Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10966–10970, December 1991
Genetics

Both activation and repression of a-mating-type-specific genes in yeast require transcription factor Mcm1

(positive and negative transcriptional regulator/general regulator of mating/pheromone/receptor transcription factor)

RANDOLPH ELBLE AND BIK-KWOON TYE

Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

Communicated by Efraim Racker, August 19, 1991 (received for review June 18, 1991)*

ABSTRACT **Mcm1 is a yeast transcription factor with homologs throughout the metazoa. *MCM1* was first identified as a gene involved in maintenance of artificial minichromosomes in yeast. More recently Mcm1 has been shown to serve as a transcriptional regulator of mating-type-specific genes. Biochemical data suggest that Mcm1 coactivates α-specific genes and corepresses a-specific genes by binding to a 10-base-pair dyad symmetry element in their upstream regions. We reported previously that an *mcm1* point mutation reduced activation of α-specific genes but had little effect on the expression of a-specific genes. We now show that another *mcm1* allele, which depletes the Mcm1 protein, affects both activation and repression of a-specific genes. The mutant strain remains capable of high levels of pheromone induction of a-specific genes, although with retarded kinetics. Mcm1 joins an increasing number of transcription factors involved in both positive and negative regulation of gene expression.**

The emerging view of gene regulation is one of combinatorial control. The specific level of expression of a given gene may result from the interplay of a multiplicity of factors, each contributing differentially to the final level of transcription. Thus, from a limited pool of DNA-binding factors, a virtually unlimited range of binding specificities and levels of expression may be generated. The same factor may act positively or negatively, depending on the context of its binding sites and/or on the other factors with which it interacts (1, 2). For example, serum response factor (SRF), the vertebrate homolog of the yeast *MCM1* gene, binds together with the ternary complex factor to activate transcription of c-*fos* (3, 4). Repression of c-*fos* is mediated by SRF acting at the same site (5, 6)—presumably in conjunction with other factors. Similarly, the glucocorticoid receptor can activate or repress transcription, depending on the relative levels of Jun and Fos available for cooperative binding (7).

In yeast the study of cell type regulation by the mating type (*MAT*) locus provides examples of combinatorial control. The ready availability of genetics to complement *in vitro* DNA-binding studies makes this system particularly amenable to study. Haploid cell type in yeast is determined by the gene cassette, *MATα* or *MAT***a**, that is resident at the *MAT* locus. The products of these alleles control batteries of genes, including those encoding pheromones and their receptors and agglutination factors (8). *MATα* encodes the α1 protein, a transcriptional activator that binds cooperatively with Mcm1 (also known as pheromone/receptor transcription factor or general regulator of mating) to the upstream regions of α-specific genes (9–11; Fig. 1). *MATα* also encodes the α2 repressor that binds cooperatively with Mcm1 to the upstream regions of **a**-specific genes and represses their transcription in α cells (ref. 11; Fig. 1). In either case Mcm1 recognizes a 10-base-pair (bp) dyad symmetry element, whereas α1 and α2 bind to adjacent sequences. We have referred to this dyad symmetry element as the Mcm1 control element (MCE). The MCE itself can mediate constitutive transcription in all cell types (9, 12). Binding-site mutagenesis studies of the MCE within the α2 operator placed upstream of reporter genes suggested that Mcm1 binding is required for both activation (11, 13) and repression (11) of **a**-specific genes. However, the α2 protein can bind to the α2 operator in the absence of Mcm1 (14). Moreover, the only known *MCM1* mutant allele, *mcm1-1*, had little detectable effect on expression of **a**-specific genes, even though it dramatically reduced α-specific gene transcription (15). This phenotype of the *mcm1-1* mutant suggests that either Mcm1 is not involved in the regulation of **a**-specific genes or that the cooperative binding of Mcm1 with other transcriptional regulators at **a**-specific upstream regulatory site is unaffected by the *mcm1-1* mutation. We now report another, tighter allele of *MCM1* based on 3′ truncation of the gene. This allele not only reduces transcription of α-specific genes in α cells but also reduces **a**-specific gene transcription in **a** cells and derepresses **a**-specific genes in α cells. This report directly demonstrates that the Mcm1 protein is involved in positive, as well as negative, regulation of the expression of an endogenous **a**-specific gene.

MATERIALS AND METHODS

Plasmid and Strain Constructions. To facilitate analysis of the phenotypic effects of *MCM1* gene 3′ truncations, we constructed haploid yeast strains bearing a deletion of the 3.4-kilobase (kb) *Xho* I–*Bam*HI fragment at the *MCM1* locus by the method of Scherer and Davis (see Fig. 2; refs. 16, 17). A *MATα* strain, C2-2μMU, and a *MAT***a** strain, 14D-2μMU, were recovered, each having the genotype *ura3-52 leu2-3,112 trp1-1 his4Δ34 mcm1-ΔXho* I/*Bam*HI. Mcm1 function in these strains is supplied by 2μMU, a clone of the same *Xho* I–*Bam*HI fragment in the 2 μm-derivative YEp24.

Plasmids bearing wild-type (YIp351-SB286) or truncated (YIp351-SB110) *MCM1* alleles, described in Fig. 2 and *Results*, were cleaved with *Bst*EII and transformed into strains C2-2μMU or 14D-2μMU, resulting in integration at *leu2* on chromosome III. Transformants were streaked onto plates containing 5-fluoroorotic acid to select for loss of 2μMU. Colonies obtained from this selection were analyzed by Southern hybridization to confirm the structure of the integrated gene and the loss of wild-type *MCM1* (data not shown). In parallel constructions, YIp5-SB286 and YIp5-XR110 were cleaved at the *Sma* I site in *URA3* and integrated at *ura3* on chromosome V in strains C2-2μML and 14D-2μML. 2μML contains the *MCM1 Xho* I–*Bam*HI fragment in YEp13. Leu$^-$ segregants were obtained by random mitotic loss of the plasmid. The resulting α or **a** strains were

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Abbreviations: MCE, Mcm1 control element; SRF, serum response factor; YPD, yeast extract/peptone/dextrose.

*Deceased September 9, 1991.

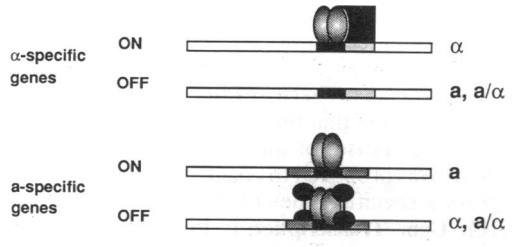

FIG. 1. Model for the role of Mcm1 in regulating mating-type-specific genes. In α cells, Mcm1 (ovals) binds cooperatively with α1 (square) to activate the transcription of α-specific genes, and it binds cooperatively with α2 (dumbbells) to repress the expression of a-specific genes. In the absence of α2 in a cells, Mcm1 behaves as a transcriptional activator of a-specific genes.

designated C2 or 14D, respectively, followed by the appropriate *MCM1* allele number as described in Fig. 2.

β-Galactosidase Assays. Assays of β-galactosidase activity were done as described (9), except that the washing step was omitted, and the blank control contained yeast cells in addition to Z buffer and *o*-nitrophenyl β-D-galactoside. Thus, the background level of β-galactosidase activity in the present study is zero, rather than the 20 units reported previously. For the experiment of Fig. 2, the *STE2-lacZ* reporter gene was contained in plasmid pCDH-DSE14 (9), from which the centromere had been removed. The resulting plasmid pCDH-DSE14Δ*BamHI/BglII* was cleaved with *Bst*EII and integrated at *leu2* in wild-type or mutant strains. Cells were grown to late logarithmic phase at 30°C before assaying.

RNA Analysis. RNA was prepared and analyzed, as described (15), using oligo(dT) columns to enrich for poly(A)$^+$ RNA and agarose/formaldehyde gels to fractionate the RNA. Autoradiograms of hybridized RNA blots were quantified by using a Betascope model 603 blot analyzer (Betagen, Waltham, MA) or by densitometric scanning. Signals were normalized to *ACT1* or other internal standards.

MFα1 was detected using nick-translated pHK2 (from I. Herskowitz, University of California, San Francisco); *ACT1* was detected using the *Bam*HI–*Eco*RI fragment from pSPACT (15); *TUB2* was detected using the 1.1-kb *Bst*EII fragment from pJT71 (D. Botstein, Stanford University, Palo Alto, CA); *HIS3* was detected using the *Sca* I–*Pst* I fragment from pRS303 (P. Hieter, Johns Hopkins Medical School, Baltimore); *MFa1* was detected using pAB161 (A. Brake, Chiron Corp., Emeryville, CA). RNA probes specific for *STE2* and *MCM1* were prepared by using pK.STE2 [3.0-kb *Bam*HI–*Pst* I fragment cloned into Bluescript (Stratagene)] and pKS323 (15), respectively, transcribed *in vitro* by T3 RNA polymerase.

RESULTS

Construction of *MCM1* 3′ Truncation Mutants. We have previously reported the cloning and sequence analysis of the *MCM1* gene (15). *MCM1* encodes a 286-amino acid protein containing a highly acidic patch beginning at residue 98 followed by a region rich in glutamine (Fig. 2*A*). The N terminus of Mcm1 is 70% identical to the DNA-binding and dimerization domain of human SRF; we infer that these functions are conserved in the corresponding region of the Mcm1 protein (refs. 3 and 15; Fig. 2). Indeed, we showed that c-*fos* serum response element, a binding site for SRF, can function as an upstream activation site in yeast in an Mcm1-dependent manner (9). Furthermore, Christ and Tye (18) have demonstrated that the 80-amino acid SRF-homologous domain of Mcm1, containing residues 18–97, is sufficient for viability, minichromosome maintenance, and general transcriptional activation, as well as repression of a-specific genes.

To further analyze the structure and function of Mcm1, we have mutagenized a plasmid containing the *Xho* I–*Eco*RI fragment, which encodes the N-terminal 187 amino acids of Mcm1, by using random insertion of *Bam*HI linkers (16). One of these insertions frameshifted Mcm1 after amino acid residue 110 (Fig. 2*A*, YIp5-XR110). To remove the frameshifted tail, we cut at the site of the linker insertion and the upstream *Sph* I site and subcloned this fragment into YIp351. This procedure introduces a termination codon shortly after the frameshift. The predicted C-terminal sequences of the truncated proteins are shown in Fig. 2. Isogenic strains were constructed containing wild-type or truncated alleles integrated at *ura3* or *leu2* and a deletion of *MCM1* at the wild-type locus. *MCM1* alleles integrated at *ura3* are denoted by a U after the allele number and those integrated at *leu2* by an L after the allele number. The *mcm1-110L* allele has a more severe phenotype and was used for all experiments, except for the reporter gene trans-activation assay. In this assay, the *mcm1-110U* allele was used because the reporter gene vector contained a *LEU2* marker. For further details, see *Materials and Methods* and Fig. 2 legend.

As expected from the functional domain analysis of Mcm1 (18), the mutant strains C2-110U and C2-110L are viable despite the removal of more than half the protein, including all of the polyglutamine tracts and part of the acidic region. Surprisingly, both of the truncation mutants grow more slowly

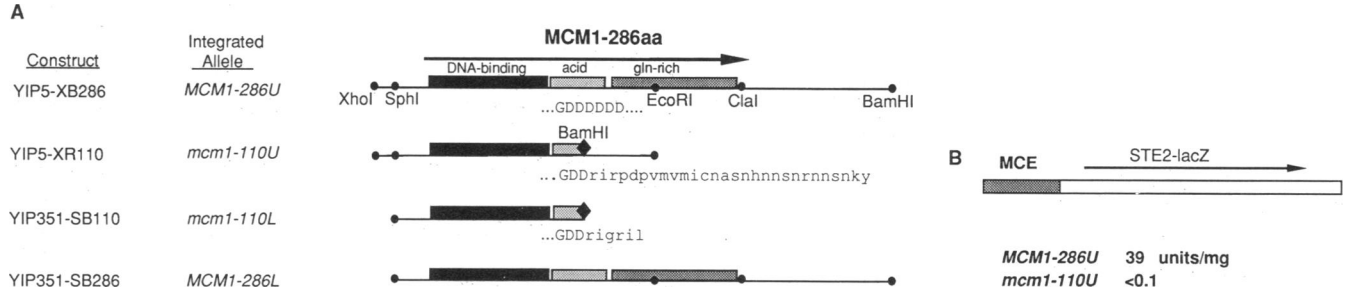

FIG. 2. Construction of C-terminal truncation mutants of *MCM1* that abolish trans-activation. (*A*) A 3.4-kb subclone of *MCM1* in YIp5 was mutagenized by random insertion of *Bam*HI linkers. An insertion was obtained that frameshifted *MCM1* after amino acid 110. This construct was transformed into *MAT***a** or *MATα* strains containing a deletion of the *MCM1* gene and integrated at the *ura3* locus. Alternatively, a subclone containing a shorter frameshifted tail was integrated at the *leu2* locus. The numerical designation following each allele name refers to the length in amino acids of the Mcm1 portion of the protein expressed by each construct. The U or L refers to the *URA3* or *LEU2* marker, respectively, borne by the plasmid construct, and, consequently, to the locus at which the *MCM1* allele is integrated. The arrow represents the *MCM1* open reading frame. The diamond represents the insertion site of the *Bam*HI linker. A portion of the amino acid sequence of wild-type and frame-shifted proteins is shown, beginning at residue 108. Lowercase letters indicate out-of-frame amino acid residues, terminating at a nonsense codon. Figure is not drawn to scale. (*B*) Effect of the truncation on transcriptional activation was assayed by using a reporter gene whose expression depends on an MCE. A plasmid containing the reporter gene was integrated at *leu2* in *MAT***a** strains containing either the *MCM1-286U* or *mcm1-110U* alleles, cells were grown at 30°C, and β-galactosidase activity was measured.

than wild-type strains at 30°C, but the growth defect of C2-110L is more severe, resulting in arrest at 37°C. This temperature-sensitive growth defect is presumably due to 3′ end truncation of the mcm1-110L mRNA, which results in a 10-fold reduction in MCM1 mRNA levels in the C2-110L strain relative to C2-286L (Fig. 3). The natural *MCM1* gene is expressed as multiple transcripts that result from 3′ heterogeneity (ref. 15; data not shown). The single band in the lane containing RNA from the 3′ truncation mutant strain in Fig. 3 results from transcription termination in vector sequences. The low mRNA level of this truncation allele coupled with the instability of the short protein (18) result in a depletion of the Mcm1 protein in the mutant strain, which manifests severe growth defects. The growth and the associated minichromosome maintenance defects of the mutant strains will be described in detail elsewhere. Here we focus on the effects of the mutation on transcriptional regulation of **a**-specific genes.

A Mutant Allele Fails to Activate Transcription of an MCE-Dependent Reporter Gene. We have previously characterized the binding specificity of purified Mcm1 protein *in vitro*. We have also shown that a reporter gene (*STE2-lacZ*) containing the consensus Mcm1-binding site (MCE) responds to varied Mcm1 levels *in vivo* (9). To determine the ability of the truncation mutant to drive transcription from the MCE, we transformed the reporter gene into wild-type (C2-286U) and mutant (C2-110U) strains and assayed β-galactosidase activity. In the wild-type strain, the MCE activated transcription at high levels, producing 39 units of activity per mg of cell protein (Fig. 2*B*). However, in the mutant strain, activity was undetectable even after long incubations. We conclude that the mutant strain is incapable of activating transcription at significant levels from this upstream activation site. These results also imply that Mcm1 is the only factor in yeast capable of activating transcription from this site. Specifically, we can rule out that Mcm1 and the related protein Arg80 have interchangeable functions (19).

The *mcm1-110L* Allele Fails to Activate α-Specific Genes in α Cells. The temperature-sensitive growth phenotype of the C2-110L mutant strain suggests that the 3′ truncation construct represents a tight mutant allele. We previously demonstrated that the original *mcm1-1* mutant has reduced levels of α-specific gene transcripts. We expected the truncated allele to have similar, if not more dramatic, effects on transcription of α-specific genes. As internal standards for our Northern (RNA) analysis, we selected the genes *ACT1*, *TUB2*, and *HIS3*, that have no apparent Mcm1-binding sites upstream. *ACT1* and *TUB2* are thought to be constitutive, whereas *HIS3* is under general amino acid control. Expression of these genes did not vary between wild type and mutant, either relative to each other or to the amount of RNA loaded (Fig. 4*A*). In contrast, MFα1 transcript levels were 15-fold lower in the mutant when cells were grown at 30°C (data not shown) and at least 50-fold lower at 37°C, the temperature at which mutant cells arrest growth. As expected, the mutant cells also display a severe mating defect as α cells, failing to mate with **a** cells but mating proficiently with α cells (Fig. 5). These results are in accord with our previous observation that the *mcm1-1* mutant shows reduced levels of MFα1 transcript and support previous models in which Mcm1 was proposed to coactivate α-specific genes and to corepress **a**-specific genes (9–11).

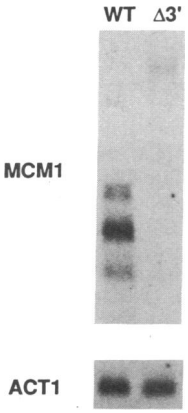

FIG. 3. Analysis of MCM1 transcripts in wild-type (WT) and *mcm1* mutant (Δ3′) strains. About 5 μg of poly(A)$^+$-enriched RNA was analyzed per lane. The gel was stained with ethidium bromide after transfer to confirm that transfer was complete from top to bottom.

a-Specific Gene Transcription Is Reduced in Mutant *MAT*a Cells. Both the *mcm1-1* and the *mcm1-110L* mutants mate poorly as α cells but mate well as **a** cells (ref. 15; Fig. 5). Therefore, we did not expect the truncated allele to greatly affect transcription of **a**-specific genes in **a** cells. Our previous observations led us to postulate a limited role for Mcm1 in activating transcription of *STE2*, an **a**-specific gene encoding the receptor for α factor. We had established that purified Mcm1 protein binds strongly to the *STE2* α2 operator *in vitro* in the absence of cofactors (9). However, *mcm1-1* **a** cells showed only a 2-fold reduction in STE2 RNA levels (15). In contrast, the *mcm1-110L* truncation allele has a considerable effect on *STE2* transcription, reducing it by ≈8-fold relative to the *ACT1* control (Fig. 4*B*). Previous experiments demonstrated that a tight *ste12* mutant similarly reduces STE2 RNA levels (20, 21). That loss of either function results in a 90% reduction of *STE2* gene transcription implies that Mcm1 and Ste12 interact synergistically rather than additively. Consistent with this view, the binding sites for Mcm1 and Ste12 are adjacent, and Ste12 binding to its site appears highly Mcm1-dependent (13, 22). To demonstrate that Mcm1 is involved in the activation of other **a**-specific genes, we examined transcription of the **a**-factor precursor gene *MFa1* (23). We observed a large decrease in transcription of *MFa1* in the *mcm1-110L* mutant (see Fig. 4*C* and below). Thus Mcm1 plays a major role in activating **a**-specific genes as well as α-specific genes.

The Mutant Strain Is Competent for Pheromone Induction. The above finding seems paradoxical because we have observed efficient mating of mutant **a** cells in patch assays (Fig. 5). It would seem unlikely that the cells should mate while expressing such low levels of pheromone receptor. However, one of the initial cell responses to pheromone is the stimulation of transcription of genes involved in mating, including *STE2*. This stimulation is mediated, in part, by Ste12 in a positive feedback loop—i.e., binding of pheromone to the Ste2 receptor stimulates a signal pathway, leading to increases in Ste12 activity, which, in turn, stimulates expression of *STE2*, leading to still more pheromone binding and stimulation of transcription (22, 24–27). Therefore, *STE2* transcription in the mutant might eventually reach levels sufficient to allow mating. We have tested this hypothesis by treating wild-type and mutant **a** cells with α factor and measuring STE2 RNA levels. Fig. 4*C* shows that after 60 min of treatment with α factor, STE2 RNA levels in the mutant approximate those in wild-type cells. Transcription of the pheromone gene *MFa1* is also stimulated, although with still more retarded kinetics (Fig. 4*C*). These observations may explain why *mcm1* **a** cells are fertile. The inability of mutant α cells to mate probably reflects the more extreme reductions in pheromone and receptor steady-state RNA levels and perhaps different requirements for pheromone response in α cells.

***MCM1* Gene Is Not Stimulated by α Pheromone.** Pheromone stimulation increases the levels of Ste12 activity, leading to increased expression of many genes regulated by Mcm1 and Ste12 (26, 27). Whether *STE12* expression is affected by pheromone has not been established. To test the possibility that *MCM1* transcription might be stimulated by pheromone, we measured *MCM1* expression in pheromone-stimulated and untreated wild-type cells (Fig. 4*D*). We found that MCM1 RNA levels were unaffected by α-factor treatment, whereas STE2 RNA levels increased by at least 5-fold relative to an *ACT1* control.

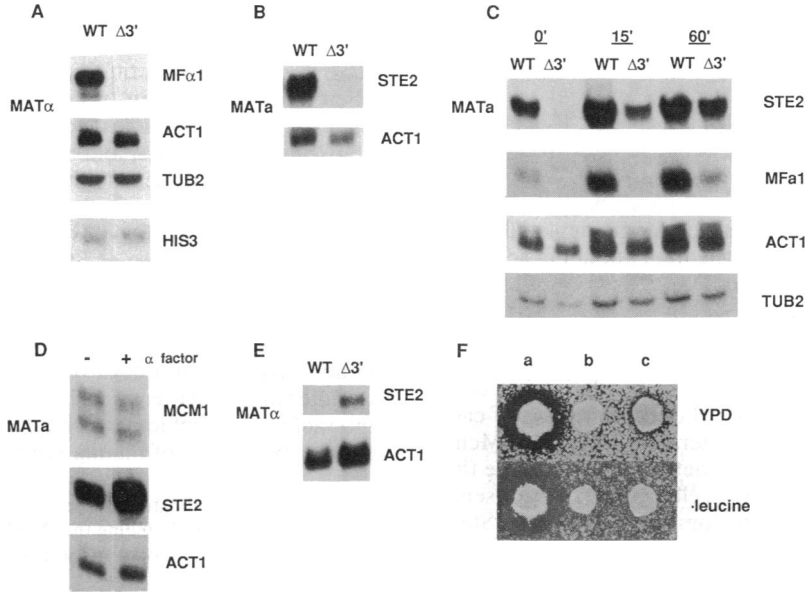

FIG. 4. Northern analysis of α-specific and a-specific gene expression in cells containing *MCM1-286L* or *mcm1-110L* alleles. (*A*) Effect of *mcm1* truncation on *MFα1* transcription. *MATα* cells containing wild-type (WT) or mutant (Δ3′) alleles were grown at 25°C in YPD broth and then shifted to 37°C for 2 hr before harvesting. Poly(A)$^+$ RNA was prepared and analyzed by electrophoresis, blotting, and hybridization. About 5 μg of RNA was loaded onto each lane. (*B*) Reduction of STE2 RNA levels in mutant cells. *MAT***a** cells were grown at 30°C in YPD to late logarithmic phase, and poly(A)$^+$ RNA was prepared and analyzed as in *A*. (*C*) Kinetics of pheromone induction in wild-type and mutant *MAT***a** cells. One hundred-milliliter cultures were grown to late logarithmic phase, and α factor was added to 1 μM. Ten-milliliter aliquots were removed at the indicated times. About 25 μg of total RNA was analyzed per lane. (*D*) Effect of pheromone induction on transcription of *MCM1*. Fifty-milliliter aliquots were taken from each culture in *C* after 60 min of pheromone treatment. Poly(A)$^+$ RNA was prepared and analyzed as in *A*. (*E*) Derepression of *STE2* in *mcm1* mutant *MATα* cells. Cells were grown at 16°C. Poly(A)$^+$ RNA was analyzed. (*F*) Halo assay showing derepression of **a**-factor secretion in α cells bearing *MCM1* on an unstable 2-μm plasmid. Lanes: a, Wild-type *MAT***a** strain 14D-286L; b, wild-type *MATα* strain C2-286L; c, *MATα* strain C2-2μML. Cells were patched onto plates containing rich medium (YPD) to allow loss of the episome or synthetic medium lacking leucine to select for retention of the episome, which bears a *LEU2* marker in addition to the *MCM1* gene. A cell suspension of *MATα sst2* strain RC757 was then sprayed onto both plates and allowed to grow up at 23°C for 4 days. Secretion of **a** factor is indicated by a halo of nongrowth surrounding the patch.

***mcm1* Mutations Can Derepress a-Specific Genes in α Cells.** Transcription of *STE2* and other **a**-specific genes is repressed in α cells by the α2 repressor. The Mcm1-binding site at *STE2* lies at the center of the α2 operator (9–11). Mcm1 binds to this site cooperatively with α2 *in vitro* (9); furthermore, mutations in the center of the α2 operator of another **a**-specific gene, *STE6*, abolish repression by the operator in α cells (11). These results implied that Mcm1 acts as a corepressor of **a**-specific genes in α cells. However, the *mcm1-1* mutation did not derepress *STE2* detectably in a Northern assay (15), even though the *mcm1-1* mutant protein has been demonstrated to have reduced DNA-binding activity (11, 18). We noticed that the C2-110L strain mated with other α cells but not with **a** cells, suggesting that **a**-specific genes must be derepressed in this strain (Fig. 5). Therefore, we asked whether the truncation allele for Mcm1 might affect repression of *STE2* in α cells.

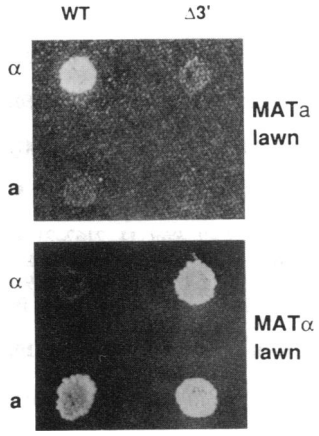

FIG. 5. Mating of *MCM1* and *mcm1-110L* strains. Wild-type and mutant strains were patched onto a YPD plate, replicated onto a lawn of α strain 6697.3 or **a** strain 6697.1, allowed to incubate 24 hr at 30°C, and then replicated onto a minimal plate to select for diploid progeny.

Indeed, C2-110L cells grown at permissive temperatures show partial derepression of *STE2* (Fig. 4*E*), whereas no derepression is detected in the wild-type strain. The difference in *ACT1* signal between the two lanes is <2-fold, whereas the difference in *STE2* signal is ≈100-fold. The C2-110U allele shows similar levels of derepression of **a**-specific functions (data not shown). We believe the derepression results from depletion of Mcm1 activity rather than removal of a region of the protein required for transcriptional regulation or addition of a heterologous tail. We have found that overproduction of the Mcm1-110U protein restores both α factor production and full repression of **a** factor production in mutant α cells, as well as complementing morphological and growth defects (data not shown). In addition, we have observed derepression of **a**-factor secretion (Fig. 4*F*), *STE2* transcription, and barrier activity (data not shown) in α cells bearing a chromosomal deletion of *MCM1* complemented by an intact *MCM1* gene borne on an unstable 2-μm plasmid, 2μML. This plasmid, which also contains the *LEU2* gene, is lost from wild-type cells at a rate of ≈15% per generation (unpublished data). Fig. 4*F* shows the results of halo assays for **a**-factor secretion by wild-type **a** cells, wild-type α cells, and the deletion strain C2-2μML. Each strain was patched onto a plate containing either rich medium, to allow loss of the plasmid, or synthetic medium lacking leucine to select for 2μML. A suspension of α cells was then sprayed onto the plates and allowed to grow up at 23°C. Secretion of **a** factor by the patched cells is indicated by a halo of nongrowth surrounding the patch because **a** factor causes growth arrest of α cells. As expected, *MAT***a** cells produce large halos on both plates, whereas wild-type *MATα* cells fail to produce halos (Fig. 4*F*, lanes a and b). In contrast, the α strain C2-2μML produces a halo on YPD but not on medium lacking

leucine (Fig. 4F, lane c), suggesting that derepression of a factor secretion in this strain is caused by loss of the plasmid bearing *MCM1*. Presumably, a-specific functions become derepressed in cells that have lost the plasmid as Mcm1 becomes depleted and before cell death ensues. Evidence for such a subpopulation depleted of Mcm1 comes from microscopic observations of C2-2μML cells grown in selective or nonselective medium. Cells with multiple elongated buds, the characteristic terminal phenotype of Mcm1-depleted cells, occur frequently in nonselective medium but not in selective medium (unpublished observations).

DISCUSSION

The emerging view of mating-type-gene regulation is a combinatorial one in which the net level of transcription of each **a**- or α-specific gene depends on interactions between Mcm1 and other factors. For instance, genetic studies indicate that expression of α-specific genes in α cells requires the presence of at least three DNA-binding factors: Mcm1, α1, and Ste12 (15, 20, 28). Mcm1 and α1 have been shown to bind to α-specific upstream activation sites cooperatively *in vitro* (9, 10), and mutations in either factor can be partially complemented by overexpression of the other (15). The role of Mcm1 is critical in the expression of α-specific genes since the *mcm1-110L* mutation described here causes a >50-fold reduction in *MFα1* transcription.

Our results indicate that Mcm1 is also involved in activation of **a**-specific genes in **a** cells. This conclusion had been suggested by DNA-binding assays in which Mcm1 was observed to bind efficiently to the α2 operator in the absence of α2 protein (9, 11, 13) and by promoter mutagenesis experiments in which disruption of the Mcm1-binding site reduced activation of **a**-specific genes (11, 13, 29). Ste12 has also been implicated in activation of **a**-specific genes, both genetically and by DNA-binding studies (13, 20–22, 24, 25). Ste12 binding at the *STE2* gene appears highly Mcm1-dependent (13).

Our observation that an *mcm1* mutation can derepress an **a**-specific gene supports the hypothesis of Keleher *et al.* (11) that **a**-specific genes are repressed by a complex of Mcm1 and α2 in α cells. This hypothesis was based originally on genetic studies showing that **a**-specific genes are derepressed in *matα2* mutants (30) and by the biochemical observation that Mcm1 and α2 bind cooperatively to the α2 operator (11, 31). However, because the *mcm1-110L* allele results in depletion of the Mcm1 protein, it is not apparent mechanistically why a reduction in Mcm1 protein levels should derepress **a**-specific genes. If Mcm1 is the principal activator of **a**-specific genes, it might be expected that lack of Mcm1 should abolish both repression and activation in the same stroke. One possible explanation is that the derepressed level of expression is due to Ste12. If Ste12 can activate *STE2*, even at very low levels in the absence of Mcm1 in mutant α cells, then this level could be further stimulated by an autocrine response, resulting from the production of both α factor and α-factor receptor in the same cell. In contrast, the mutant Mcm1-1 protein can function as a corepressor with α2 (15), even though it has reduced binding activity. This observation suggests that cooperativity of these two proteins in their binding to operator sites can partially overcome the binding defect of the mutant Mcm1-1 protein. This explanation is consistent with the conclusion drawn from the functional domain analysis of the Mcm1 protein by Christ and Tye (18) that the α2 interaction site lies within the 80-amino acid SRF homologous domain and presumably is unaffected by the *mcm1-1* mutation.

Several observations suggest that Mcm1 plays a broader role in the cell than simply as a regulator of genes involved in mating. For instance, Mcm1 has been implicated in the regulation of arginine metabolic genes by DNA-binding studies that show the presence of Mcm1 in protein complexes bound to the upstream regions of these genes (32). The *MCM1* gene is essential for mitotic growth in all cell types. Its expression is constitutive with respect to cell type and pheromone stimulation (ref. 15; Fig. 4D). In contrast, the mating regulator Ste12 is encoded by a nonessential gene whose expression is haploid-specific (33). In addition, *MCM1* is required for mitotic stability of minichromosomes, endogenous 2-μm plasmid, and natural chromosomes (unpublished data). Potential binding sites for Mcm1 exist within the Ty*1* transposon and upstream of several genes with important cell-cycle functions—namely, *CDC28, CLN3, MCM3*, and *CDC31* (9, 34–37). We have preliminary data suggesting that the expression of these genes may be regulated by Mcm1 in either a positive or a negative manner. It will be interesting to determine whether Mcm1 also regulates these genes by interacting with cofactors, which has been so well illustrated for the expression of mating-type genes.

This paper is dedicated to the memory of Ef Racker. We thank Chantal Christ for sequencing the *Bam*HI linker junction. This work was supported by National Institutes of Health Grant GM34190.

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