphically as $P(\text{PAL-16})_{\text{bound}}$ versus $P(\text{PAL-16})_{\text{bound}}/P(\text{PAL-16})_{\text{free}}$ (see Fig. 4 for an example). The y intercept of the plot yields the concentration of active protein in the reaction, while the negative slope of the line yields the dissociation constant (K_d). This type of analysis assumes that the DNA-binding protein binds as a monomer or a preformed multimer, and so it was necessary to establish the state of MCM1 in solution and when bound to DNA.

Evidence from several groups suggests that MCM1 binds DNA as a dimer (1, 13, 21; this study). To confirm this and to determine the state of MCM1 in solution, we used the fact that band shift complexes containing heterodimers of MCM1 (1-286) and MCM1(1-98) should migrate at a position intermediate between those of complexes containing either homodimer. Lanes 1 through 3 of Fig. 3 show band shift reactions with extracts from cells expressing either MCM1 (1-286)_R (lane 1), MCM1(1-98)_L (lane 2), or both constructs (lane 3). The mobility of the intermediate complex formed in lane 3 corresponds to that expected of heterodimers consisting of full-length MCM1 and MCM1(1-98). The fact that only three discrete complexes were observed demonstrates that MCM1 binds as a dimer. When equal amounts of two separate extracts from strains expressing MCM1(1-286)_R or MCM1(1-98)_L were incubated together for up to 60 min prior to addition of P(PAL-16) fragment, no intermediate complexes were detectable (Fig. 3, lanes 4 to 7). Thus, no observable exchange of MCM1 monomers occurs under the conditions used in these experiments, as expected if MCM1 exists as a stable dimer in solution prior to DNA binding.

Given this observation, we then determined the relative amount of MCM1 dimers and the K_d of the different versions of MCM1 by quantitative band shift analysis. The inset of Fig. 4 shows a representative example of a band shift gel in which 5 μ g of extract from cells expressing MCM1(1-187)_R

FIG. 3. MCM1 exists as a stable dimer in solution prior to DNA binding. P(PAL-16) fragment (100 pM) was incubated with 5 μ g of extract from strains expressing MCM1(1-286)_R (lane 1), MCM1(1-98)_L (lane 2), or both proteins (lane 3). In lanes 4 through 7, 5 μ g of extract from the strain expressing MCM1(1-286)_R was incubated with 5 μ g of extract from the strain expressing MCM1(1-98)_L for 0, 20, 40, or 60 min, respectively, at room temperature prior to addition of 100 pM P(PAL-16) fragment. MCM1-P(PAL-16) complexes were separated from free DNA, indicated by the arrowhead, in a polyacrylamide gel in 1 $\times$ TGE.

(lanes 2 to 7) or MCM1(1-187)_L (lanes 9 to 15) was added to a range of P(PAL-16) fragment concentrations. According to these data (Fig. 4), dimers of MCM1(1-187) from strains expressing the R orientation were present at a concentration of 90 pM in the band shift reaction and dimers of MCM1(1-187) from strains expressing the L orientation were present at 53 pM. The dissociation constants for binding to P(PAL-16) were 5.5×10^{-11} M for MCM1(1-187)_R and 6.3×10^{-11} M for MCM1(1-187)_L.

Similar quantitative band shift experiments were done with extracts from cells expressing the other versions of MCM1, and the values obtained for MCM1 protein concentration and K_d are shown in Table 1. Although there are some slight differences, the deleted versions of MCM1 do not differ much from MCM1(1-286)_R in either protein concentration or binding affinity to P(PAL-16). As discussed above, it seems unlikely that the slight differences in the amount and/or K_d of the deleted versions of MCM1 (Table 1) could result in the ~ 2.5 -fold reduction in expression of P(PAL-16)-*lacZ* observed for the C-terminal deletions (Fig. 1). These results imply that the C-terminally deleted versions of MCM1 are deficient in activation of P(PAL-16)-*lacZ* because the region from residues 188 to 286 contributes to the transcriptional activation function of MCM1 rather than to DNA-binding affinity or protein stability.

It should be noted that the quantitative band shift experiments were done with crude extracts, not purified protein, and so the values obtained for the dissociation constant of MCM1 should be viewed in relative terms. These values do agree well, however, with the dissociation constant of 5.9×10^{-11} M obtained for purified MCM1 binding to the STE2 UAS by Tan et al. (36).

Transcription activation by LexA-MCM1 fusions. The experiments described above indicate that MCM1(1-96) is capable of bringing about transcription activation. To exam-

FIG. 4. Quantitative band shift analysis of MCM1(1-187). The inset shows band shift experiments in which 5 μ g of extract from strains expressing MCM1(1-187)_R (lanes 2 through 8) or MCM1(1-187)_L (lanes 9 through 15) was incubated with a series of P(PAL-16) fragment concentrations. Each reaction mix contained 100 pM radiolabeled P(PAL-16) fragment and an additional amount of unlabeled P(PAL-16) fragment, so that the final concentration in each lane ranged from 100 pM (lanes 2 and 9) to 400 pM (lanes 8 and 15). MCM1-P(PAL-16) complexes were separated from free DNA in a polyacrylamide gel in 1 $\times$ TGE. The concentration of bound and free DNA in each lane was determined as described in Materials and Methods and plotted as shown. The negative slopes of the lines give the dissociation constants for the binding: 5.5×10^{-11} M for MCM1(1-187)_R and 6.3×10^{-11} M for MCM1(1-187)_L. The y intercept gives the concentration of active MCM1 dimers in the band shift reactions: 90 pM for MCM1(1-187)_R and 53 pM for MCM1(1-187)_L.

ine the ability of this segment and other regions of MCM1 to activate transcription independent of MCM1's DNA-binding function, we have constructed a series of hybrid proteins consisting of the DNA-binding domain of LexA (residues 1 to 87) fused in frame to different regions of MCM1 (Fig. 5

TABLE 1. Concentrations and dissociation constants of wild-type MCM1 and the deletion derivatives^a

Construct	MCM1 (pM)	K_d (M)
MCM1(1-286) _R	71	5.3×10^{-11}
MCM1(1-187) _L	57	6.4×10^{-11}
MCM1(1-157) _L	56	6.7×10^{-11}
MCM1(1-117) _L ^b	89	3.5×10^{-11}
MCM1(1-98) _L	71	8.2×10^{-11}
MCM1(1-96) _L	55	6.0×10^{-11}
MCM1(Δ 99-117) _R	53	8.0×10^{-11}

^a The concentrations and dissociation constants were determined as described in the legend to Fig. 4, and each value is the mean of two separate determinations.

^b P(PAL-16)-MCM1(1-117) complexes tended to dissociate during electrophoresis in gels containing 1 $\times$ TGE (see Fig. 6, lane 20). Therefore, band shift reaction mixes containing MCM1(1-117) were electrophoresed in 0.5 $\times$ TBE. Quantitative experiments with other versions of MCM1 showed that the two different gel conditions had no effect on the values obtained for concentration or K_d (data not shown).

and Materials and Methods). The fusions were expressed on high-copy-number plasmids from the *ADHI* promoter in strains that contain *lacZ* reporter constructs with 0 (control), 4, or 8 LexA binding sites to serve as potential upstream activation sequences. The LexA fusion vector, the LexA-GAL4 fusion, and the LexA reporter gene constructs were generously provided by Roger Brent's laboratory (see Materials and Methods).

Figure 5 shows the β -galactosidase activity from strains expressing the different hybrid proteins. Although there were differences in the activity of the fusion proteins, these could potentially be ascribed to differences in the stability or structure of the hybrid proteins rather than to the activity of the MCM1 moiety. Therefore, we have interpreted the data only in a qualitative or semiquantitative manner, and three general conclusions emerged. First, the most active MCM1 fusion proteins were about one-fifth as active as a LexA-GAL4 fusion. Second, all fusion proteins that included MCM1 residues 1 to 96 had transcriptional activity. This finding complements the results obtained when the activity of MCM1 mutants was assessed by using reporter constructs based on the MCM1 binding site, P(PAL-16). Finally, four different LexA fusions containing C-terminal regions of MCM1 including residues 187 to 286 [LexA-MCM1(32-286), -(118-286), -(158-286), and -(187-286)] were able to activate transcription, albeit less well than the fusion proteins that contained residues 1 to 96. The only fusion protein containing residues 187 to 286 that was unable to activate transcription was LexA-MCM1(92-286). This hybrid protein contains a very short, two-amino-acid linker between LexA and MCM1, whereas the other constructs include longer linker regions of 7 or 15 residues (see Materials and Methods). Perhaps the close juxtaposition of the highly acidic region of MCM1 to the LexA moiety in this construct interferes with its ability to bind DNA.

The strain in which the LexA-MCM1 hybrid constructs were tested contains a chromosomal copy of wild-type *MCM1*. Wild-type MCM1 could potentially contribute to the activity of the LexA-MCM1 hybrid constructs by interacting with the fusion proteins (e.g., through dimerization). To test this possibility, the LexA-MCM1 plasmids which could maintain viability [LexA-MCM1(1-286), -(Δ 99-117), -(1-187), -(1-98), and -(1-96)] were assayed in strains lacking a chromosomal copy of *MCM1*. In all cases, the ability of the hybrid proteins to activate expression of the *lexA* reporter genes was the same in strains containing and lacking the native copy of *MCM1* (data not shown). These observations support the conclusion that the activities we observed for the fusion proteins were due to direct interaction of the LexA-MCM1 hybrids with the general transcription machinery rather than indirect interactions mediated by wild-type MCM1.

Expression of QP box-controlled genes and interaction with α 1 in vitro. In order to determine which regions of MCM1 are important for interaction with α 1, we first assayed the different mutants for their relative ability to activate an α -specific reporter gene, QP(*STE3*)-*lacZ*, in *MAT α* cells. Mutants with a greater defect in QP(*STE3*)-*lacZ* expression than in P(PAL-16)-*lacZ* expression may be specifically deficient in the ability to interact with α 1. As seen for the P(PAL-16)-*lacZ* assays, deletion mutants MCM1(1-187)_L, MCM1(1-157)_L, and MCM1(1-117)_L showed a roughly two-fold reduction in expression of QP(*STE3*)-*lacZ* compared with wild-type MCM1(1-286)_R (Table 2). This finding is again consistent with the idea that residues 187 to 286 contribute to transcription activation. An additional ca.

		β -Galactosidase activity		
		control	4 LexA binding sites	8 LexA binding sites
LexA		0.3	0.5	0.6
LexA-GAL4		0.3	822	1348
LexA-MCM1(1-286)		0.4	131	286
LexA-MCM1(Δ 99-117)		0.5	185	179
LexA-MCM1(1-187)		0.5	28	50
LexA-MCM1(1-98)		0.5	157	185
LexA-MCM1(1-96)		1.0	158	272
LexA-MCM1(1-91)		0.4	2.5	3.4
LexA-MCM1(32-286)		0.3	9.3	17
LexA-MCM1(92-286)		0.3	1.3	3.5
LexA-MCM1(118-286)		0.4	19	20
LexA-MCM1(158-286)		0.5	24	33
LexA-MCM1(187-286)		0.4	27	53
LexA-MCM1(32-187)		0.2	2.8	2.7
LexA-MCM1(92-187)		0.3	1.0	1.0

FIG. 5. Activities of LexA-MCM1 fusions. The segment of MCM1 present in each LexA fusion is represented schematically with the same diagram shown in Fig. 1. Each *lexA-MCM1* construction was introduced into strain SY2158 along with a reporter plasmid: LR1 Δ 1 (control), pSH18-8 (four LexA binding sites), or pSH18-34 (eight LexA binding sites). The transformants were grown in S-Raf-His-Ura medium and assayed for β -galactosidase activity. Each value reported is the mean for at least three independent transformants, and the standard deviation was not more than 50% of the mean value.

twofold reduction in expression is seen for deletion mutants MCM1(1-98)_L, MCM1(1-96)_L, and MCM1(Δ 99-117)_R. This contrasts with the behavior of these mutants in the P(PAL-16)-*lacZ* assays. For example, MCM1(Δ 99-117)_R gave 17% of the β -galactosidase activity of MCM1(1-286)_R when QP(*STE3*)-*lacZ* was the reporter but gave 68% when P(PAL-16)-*lacZ* was the reporter. These findings imply that the acidic region from residues 98 to 117 contributes to MCM1's ability to interact with α 1 and activate α -specific genes.

To test this hypothesis, we assayed the ability of the deletion mutants to interact with α 1 protein in vitro by using

band shift assays. As shown previously by Bender and Sprague (2), MCM1 is unable to bind a QP(*STE3*)-containing DNA fragment unless exogenous α 1 is added, in which case a specific QP(*STE3*)-MCM1- α 1 complex is formed with an electrophoretic mobility close to that of P(PAL)-MCM1 complexes. Figure 6 shows a series of band shift experiments in which we compared the ability of the different versions of MCM1 to bind to QP(*STE3*) with and without added α 1 protein. Wild-type MCM1 prepared from a cells readily bound to P(PAL-16) DNA, and as expected, no complex with QP(*STE3*) was detected in the absence of α 1 (Fig. 6, lanes 4 and 5). Addition of α 1 to the reaction mixture allowed binding to QP(*STE3*), and the amount of complex formed was greater when 9 units of α 1 (an arbitrary designation for the amount of α 1 used) was added to the reaction than when 3 units was added (Fig. 6, lanes 6 and 7). As presented in Fig. 6, each subsequent set of four lanes shows a similar analysis for each of the MCM1 deletions. These data were quantified, and the amount of QP(*STE3*)-MCM1- α 1 complex formed in the presence of 3 and 9 units of α 1 was compared with the amount of P(PAL-16)-MCM1 complex formed for each version of MCM1 (Fig. 7). Wild-type MCM1 formed roughly an equivalent amount of complex on P(PAL-16) as it did on QP(*STE3*) in the presence of 9 units of α 1 protein, and MCM1(1-187), MCM1(1-157), and MCM1(1-117) showed a similar ability to interact with α 1 and bind QP(*STE3*). In contrast, MCM1(Δ 99-117), MCM1(1-98), and MCM1(1-96) formed substantially more (ca. twofold) complex on P(PAL-16) than they did on QP(*STE3*) in the presence of 9 units of α 1. These results support the idea that the acidic region contributes to MCM1's ability

TABLE 2. Expression of QP(*STE3*)-*lacZ* and P(*STE3*)-*lacZ* reporter genes^a

Construct	β -Galactosidase activity				
	P(PAL-16), α^b	QP(<i>STE3</i>)		P(<i>STE3</i>)	
		α	a	α	a
MCM1(1-286) _R	22.2	9.3	1.4	1.6	2.1
MCM1(1-187) _L	9.0	3.3	0.6	0.7	0.8
MCM1(1-157) _L	9.1	3.3	0.5	0.7	0.8
MCM1(1-117) _L	9.4	4.1	0.6	0.9	0.9
MCM1(1-98) _L	9.2	2.3	0.4	0.6	0.6
MCM1(1-96) _L	8.2	2.1	0.4	0.6	0.5
MCM1(Δ 99-117) _R	15.1	1.6	0.8	1.2	1.1

^a Constructs expressing the different versions of MCM1 were transformed into strains carrying the reporter genes (see Materials and Methods) by the plasmid shuffle technique (3), and β -galactosidase activities were determined as described in the legend to Fig. 1.

^b Assay values are repeated from Fig. 1.

FIG. 6. Interaction of the MCM1 deletion derivatives with $\alpha 1$ in vitro. Band shift reactions were performed with 100 pM DNA fragment of the type shown in each lane of the figure. P represents P(PAL-16), and Q represents the QP(STE3) fragment. Slightly different amounts of extract containing the different versions of MCM1 were used in each set of reactions, so that the concentration of MCM1 dimers was ~ 70 pM, based on the Scatchard analysis results shown in Table 1. Buffer (–) or 3 or 9 units of $\alpha 1$ protein was mixed with the extracts prior to addition of DNA. Protein-DNA complexes were separated from free DNA in a polyacrylamide gel in 1 $\times$ TGE. Note that the P(PAL-16)-MCM1(1–117) complexes dissociate somewhat during electrophoresis, as indicated by the smear between the bound and free DNAs in lane 20. QP(STE3)-MCM1(1–117)- $\alpha 1$ complexes, however, seem to be stable (lanes 22 and 23).

to interact cooperatively with $\alpha 1$. However, they also clearly demonstrate that versions of MCM1 lacking the acidic region retain the ability to bind QP(STE3) in conjunction with $\alpha 1$ in vitro, indicating that the region from residues 1 to 96 is competent to interact with $\alpha 1$.

Interestingly, unlike the other versions of MCM1, MCM1($\Delta 99$ –117), MCM1(1–98), and MCM1(1–96) each show a small amount of complex formed with QP(STE3) in the absence of $\alpha 1$ protein under the conditions used for these experiments. They also form a similar amount of MCM1-DNA complex when a P(STE3)-containing DNA fragment, which lacks the 10-bp Q box, was used in band shift assays (data not shown). To test whether this amount of $\alpha 1$ -independent binding is functionally significant in vivo, we

assayed the ability of the different versions of MCM1 to activate reporter genes when $\alpha 1$ protein and/or the Q box was not present. As shown in Table 2, these three deletion mutants did not confer expression from QP(STE3)-*lacZ* or P(STE3)-*lacZ* in a cells, implying that the mutant MCM1 proteins do not bind to these sequences in vivo or that the complexes are functionally inert.

DISCUSSION

MCM1 is a 286-residue protein with several functions necessary for its activity in regulating cell type-specific gene expression in *S. cerevisiae*. We sought to define the regions of MCM1 necessary for three of these functions—DNA binding, transcriptional activation, and interaction with $\alpha 1$ —by analyzing a series of MCM1 deletions and LexA-MCM1 hybrid proteins using both in vivo reporter gene assays and in vitro DNA-binding studies. In accord with Christ and Tye (5) and Primig et al. (23), we find that a small region of MCM1 contained within the first 96 amino acids is competent to bind DNA, activate transcription, and interact with $\alpha 1$. In addition, we provide direct evidence that a glutamine-rich region in the C terminus of MCM1 contributes to transcription activation and that the acidic region in the middle of the protein contributes to MCM1's ability to interact with $\alpha 1$ protein.

The N-terminal third of MCM1 is sufficient for DNA binding and transcription activation. MCM1(1–96) is sufficient for high-affinity DNA binding to palindromic P boxes in vitro, as expected from the presence of homology between residues 18 to 98 of MCM1 and the region of SRF shown to bind the serum response element. This segment of MCM1 does not contain sequences that resemble known DNA-binding motifs. Therefore, the continued study of MCM1 may reveal a new structural motif capable of sequence-specific binding to DNA.

This small segment of MCM1 is also sufficient to promote transcription activation. MCM1(1–96) and MCM1(1–98) mediate expression of reporter genes controlled by P(PAL-16) to levels only modestly diminished ($\sim 40\%$) from levels promoted by wild-type MCM1. This finding is in general

FIG. 7. Relative binding of MCM1 derivatives to P(PAL-16) and QP(STE3). The amounts of bound and free DNA in the band shift experiments shown in Fig. 6 were quantified as described in Materials and Methods. The percentage of DNA bound in the lanes containing P(PAL-16), QP(STE3) plus 3 units of $\alpha 1$, and QP(STE3) plus 9 units of $\alpha 1$ is represented by a bar for each version of MCM1.

agreement with the results of Christ and Tye (5) and Primig et al. (23), although the precise activity of similar constructions differs slightly between laboratories. For example, in contrast to our results, Christ and Tye found that a deletion derivative containing the N-terminal 97 residues of MCM1, MCM1- Δ DEQ, shows a fivefold reduction in activation of a P(PAL) reporter gene compared with wild-type MCM1 (5). The discrepancy in the activities of MCM1(1-96) and MCM1- Δ DEQ may result from differences in the genetic background of the strains used or from slight differences in the mutant proteins. In particular, MCM1- Δ DEQ contains two non-MCM1 amino acids, VY, at its C terminus (5), whereas MCM1(1-96) and MCM1(1-98) terminate at the specified MCM1 residue. In any case, the salient point is that these severely truncated forms of MCM1 are capable of activating transcription. This observation, coupled with our finding that a LexA-MCM1(1-96) hybrid is able to mediate expression of a reporter gene controlled by LexA binding sites, indicates that MCM1(1-96) is capable of direct interaction with the general transcription machinery.

The transcriptional activation domains of many eukaryotic regulatory proteins are characterized by a high content of a particular amino acid (e.g., acidic residues, glutamines, and prolines), but inspection of residues 1 to 96 does not reveal features common to known activation domains. Thus, as for DNA binding, the study of MCM1 may reveal new mechanisms by which a transcription factor interacts with the general transcription machinery.

A glutamine-rich region at the C terminus of MCM1 contributes to transcriptional activation. In contrast to the N-terminal third of MCM1, the rest of the protein is rich in residues commonly found in eukaryotic activation domains and includes a region in which 19 of 20 residues are negatively charged followed by a region highly enriched for glutamine. Deletion analysis shows that the acidic region, residues 98 to 117, is not important for transcriptional activation but rather contributes to MCM1's ability to interact with α 1 (see below). The region from residue 187 to the C terminus, a segment particularly rich in glutamine residues (49%), does appear to contribute, albeit weakly, to the transcription activation function of MCM1. This conclusion follows from two complementary findings. Deletion mutants lacking this region show reduced expression of reporter genes even though the mutant proteins are present at normal concentrations and bind to DNA with wild-type affinity. In addition, residues 187 to 286 seem to be capable of activating transcription when delivered to DNA by a heterologous DNA-binding domain, a hallmark of many eukaryotic activation domains.

The mammalian glutamine-rich activator Sp1 does not appear to function in *S. cerevisiae* (25), raising the possibility that this class of activator cannot interact with the yeast transcription machinery. However, our observation that a glutamine-rich segment of MCM1 seems to act as an independent activation domain supports the idea that these domains, like acidic activation domains (19, 24), can activate transcription in *S. cerevisiae*.

Although the glutamines within residues 187 to 286 of MCM1 are most prominent, this region is also proline-rich (9%), another feature associated with some transcription activation domains (19). The mechanisms by which glutamine- or proline-rich activation domains function remain unclear, but it has been postulated that these domains fold into defined structures which provide an activation surface capable of interacting with some part of the general transcription machinery. Although further studies will be neces-

sary to determine exactly what features of MCM1 from residues 187 to 286 are necessary for activation, it seems possible that formation of the activation surface requires a combination of glutamine and proline residues. In this regard, it is interesting that the amino acid sequences of other glutamine-rich regions shown to be important for activation (e.g., the two from Sp1) also contain many prolines.

Interaction with α 1. The ability of MCM1 to interact with α 1 cannot be neatly attributed to one of the striking features of the MCM1 sequence. Primig et al. (23) found that residues 1 to 98 were sufficient for interaction with α 1 but suggested that this derivative interacted less well with α 1 than did wild-type MCM1. By analysis of a series of deletions, we find that the region important for interaction with α 1 encompasses the acidic segment and some residues within the N-terminal third of the protein. In particular, we find that versions of MCM1 lacking the acidic region [MCM1(Δ 99-117), MCM1(1-98), and MCM1(1-96)] are more deficient for activation from QP(STE3) than from P(PAL-16). This is the phenotype expected for mutations that specifically interfere with MCM1's ability to interact with α 1, and indeed, these mutant versions of MCM1 are deficient for interaction with α 1 in vitro. Thus, part of the interaction between MCM1 and α 1 may involve electrostatic interactions between the very acidic segment of MCM1 and a basic region of α 1, for instance, the N-terminal half, which has a net charge of +21. However, there must also be another basis for MCM1's ability to interact with α 1 because the versions of MCM1 lacking the acidic region all retain some capacity to activate transcription from QP(STE3) and to complex with α 1 in vitro.

In summary, we have found that a small version of MCM1, containing less than 100 amino acids, is sufficient for viability and retains all the functions of MCM1 that we have assayed, including DNA binding, transcription activation, and interaction with α 1. Additionally, observations by other researchers indicate that this small region of MCM1 is also capable of interaction in vitro with two other coregulators, α 2 and STE12 (23, 37a). Because α 1, α 2, and STE12 do not exhibit significant homologies, they may interact with MCM1 through different mechanisms. This possibility suggests that the N-terminal region of MCM1 may be even more versatile than has been demonstrated to date.

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