MCM1 Binds to a Transcriptional Control Element in Ty1

BEVERLY ERREDE

Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599

Received 16 June 1992/Returned for modification 13 August 1992/Accepted 14 September 1992

Some Ty1 transposable-element insertion mutations of *Saccharomyces cerevisiae* activate adjacent-gene expression. These Ty1-activated genes are regulated similarly to certain mating genes. This report shows that the MCM1 protein, which binds to several mating genes, also binds to a transcriptional regulatory sequence in Ty1. The binding of MCM1 to Ty1 correlates with the ability of its binding site to function as a component of the Ty1 transcriptional activator. This correlation supports the idea that MCM1 is important for Ty1-activated gene expression. At mating-gene promoters, MCM1 binds with coactivators or repressors such as STE12, $\alpha 1$, or $\alpha 2$. In contrast, MCM1 binds without these associated DNA-binding proteins at its site in Ty1. This finding suggests that its role in Ty1-mediated transcription is different from that at mating genes.

Expression of the Ty retrotransposon of *Saccharomyces cerevisiae* is 5 to 20 times greater in either a or α haploid cells than in a/ α diploid cells (8). Some Ty promoter insertion mutations cause cell-type-specific expression of the adjacent gene (10, 33). This pattern of expression occurs because Ty transcriptional control sequences respond to cellular regulators whose physiological roles are in the mating response.

The proteins encoded by the mating-type locus, *MAT*, establish the cell type of yeast. *MAT α* encodes the $\alpha 1$ and $\alpha 2$ DNA-binding proteins, and *MATa* encodes the a1 DNA-binding protein. Depending on which of these three proteins are present, a-, α -, or diploid-specific genes will be expressed (17, 31). In addition, haploid-specific genes (expressed in a and α cells but not in a/ α cells) such as *HO* and Ty elements are regulated by these *MAT*-encoded DNA-binding proteins (8, 10, 19, 33). Other DNA-binding proteins are also important for the transcriptional regulation of cell-type-specific genes. For example, the MCM1 transcription factor cooperates with the $\alpha 1$ protein for expression of α -specific genes and with the $\alpha 2$ protein for repression of a-specific genes (1, 2, 18, 20, 26). At some cell-type-specific promoters, MCM1 cooperates with the STE12 transcription factor (9, 25, 28). STE12 is the proposed target of the pheromone response pathway that induces expression of some genes involved in mating (7, 9, 30).

The *CYC7-H2* allele is a Ty1 promoter insertion mutation that responds to cell-type-specific regulators. The Ty1 element causes a 20-fold overproduction of the *CYC7* gene product in a and α haploid cell types. Expression of both *MATa1* and *MAT $\alpha 2$* in the a/ α diploid cell type represses this overproduction (10, 29). Mutations that cause loss of function of the *STE7*, *STE11*, or *STE12* genes similarly abolish *CYC7-H2* expression in haploid cell types (10, 11). The *STE7* and *STE11* genes encode protein kinases that are assumed to affect the activity of the *STE12*-encoded transcription factor (see reference 32 for a review).

There are at least two distinct transcriptional control elements within the 5.6-kb Ty1 element that are responsible for haploid-specific and *STE*-dependent regulation of *CYC7-H2* expression (5). One of these is the sterile responsive element (SRE), which is proximal to the 5' delta of Ty1 (Fig. 1A). The SRE causes *STE7*-, *STE11*-, and *STE12*-dependent activation of the *CYC7* reporter gene and has a binding site for the STE12 protein (4, 8a, 9). The second control element is more distal to the 5' delta element. Two to four tandem copies of a sequence from the Ty1 distal

regulatory region activate expression of the *CYC7* reporter gene in haploid cells but not in diploid cells (Fig. 1A, fragment H) (6).

Footprint analysis identified binding sites for proteins that form complexes with the distal Ty1 regulatory sequence (Fig. 1B, sites 1 and 2) (6). One of these binding sites (site 2) is within a 28-bp oligonucleotide that functions as a component of the Ty1 transcriptional activator of adjacent gene expression (6, 13). One candidate for the binding factor associated with the site 2 footprint is MCM1. A perfect palindrome sequence (Fig. 1C, PAL) was deduced from the naturally occurring MCM1-binding elements found in the promoter region of several a- and α -specific genes (2). A comparison of the Ty1 sequence and the PAL sequence shows that the two are similar (12 of 16 match). This report shows that MCM1 is the protein that binds to Ty1 at this sequence. Additional evidence supports the idea that MCM1 is important for this sequence to function as a component of the Ty1 transcriptional activator.

MATERIALS AND METHODS

Purified MCM1, MCM1 extracts, and in vitro-synthesized MCM1. The purified MCM1 in these studies was the 1 $\times$ P Sepharose material that was described and kindly provided by G. Ammerer (1). Yeast extracts containing either MCM1 or mcm1-98 were prepared by the method of Pfeifer et al. (27) with modifications as described by Company et al. (4). Strains used for these preparations were BJ2168 (*MATa MCM1 leu2 trp1 ura3 prb1 pep4 pcr1 gal2*), SL3-2C (*MAT α MCM1*), and SL3-2A (*MAT α mcm1::LEU2 ADH-mcm1-98::URA3*) (1, 28). Plasmids pGA1761 and pGA1797 were the templates for in vitro synthesis of MCM1 and mcm1-98 mRNA, respectively (1). pGA1797 is identical to pGA1761 except that it includes only the first 98 codons of MCM1 followed by a TGA stop codon (1a). RNA was synthesized by the method of van der Werf et al. (34) with modifications as specified by Primig et al. (28). RNA was translated into protein by using a rabbit reticulocyte lysate system (Promega L4210) in the presence of cold methionine under conditions specified by the vendor.

Probe and competitor DNAs. Ty1 fragment D was isolated from pNC121 by using the flanking *Xba*I and *Sal*I sites in the polylinker cloning region (6). Ty1 fragment H was isolated from pNC139 by using the flanking *Xba*I and *Bgl*II sites in the polylinker cloning region (6). The *STE2* upstream acti-

FIG. 1. (A) Location of transcriptional control regions in the *CYC7-H2* Ty1. The *CYC7* coding sequence is shown by the solid box. The solid lines represent *CYC7* flanking sequences. The arrow above *CYC7* indicates the transcription unit. Delta sequences are shown by stippled boxes. Triangles indicate the U5 domain of delta. (The 5' delta of the *CYC7-H2* Ty1 is abnormal in that it consists of an inverted repeat of the U5 domain.) The horizontally hatched box identifies the SRE. The diagonally hatched boxes identify block I and block II sequences (I and II). Ty1 fragment D boundaries are the *Hpa*I (Hp) and *Sau*3A (Sa) restriction sites. Ty1 fragment H is a synthetic DNA and does not have restriction site boundaries (6). (B) Nucleotide sequence of Ty1 fragment D. Numbers above the sequence are for reference to the nucleotide positions of the published *CYC7-H2* Ty1 sequence (12). The Ty1 fragment H sequence extends from positions 666 to 722. Block II (positions 673 to 700) is indicated by the bar above the sequence. The hatched lines above the sequence (positions 682 to 696 and 696 to 712) delimit regions with homology to the α 1- α 2 site from *MAT* α . The hatched line below the sequence (positions 675 to 691) delimits the region with homology to the simian virus 40 enhancer. The locations of site 1 and site 2 footprints for DNA-binding factors are indicated by brackets below the sequence (6). (C) Alignment of the Ty1 site 2 footprint sequence with the "perfect" PAL sequence that is the consensus recognition sequence for MCM1 and with the P-box that is the MCM1-binding site in the *STE2* UAS (1, 2, 9).

vation sequence (UAS) fragment was isolated from pGA1682 by using the flanking *Xba*I and *Eco*RI sites in the polylinker cloning region (9). For use as radiolabeled probes, the *Xba*I ends of these various fragments were filled in, using Klenow polymerase (Boehringer Mannheim), with dATP, dGTP, dTTP and [α - 32 P]dCTP (NEN). Plasmid pGA1667 (provided by G. Ammerer) was used as PAL competitor DNA. Complementary strands of the synthetic oligonucleotides with the PAL sequence (called MFa-1-C') were annealed and cloned into pUC18 to give pGA1667 (1). For PAL competitions, plasmid pGA1677 (provided by G. Ammerer) was used as nonspecific competitor DNA. pGA1677 is pUC18 with an insert of unrelated DNA that is the same size as the PAL oligonucleotide. Synthesis and cloning of the T28 and T10 block II oligonucleotides have been described previously (6,

13). The *Xho*I-*Bam*HI fragments from the corresponding pNC97 recombinant clones were isolated and used as competitor DNA. pNC97 is identical to the previously described pNC83 reporter gene plasmid except that it includes the intergenic region from M13 as found in pUC118 (6, 35). In block II competition experiments the corresponding fragment from the vector pNC97 was used as the nonspecific competitor DNA.

DNA-binding assays. Specific protein-DNA complexes were detected by gel mobility shift assays (15, 16). Protein-DNA-binding reactions were carried out in a 20- μ l volume containing $\sim$ 0.5 ng of probe DNA and various amounts of protein as specified for each experiment. The binding reactions were performed with 20 mM Tris (pH 8), 40 mM NaCl, 4 mM MgCl₂, 1 mM dithiothreitol, 5% glycerol, and 2 μ g of salmon sperm DNA. Binding mixtures were incubated for 10 min at room temperature. Conditions for competition assays were exactly the same as those used for binding assays, except that competitor DNAs were added to the reaction mix before protein was added. Gels for resolving protein-DNA complexes consisted of 5% polyacrylamide-bisacrylamide (19:1) in 0.5 $\times$ TBE. (1 $\times$ TBE is 89 mM Tris, 89 mM borate, and 2.4 mM EDTA). Gels were run in 0.5 $\times$ TBE at 150 V for 2 to 4.5 h without preelectrophoresis. After electrophoresis, the gels were dried and autoradiographed with an intensifying screen at -70°C .

DNase I protection analysis. The MCM1-binding reaction was carried out at room temperature in a 50- μ l volume containing 5 μ l of affinity-purified MCM1 and 5 ng of Ty1 probe D in binding buffer as described above. Protein-DNA complexes were allowed to form during a 10-min incubation at room temperature. After this incubation, 5 μ l of a DNase I solution (4 ng/ μ l in 25 mM CaCl₂-25 mM MgCl₂) was added to the reaction mixture and incubated for 45 s at room temperature. The DNase I reaction was stopped by addition of ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) to a final concentration of 20 mM. The protein-DNA complex was separated from free probe DNA by electrophoresis as described above. All subsequent manipulations for the recovery and analysis of DNase I cleavage products were carried out as previously described (4).

RESULTS

Affinity-purified MCM1 binds to the distal Ty1 regulatory region. Gel mobility shift assays showed that two complexes of differing mobilities form in binding reactions with Ty1 probe D and yeast whole-cell extracts (Fig. 2, lane 1) (6). Previous analysis of the two complexes by DNase I protection assays identified the factor-binding sites in each (6). There is a single protected site in the lower-mobility complex (Fig. 1B, site 1; Fig. 2, complex D1) (6), and there are two protected sites in the higher-mobility complex (Fig. 1B, sites 1 and 2; Fig. 2, complex D2) (6). Most generally, the complex with a single binding factor will have a higher mobility than the complex with two factors. This is not always the case because, in addition to its mass, the conformation and net charge of the complex influences its mobility in gel electrophoresis. For example, DNA-binding proteins can affect the degree and location of DNA flexure, which in turn have a strong effect on the mobility of the DNA-protein complex (22, 36).

The site 2 footprint sequence from complex D2 has a 12-of-16-bp match to the PAL sequence that is a consensus recognition site for the MCM1 transcription factor (Fig. 1C). The similarity raises the possibility that MCM1 binds to Ty1

FIG. 2. MCM1 binding to Ty1 fragment D as detected by gel mobility shift assays. Binding-reaction mixtures contained end-labeled Ty1 fragment D (0.5 ng) as probe DNA. (See Fig. 1 for the sequence and location of fragment D in Ty1.) Protein for binding reactions was either 25 μ g of yeast extract from strain BJ2168 or 2 μ l of affinity-purified MCM1, as indicated. The positions of free probe and different protein-DNA complexes are also indicated. Schematic diagrams represent the probe DNA and the inferred compositions of the different complexes. The site 1 and site 2 footprint regions of the probe are distinguished by the stippled and vertically hatched boxes, respectively. Evidence that MCM1 is the site 2-binding protein in yeast whole-cell extracts is given in Fig. 4. The factor in yeast extracts that binds at site 1 is not defined.

probe D. To test this, I used affinity-purified MCM1 protein (provided by G. Ammerer) in the gel shift assays (1). A single complex (D3) formed with Ty1 probe D and purified MCM1 (Fig. 2, lane 2). The MCM1 complex (D3) has a higher mobility than complex D2 from yeast extracts. If other parameters such as DNA flexure remain constant, this mobility is consistent with the notion that complex D2 has proteins bound at two sites whereas complex D3 is formed by MCM1 binding at only one of the sites.

DNase I protection analysis confirmed that affinity-purified MCM1 binds at the predicted sequence in Ty1 fragment D (Fig. 3). The footprint for the MCM1 complex D3 coincides with the site 2 footprint of complex D2 as reported by Company and Errede (6). Although the footprint with affinity-purified MCM1 shows two hypersensitive sites that were not apparent with the footprint from yeast extracts, the patterns of protected bases for the two determinations are very similar (Fig. 3) (6). If the same protein is responsible for the two footprints, one explanation for the minor differences is that the DNase I protection assay may have greater sensitivity with complexes involving purified protein than with whole-cell extracts.

MCM1 is the protein in whole-cell extracts that binds to Ty1 probe DNA. To test whether MCM1 is the protein from whole-cell extracts that binds to the Ty1 probe, I compared protein extracts from *S. cerevisiae* expressing either the full-length MCM1 or a truncated derivative, mcm1-98. The full-length MCM1 is a 286-amino-acid polypeptide. The mcm1-98 protein is the N-terminal 98 amino acids that includes the DNA-binding and oligomerization domains of the protein (28). If MCM1 is the protein present in the Ty1 DNA complex, its mobility will be different for the two extracts. The DNA probe in these experiments was the 57-bp Ty1 fragment H. This fragment encompasses only the protein-binding region with similarity to the MCM1 recognition sequence. Additionally, fragment H is sufficient for the

FIG. 3. DNase I protection analysis of the Ty1 probe D complex with affinity-purified MCM1. Lanes display DNase I cleavage products from free probe (Free) and complex D3 (MCM1) as shown and defined in Fig. 2. The cleavage products from purine-specific reactions with probe DNA (A+G) provide a reference ladder. The sequence of the region encompassing the DNase I-protected sites in the MCM1 complex is shown at the left. The base positions showing strong protections (•) or hypersensitive cleavage sites (←) in the complex with purified MCM1 are indicated to the right of the sequence. Base positions showing strong (•) and weak (○) protections at the site 2 footprint that were previously reported for the complex with yeast extract are shown to the left of the sequence (6).

transcriptional activation associated with the larger Ty1 fragment D (6). The gel mobility shift assay detected a single complex that forms with Ty1 probe H and either the MCM1 or mcm1-98 protein extract. Importantly, the complex with the mcm1-98 extract has a mobility different from that with the full-length MCM1 protein (Fig. 4, lanes YE). Therefore, MCM1 is the yeast extract protein that is a component of the complex that forms with Ty1 DNA probes from the distal regulatory region.

If MCM1 is the only protein present in the Ty1 complex, the mobility of the complexes that form with yeast extracts should be identical to those of complexes that form with the corresponding MCM1 or mcm1-98 protein alone. Although meeting this condition does not eliminate the possibility that proteins in addition to MCM1 are present, any differences between the two would require consideration of their presence. To obtain MCM1 or mcm1-98 free from other yeast proteins, the corresponding open reading frames were translated in vitro from RNA generated with T7 polymerase by using a rabbit reticulocyte system (1, 28). There is not significant difference in the mobility of complexes with Ty1 probe H when using yeast extract (Fig. 4, lanes YE) or the reticulocyte lysate (Fig. 4, lanes RL) for MCM1- or mcm1-98-binding activities. This result is consistent with the suggestion that MCM1 is the only protein present in the complex that forms at Ty1 site 2 under conditions of the in vitro gel shift assay. Still, MCM1 may interact with other proteins

FIG. 4. Comparison of gel mobility shift complexes with either in vitro-synthesized MCM1 or whole-cell yeast extracts. Comparisons were made with the full-length MCM1 and an N-terminal peptide encompassing amino acids 1 to 98 (mcm1-98). Each binding-reaction mixture contained end-labeled Ty1 probe H (0.5 ng). (See Fig. 1 for the sequence and location of fragment H in Ty1.) Sources of binding protein were 7.5 μ g of yeast extract (lanes YE) from strains SL3-2C (MCM1) or SL3-2A (mcm1-98) or 1 μ l of a reticulocyte lysate (lanes RL) containing the in vitro-synthesized MCM1 or mcm1-98 proteins.

in vivo, when other parameters can influence the stability of protein DNA complexes.

MCM1 binds more weakly to its site in Ty1 than to its consensus-binding site. Competition-binding experiments compared the binding of purified MCM1 to Ty1, the *STE2* UAS, or PAL oligonucleotide (Fig. 1C). The PAL sequence is a perfect palindrome site with high affinity for MCM1 (1, 2). As expected, addition of increasing amounts of unlabeled Ty1 fragment D to the binding-reaction mixtures caused increasing competition for MCM1 complex formation with the radiolabeled probe DNA. A 200-fold molar excess of the unlabeled fragment substantially decreased the signal for the MCM1 complex (Fig. 5). Unlabeled Ty1 fragment D was also a competitor for the MCM1 complex that forms with the *STE2* probe (Fig. 5). As little as a 10-fold molar excess of unlabeled PAL DNA was sufficient to compete for either the MCM1-*STE2* or the MCM1-Ty1 complex. On this basis, the PAL sequence is significantly more effective in binding MCM1 than either the *STE2* or Ty1 sequence.

MCM1 binding to Ty1 correlates with reporter gene activation. A 28-bp sequence that encompasses the MCM1-binding site functions as one component of the Ty1 activator region (6, 13). This conclusion was based on the finding that the 28-bp oligonucleotide (T28) supported 50% of reporter gene activation associated with a 983-bp region of Ty1 whereas a single-base-pair substitution derivative (T10) did not support this activity. Further, the ability of these oligonucleotides to compete for protein complex formation with yeast extracts correlated with their ability to activate gene expression (6).

If MCM1 is important for activator function of the 28-bp sequence, the ability of purified MCM1 to bind to wild-type and mutant sequences should similarly correlate with in vivo function. Therefore, I tested the relative binding efficiency of the T28 and T10 oligonucleotides for affinity-purified MCM1

FIG. 5. Competition analysis for MCM1 binding to Ty1, *STE2*, and PAL DNA. Binding reactions with radiolabeled Ty1 probe D (0.5 ng) and 2 μ l of affinity purified MCM1 were performed without competitor DNA (none) or in the presence of the specified molar excess of competitor DNA. Competitor DNAs were the Ty1 D fragment or the PAL oligonucleotide, as indicated.

by competition-binding experiments. Similar to our previous findings with yeast extracts, the wild-type oligonucleotide (T28) competed with Ty1 probe D for MCM1 binding (Fig. 6) (6). Also similar to results with yeast extracts, the nonfunctional mutant oligonucleotide (T10) was a weak competitor for MCM1 binding (Fig. 6) (6). This correlation adds further support to the idea that MCM1 is the protein that mediates the transcriptional effects of this sequence within Ty1.

FIG. 6. (A) Competition analysis for MCM1 binding to normal and mutant block II DNA. Binding reactions with radiolabeled Ty1 probe D (0.5 ng) and 2 μ l of affinity-purified MCM1 were performed in the absence (none) or presence of the specified molar excess of unlabeled competitor DNA. Competitor DNAs were wild-type block II (T28) or mutant block II (T10) sequences as specified. (B) Sequence of the functional wild-type block II element (T28) and its inactive single-base-pair substitution mutant (T10) (6).

DISCUSSION

MCM1 binds to a transcriptional regulatory site in Ty1. Multiple sequences within the Ty1 retrotransposon contribute to its transcriptional effects on adjacent genes (5). One of the regions that has been analyzed in some detail includes the block II sequence (Fig. 1, fragment H) (6, 13). This report shows that MCM1 binds to the block II sequence in Ty1. Also, MCM1 binding to mutant block II elements correlates with their transcriptional effects in vivo. This correlation supports the idea that MCM1 mediates the transcriptional activation effects associated with the block II sequence.

Usually MCM1 cooperates with other regulatory proteins in its binding to various transcriptional control elements (1, 2, 9, 21, 24, 25, 28). However, the MCM1-binding site in Ty1 is not near recognition sequences for DNA proteins known to interact with MCM1. This suggests that MCM1 may mediate transcriptional effects of the Ty1 sequence without associated DNA-binding proteins. The observation that the consensus MCM1-binding site (PAL) is a UAS shows that MCM1 can mediate transcriptional activation in the absence of additional DNA-binding proteins (18). Thus, there is a precedent for the suggested role of MCM1 in Ty1-mediated transcriptional activation.

Although MCM1 is the proposed transcriptional activator associated with the distal Ty1 regulatory region, there are differences in the transcriptional activation properties of the Ty1 site and the PAL sequence. A single copy of the PAL sequence functions as a UAS (18). In contrast, significant activation of reporter gene expression has been demonstrated only with multiple tandem copies of a 57-bp Ty1 fragment that encompasses the MCM1-binding sequence (Fig. 1A and B, fragment H) (6). Multiple copies of the Ty1 sequence may be required for UAS function because MCM1 binds less well to its site in Ty1 than to the PAL sequence.

Another difference is that the PAL sequence activates transcription in all cell types whereas the tandem copies of Ty1 fragment H activate transcription in the haploid but not diploid cell type (6, 18). The additional sequence features of the Ty1 fragment provide a reasonable explanation for the cell type specificity imposed on the MCM1-mediated transcriptional effects. Fragment H includes a sequence related to an $\alpha 1$ - $\alpha 2$ site immediately adjacent to the MCM1-binding site (Fig. 1B). The $\alpha 1$ - $\alpha 2$ site from *MAT α* can repress transcription in diploid cells when it is positioned either 5' or 3' to a UAS element (20). Therefore, the additional transcriptional control element within fragment H could mediate repression of reporter gene expression in diploid cells.

Ty1 transcriptional activating sequences are organized differently than promoters of mating genes. The pattern of transcriptional regulation for some Ty1 insertion mutations is similar to that for certain genes whose products are directly involved in mating. Therefore, it is not surprising to find that Ty1 elements use transcription factors associated with mating-specific genes. Interestingly, the combinatorial interactions between the various transcription factors that accomplish this regulation are different for Ty1 and some well-characterized mating-gene promoters. A good illustration of this difference comes from the comparison of Ty1 with the *STE2* receptor gene promoter. MCM1 and STE12 are required for the transcriptional activation mediated by the two sequences. For the *STE2* promoter, the binding sites for STE12 and MCM1 are within a single 47-bp UAS. Also, binding of STE12 at the *STE2* UAS requires direct cooperation with MCM1 (9, 28). In contrast, STE12 and MCM1

bind at different UAS sites in Ty1. STE12 binds at the SRE that is 435 bp away from the MCM1-binding site at block II (Fig. 1A). STE12 binding at the SRE is independent of MCM1 but requires cooperation with an unrelated DNA-binding protein (1b, 9). Additionally, the UAS activity associated with the MCM1-binding region is not affected by the presence or absence of STE12 (6).

The different sequence contexts for the mating-specific transcription factors in Ty1 could also account for differences in the effects of other transcriptional regulators. For example, SPT13 (*GAL11*) is a negative regulator of Ty1 transcriptional activity but a positive regulator of some mating-specific genes such as *MF α 1* (14). (The *SPT13* and *GAL11* genes were identified by different mutational screens but are the same gene [14].) Although it is not known how SPT13 (*GAL11*) acts to modulate transcriptional activation, studies by Yu and Fassler reported in the accompanying article (37) show that SPT13 exerts its negative effects through the regulatory sequence in Ty1 that binds to MCM1. It is tempting to speculate that SPT13 (*GAL11*) will also act through the MCM1-dependent sites at the promoters of mating-specific genes. Further, SPT13 (*GAL11*) may have opposite effects on Ty1 and mating-gene transcription because MCM1 is associated with coactivators such as $\alpha 1$ or STE12 at the mating-gene promoters but not at the Ty1 regulatory sequence.

A common question is why Ty1 elements are under mating-type regulation. Although there is not a clear answer, it seems that Ty1 elements have evolved to respond to this cellular regulatory system. One possibility is that the Ty1 element adapted its regulatory sequences to recognize mating-specific transcriptional regulators. An alternative is that the Ty1 element incorporated the promoter region of some mating-specific gene by a recombination event. The former possibility seems more likely because the recognition sites and combinatorial interactions for mating-specific transcription factors are different for Ty1 and for the promoters of mating genes.

The *TEC1* regulatory protein is not likely to act at the block II sequence. Not all regulators of Ty1 are mating specific. One example is the *TEC1* gene product, which is required for Ty1 activation of adjacent gene expression but is not a regulator of the mating process (23). The predicted *TEC1* protein has a TEA domain that defines a DNA-binding motif (3). Another protein that has a TEA DNA-binding domain is TEF-1, a simian virus 40 enhancer-binding protein. Because the block II sequence is similar to the simian virus 40 enhancer sequence, a logical prediction was that *TEC1* is the block II DNA-binding protein (Fig. 1B) (3). This prediction is not supported by the finding that MCM1 is the block II DNA-binding protein. As yet, the sequences within Ty1 that may be the recognition site for the presumed *TEC1* DNA-binding protein have not been identified.

ACKNOWLEDGMENTS

I am grateful to G. Ammerer for generously providing purified MCM1; plasmids pGA1667, pGA1677, pGA1761, and pGA1797; and strains SL3-2C and SL3-2A which made these studies possible. I am also grateful to K. Nasmyth for providing space in his laboratory. I thank M. Primig for his assistance with in vitro transcription and translation of MCM1. I thank J. Fassler for helpful discussions and K. Esch, T. Schuster, A. Amon, and L. Dirick for comments on the manuscript.

This work was supported by Public Health Service research grant GM30619 from the National Institutes of Health.

REFERENCES

1. Ammerer, G. 1990. Identification, purification, and cloning of a polypeptide (PRTF/GRM) that binds to mating-specific promoter elements in yeast. *Genes Dev.* 4:299–312.
- 1a. Ammerer, G. Unpublished data.
- 1b. Baur, M., and B. Errede. Unpublished data.
2. Bender, A., and G. F. Sprague, Jr. 1987. MAT α 1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. *Cell* 50:929–937.
3. Burglin, T. R. 1991. The TEA domain: a novel, highly conserved DNA-binding motif. *Cell* 66:11–12.
4. Company, M., C. Adler, and B. Errede. 1988. Identification of a Ty1 regulatory sequence responsive to *STE7* and *STE12*. *Mol. Cell. Biol.* 8:2545–2554.
5. Company, M., and B. Errede. 1987. Cell-type-dependent activation by yeast transposon Ty1 involves multiple regulatory determinants. *Mol. Cell. Biol.* 7:3205–3211.
6. Company, M., and B. Errede. 1988. A Ty1 cell-type-specific regulatory sequence is a recognition element for a constitutive binding factor. *Mol. Cell. Biol.* 8:5299–5309.
7. Dolan, J. W., C. Kirkman, and S. Fields. 1989. The yeast *STE12* protein binds to the DNA sequence mediating pheromone induction. *Proc. Natl. Acad. Sci. USA* 86:5703–5707.
8. Elder, R. T., T. P. St. John, D. T. Stinchcomb, and R. W. Davis. 1981. Studies on the transposable element of yeast. I. RNA homologous to Ty1. Cold Spring Harbor Symp. Quant. Biol. 45:581–584.
- 8a. Errede, B. Unpublished data.
9. Errede, B., and G. Ammerer. 1989. *STE12*, a protein involved in cell-type-specific transcription and signal transduction in yeast, is part of protein-DNA complexes. *Genes Dev.* 3:1349–1361.
10. Errede, B., T. S. Cardillo, F. Sherman, E. Dubois, J. Deschamps, and J. M. Wiame. 1980. Mating signals control expression of mutations resulting from insertion of a transposable repetitive element adjacent to diverse yeast genes. *Cell* 22:427–436.
11. Errede, B., T. S. Cardillo, G. Wever, and F. Sherman. 1981. Studies on transposable elements in yeast. I. ROAM mutations causing increased expression of yeast genes: their activation by signals directed toward conjugation functions and their formation by insertion of Ty1 repetitive elements. Cold Spring Harbor Symp. Quant. Biol. 45:593–602.
12. Errede, B., M. Company, J. D. Ferchak, C. A. Hutchison III, and W. S. Yarnell. 1985. Activation regions in a yeast transposon have homology to mating type control sequences and to mammalian enhancers. *Proc. Natl. Acad. Sci. USA* 82:5423–5427.
13. Errede, B., M. Company, and C. A. Hutchison III. 1987. Ty1 sequence with enhancer and mating-type-dependent regulatory activities. *Mol. Cell. Biol.* 7:258–265.
14. Fassler, J. S., and F. Winston. 1989. The *Saccharomyces cerevisiae* *SPT13/GAL11* gene has both positive and negative regulatory roles in transcription. *Mol. Cell. Biol.* 9:5602–5609.
15. Fried, M., and D. M. Crothers. 1981. Equilibria and kinetics of *lac* repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res.* 9:6505–6525.
16. Garner, M. M., and A. Revzin. 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions. Application to components of the *Escherichia coli* lactose operon regulatory system. *Nucleic Acids Res.* 9:3047–3060.
17. Herskowitz, I. 1989. A regulatory hierarchy for cell specialization in yeast. *Nature (London)* 342:749–757.
18. Jarvis, E. E., D. C. Hagen, and G. F. Sprague, Jr. 1988. Identification of a DNA segment that is necessary and sufficient for α -specific gene control in *Saccharomyces cerevisiae*: implications for regulation of α -specific and a-specific genes. *Mol. Cell. Biol.* 8:309–320.
19. Jensen, R., G. F. Sprague, Jr., and I. Herskowitz. 1983. Regulation of yeast mating-type interconversion: feedback control of *HO* gene expression by the mating-type locus. *Proc. Natl. Acad. Sci. USA* 80:3035–3039.
20. Johnson, A. D., and I. Herskowitz. 1985. A repressor (*MAT α 2* product) and its operator control expression of a set of cell type specific genes in yeast. *Cell* 42:237–247.
21. Keleher, C. A., S. Passmore, and A. D. Johnson. 1989. Yeast repressor α 2 binds to its operator cooperatively with yeast protein Mcm1. *Mol. Cell. Biol.* 9:5228–5230.
22. Kerppola, T. K., and T. Curran. 1991. Fos-Jun heterodimers and Jun homodimers bend DNA in opposite orientations: implications for transcription factor cooperativity. *Cell* 66:317–326.
23. Laloux, I., E. Dubois, M. Dewerchin, and E. Jacobs. 1990. *TEC1*, a gene involved in the activation of Ty1 and Ty1-mediated gene expression in *Saccharomyces cerevisiae*: cloning and molecular analysis. *Mol. Cell. Biol.* 10:3541–3550.
24. Lydall, D., G. Ammerer, and K. Nasmyth. 1991. A new role for MCM1 in yeast: cell cycle regulation of *SWT5* transcription. *Genes Dev.* 5:2405–2419.
25. Mueller, C. G. F., and A. Nordheim. 1991. A protein domain conserved between yeast MCM1 and human SRF directs ternary complex formation. *EMBO J.* 10:4219–4229.
26. Passmore, S., R. Elble, and B.-K. Tye. 1989. A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. *Genes Dev.* 3:921–935.
27. Pfeifer, K., B. Arcangioli, and L. Guarente. 1987. Yeast *HAP1* activator competes with the factor RC2 for binding to the upstream activation site UAS1 of the *CYC1* gene. *Cell* 49:9–18.
28. Primig, M., H. Winkler, and G. Ammerer. 1991. The DNA binding and oligomerization domain of MCM1 is sufficient for its interaction with other regulatory proteins. *EMBO J.* 10:4209–4218.
29. Rothstein, R. J., and F. Sherman. 1980. Dependence on mating type for the overproduction of iso-2-cytochrome *c* in the yeast mutant *CYC7-H2*. *Genetics* 94:891–898.
30. Song, O.-K., J. W. Dolan, Y. O. Yuan, and S. Fields. 1991. Pheromone-dependent phosphorylation of the yeast *STE12* protein correlates with transcriptional activation. *Genes Dev.* 5:741–750.
31. Sprague, G. F., Jr. 1990. Combinatorial associations of regulatory proteins and the control of cell type in yeast. *Adv. Genet.* 27:33–62.
32. Sprague, G. F., Jr. 1991. Signal transduction in yeast mating. *Trends Genet.* 7:393–398.
33. Taguchi, A. K. W., M. Ciriacy, and E. T. Young. 1984. Carbon source dependence of transposable element-associated gene activation in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 4:61–68.
34. van der Werf, S., J. Bradley, E. Wimmer, F. W. Studier, and J. J. Dunn. 1986. Synthesis of infectious poliovirus RNA by purified T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA* 83:2330–2334.
35. Vieira, J., and J. Messing. 1987. Production of single-stranded plasmid DNA. *Methods Enzymol.* 153:3–11.
36. Wu, H.-M., and D. M. Crothers. 1984. The locus of sequence-directed and protein-induced DNA bending. *Nature (London)* 308:509–513.
37. Yu, G., and J. S. Fassler. 1993. *SPT13 (GAL11)* of *Saccharomyces cerevisiae* negatively regulates activity of the MCM1 transcription factor in Ty1 elements. *Mol. Cell. Biol.* 13:63–71.