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Yeast Repressor α2 Binds to Its Operator Cooperatively with Yeast Protein Mcm1

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To bring about repression of a family of genes in *Saccharomyces cerevisiae* called the a-specific genes, two transcriptional regulatory proteins, α2 and GRM (general regulator of mating type), bind cooperatively to an operator found upstream of each a-specific gene. To date, GRM has been defined only biochemically. In this communication we show that the product of a single yeast gene (*MCM1*) is sufficient to bind cooperatively with α2 to the operator. We also show that antiserum raised against the *MCM1* gene product recognizes GRM from yeast cells. These results, in combination with previous observations, provide strong evidence that *MCM1* encodes the GRM activity.

The expression of many eucaryotic genes appears to be controlled by combinations of regulatory proteins (for a recent review, see reference 2). An example of this phenomenon is found in the repression of a group of cell-type-specific genes (called the **a**-specific genes) in the α cell type of the yeast *Saccharomyces cerevisiae*. This repression requires the activity of two proteins that bind cooperatively to an operator located upstream of each **a**-specific gene. Either protein alone can recognize the operator, but protein-protein interactions between the two couple their affinities for the DNA and ensure tight binding by the complex. The proteins are α2, a cell-type-specific protein, and GRM, a non-cell-type-specific protein. GRM has been defined only in terms of biochemical function, as follows: GRM is an activity that is (i) capable of binding alone to the **a**-specific gene operator and (ii) able to bind the operator cooperatively with α2 (4).

Considerable evidence suggests that GRM is encoded by the *MCM1* gene. The *MCM1* gene was originally identified by the isolation of a mutation, *mcm1-1*, that rendered strains unable to maintain certain plasmids (hence its name, from minichromosome maintenance) (6; G. R. Maine, Ph.D. thesis, Cornell University, 1984). However, α cells that carried the *mcm1-1* allele exhibited an additional phenotype; they mated poorly (9), a property expected for a cell deficient in GRM. This connection was strengthened by the observation that GRM-binding activity in extracts derived from *mcm1-1* strains was lower than that in extracts from wild-type strains (4). Recently, the *MCM1* gene product (produced in *Escherichia coli*) has been shown to meet the first criterion for GRM activity, namely, it recognizes the **a**-specific gene operator (8).

To determine whether Mcm1 meets the second criterion of GRM activity, we tested the ability of the *MCM1* gene product produced in *E. coli* to bind to an **a**-specific gene operator cooperatively with α2. A portion of the *MCM1* coding sequence (amino acids 1 through 188 [total, 286) fused to 13 amino acids of a bacteriophage protein was overexpressed in *E. coli* and partially purified (30% of the preparation was Mcm1 [see reference 8 for details]). The gel mobility shift experiment shown in Fig. 1 was set up with a ^{32}P-labeled DNA fragment that contained a synthetic copy of the *STE6* **a**-specific gene operator. Partially purified GRM from yeast cells (purified approximately 1,300-fold [see reference 4 for details]) provided a positive control for cooperativity. Low levels of the *MCM1* gene product (from *E. coli*) and purified α2 (from *E. coli* as described in reference 10) were used such that added separately, each occupied only a small fraction of the operators (Fig. 1, lanes 2 and 3, respectively). Figure 1, lane 5 shows the result obtained when the same quantities of Mcm1 protein and α2 were added together to the operator. Here, virtually all of the operator migrates in new, slower-mobility complexes; the fraction of operators occupied by both Mcm1 and α2 is considerably greater than that occupied by either protein added singly. This enhanced binding of the operator by Mcm1 and α2 together is very similar to that obtained upon addition of bona fide GRM (from yeast cells) and α2 together (Fig. 1, lane 4). Also as in the case of GRM, formation of the Mcm1-α2-operator complex required the GRM-binding sequences of the operator. Formation of the complex can be competed against only by operators containing a functional GRM-binding site (data not shown). Given these results, we conclude that Mcm1 and α2 bind to the operator cooperatively.

Using a standard statistical thermodynamic analysis (for an example, see reference 1) of data obtained from densitometric scans of Fig. 1 and of autoradiographs from other experiments, we estimate that the *MCM1* gene product raises the affinity of α2 for the operator approximately 500-fold, corresponding to a cooperative interaction energy of 3 to 4 kcal (12 to 16 kJ)/mol. Additional experiments (data not shown) demonstrate that the *MCM1* gene product, like GRM, cobinds but does not cooperate with the purified C-terminal DNA-binding domain of α2. Thus, cooperative binding of the *MCM1* gene product and α2 requires the amino-terminal domain of α2 and is not simply an indirect consequence of contiguous binding. Because a truncated *MCM1* gene product was used in this experiment, we also know that the C-terminal 98 amino acids of Mcm1 are not required for cooperative binding with α2. It is not known why the Mcm1 hybrid product used in this experiment gives rise to two minor DNA-protein complexes in addition to the major complex (Fig. 1, lane 2). One likely possibility is that the Mcm1 preparation contains minor degradation products.

Because the Mcm1 protein produced in *E. coli* can provide

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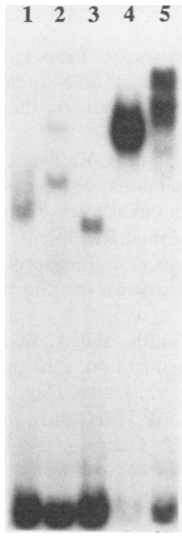

FIG. 1. Gel mobility shift assay of protein-DNA complexes formed by adding purified α2, partially purified Mcm1 (made in *E. coli*), partially pure GRM, or combinations of these proteins to labeled operator. The protein-DNA complexes were separated from free DNA by mobility shift on native polyacrylamide gels and visualized by autoradiography. Lanes: 1, 10 μg of partially pure GRM (total protein) per ml; 2, 24 ng of partially pure Mcm1 per ml; 3, 0.24 μg of purified α2 per ml; 4, 10 μg of GRM and 0.24 μg of α2 per ml; 5, 24 ng of Mcm1 and 0.24 μg of α2 per ml. All reactions contained 4 ng of labeled operator and 1 μg of sheared salmon sperm DNA per ml. No operator binding, alone or cooperatively with α2, was observed in extracts of *E. coli* that did not contain *MCM1* (not shown).

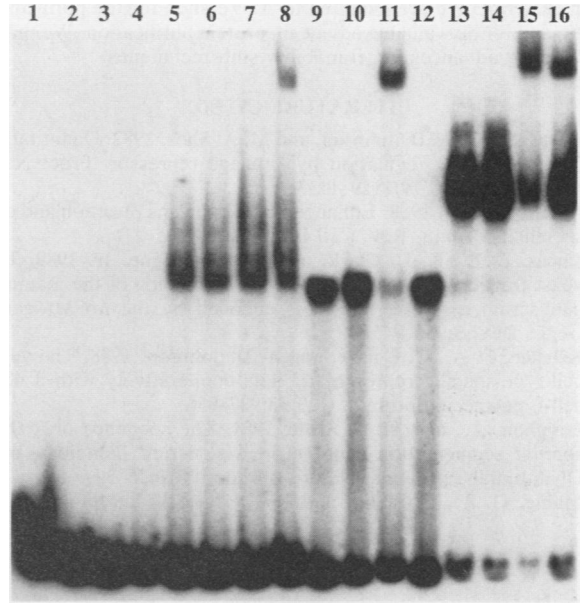

FIG. 2. Gel mobility shift assay of protein-DNA complexes formed by adding purified α2, partially pure GRM, or both to labeled operator in the presence or absence of anti-Mcm1 or anti-α2 antiserum. The protein-DNA complexes were separated from free DNA by mobility shift on native polyacrylamide gels and visualized by autoradiography. Lanes: 1 through 4, no protein; 5 through 8, 11 μg of partially pure GRM (total protein) per ml; 9 through 12, 0.8 μg of purified α2 per ml; 13 through 16, 5.6 μg of partially pure GRM and 0.4 μg of purified α2 per ml; 3, 7, 11, and 15, anti-α2 antiserum; 4, 8, 12, and 16, anti-Mcm antiserum; 2, 6, 10, and 14, preimmune serum; 1, 5, 9, and 13, no antiserum added. All reactions contain 10 ng of labeled operator per ml, and no nonspecific DNA was added.

both of the functions that define GRM, we performed a gel mobility shift experiment (Fig. 2) to test for the presence of Mcm1 in the GRM-operator complex formed using partially pure GRM from yeast cells. Polyclonal antiserum that recognizes the *MCM1* gene product (8) was incubated with GRM prior to the addition of ^{32}P-labeled operator. If the anti-Mcm1 antibodies recognize GRM, they would be expected either to prevent GRM binding to the operator or to form an antibody-GRM-operator complex that migrates more slowly than the GRM-operator complex. The latter result was obtained (Fig. 2). Addition of anti-Mcm1 antiserum gave rise to a shifted complex (lane 8) which migrated more slowly than that given by GRM plus operator alone (lane 5). This recognition is specific, since addition of preimmune or anti-α2 serum (Fig. 2, lanes 6 and 7, respectively) had no effect on the mobility of the GRM-operator complex. Thus, the anti-Mcm1 antibodies do recognize GRM, showing that the *MCM1* gene product (or a related protein) is part of the GRM-binding activity from yeast.

We next tested whether the protein recognized by the anti-Mcm1 antiserum was present in the cooperative three-part complex made up of α2, GRM, and the operator. To this end, purified α2 and partially purified GRM were incubated alone or with anti-Mcm1, anti-α2, or preimmune antiserum before addition of labeled operator DNA. The cooperative GRM-α2-operator complex is shown in Fig. 2, lane 13; it migrated more slowly than either GRM (lane 5) or α2 (lane 9) alone bound to the operator. As expected, addition of either anti-α2 antiserum (lane 15) or anti-Mcm1 antiserum (lane 16) but not preimmune antiserum (lane 14) further reduced the mobility of the GRM-α2-operator complex. This indicates that both the α2 protein and the *MCM1* gene product are present in the ternary complex.

In this paper, we show that Mcm1 binds cooperatively with α2 to the operator and that a protein recognized by anti-Mcm1 antiserum is present in the GRM activity isolated from yeast cells. In addition, preliminary evidence suggests that the *MCM1* gene product is required for repression by α2 in yeast cells: α cells expressing a truncated *MCM1* gene are deficient in repression of at least one a-specific gene (R. Elble and B.-K. Tye, unpublished results). The results presented in this paper add to those previously obtained (see above) (4, 8) to strongly suggest that GRM is encoded by *MCM1* and that Mcm1 and α2 function together in vivo to bring about repression of transcription. It should be noted that recent work (3) indicates that *MCM1* encodes pheromone-receptor transcription factor (PTRF) that, with α1, coactivates the α-specific genes (a family of genes transcribed only in the α cell type of *S. cerevisiae*). Thus, it appears that as previously suggested (3, 4, 8), *MCM1* encodes a protein involved in either repression or activation, depending on its interaction with α2 or α1, respectively.

Mcm1 and α2 are members of two general classes of eucaryotic regulatory proteins. A portion of α2 has been shown to have homology with the homeodomain, a region found in many proteins known to specify cell identity (5, 11). Mcm1 has a region of strong homology with the serum response factor, a mammalian transcription activator that appears to be present in other eucaryotes as well (7). Therefore, we feel that it is reasonable to expect that gene regulation by pairwise combinations of homeodomain proteins and SRF-like proteins will be found in other cases.

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