A new role for MCM1 in yeast: cell cycle regulation of *SWI5* transcription

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In the yeast *Saccharomyces cerevisiae* cell cycle-regulated *SWI5* transcription is essential for ensuring that mother and not daughter cells switch mating type. We have identified a 55-bp promoter sequence that appears to be responsible for restricting transcription to the late S, G₂, and M phases of the cell cycle. Two proteins, MCM1, a transcription factor described previously, and SFF (SWI five factor, a newly identified factor) bind this sequence in vitro. MCM1 binds the DNA tightly on its own, but SFF will only bind as part of a ternary complex with MCM1. We observe a strong correlation between the ability of mutated *SWI5* promoter sequences to form a ternary MCM1-SFF-containing complex in vitro and to activate transcription in vivo, which suggests that efficient transcription requires that both proteins bind DNA. Through its interactions with cell type-specific coactivators and corepressors, MCM1 controls cell type-specific expression of pheromone and receptor genes. By analogy, we propose that SFF enables MCM1 to function as a part of a cell cycle-regulated transcription complex.

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As cells of the budding yeast *Saccharomyces cerevisiae* proceed through the cell cycle, they transiently activate the transcription of several genes. For example, in late G₁ at START when cells become committed to enter S phase, many of the genes involved in DNA replication become transcriptionally activated (for review, see Andrews and Herskowitz 1990). However, other genes respond to different types of cell cycle control. *SWI5*, which encodes a transcription factor for the *HO* endonuclease gene, and *CLB1* and *CLB2*, which encode B-type cyclins, are only transcribed at later stages of the cell cycle during S, G₂, and M phases (Nasmyth et al. 1987; Ghiara et al. 1991; Surana et al. 1991).

SWI5 is required for mating-type switching in *S. cerevisiae*. Mating-type switching is induced when the *HO* endonuclease introduces a double-strand break to the *MAT* locus of a haploid yeast cell. The process is regulated tightly, owing to control over *HO* gene transcription, and *SWI5* is just one of several genetically identified regulators of *HO* transcription (for review, see Herskowitz 1989). *S. cerevisiae* cells divide asymmetrically to produce a mother cell, which transcribes *HO* and switches, and a smaller daughter cell, which does not transcribe *HO* and, therefore, does not switch. Mother cells only transcribe *HO* in a brief post-START window of their cell cycle, that is, after they are committed to cell division but before they have replicated their DNA (Nasmyth 1983); thus, *HO* is one of the large class of yeast genes that is only transcribed in late G₁ as cells become committed to enter S phase. One of the reasons why mother but not daughter cells express *HO* is that

SWI5 gene expression is cell cycle regulated. *SWI5* RNA begins to accumulate at some point, appreciably later than START, probably in middle or late S phase, and declines as cells complete mitosis and enter G₁. If *SWI5* is expressed ectopically during G₁, then daughter cells are able to switch mating type (Nasmyth et al. 1987).

This paper concerns the mechanism by which the cell cycle influences *SWI5* transcription. We have identified a 55-bp DNA sequence that is both necessary for transcription and sufficient to impose cell cycle-regulated transcription on a reporter gene. We can detect two proteins binding to this sequence in vitro: One, MCM1, is a well-characterized yeast transcription factor, involved in the transcriptional control of yeast cell mating type (Sprague 1990); the other protein SFF (SWI five factor) is less well characterized and only binds to the *SWI5* upstream activation sequence (UAS) with the aid of MCM1. We suggest that *SWI5* transcription requires that both proteins bind DNA as a ternary complex.

Results

The SWI5 promoter and not SWI5 RNA is cell cycle regulated

To determine whether the cell cycle periodicity in *SWI5* RNA levels is the result of regulated transcription or regulated RNA degradation, we tested whether RNA abundance during the cell cycle could be affected by manipulating the promoter. The natural UAS of *SWI5* was replaced by the *RP39* gene UAS (the *RP39* UAS is able to

confer constitutive activation on a heterologous gene and is derived from a ribosomal protein-encoding gene). The *RP39-SWI5* promoter fusion produces twofold more RNA than the wild-type promoter in cycling cells; but unlike the wild type, it is still active during G_1 arrest (see Materials and methods). This observation implies that the *SWI5* promoter is usually not active in G_1 and that *SWI5* RNA levels are transcriptionally regulated. The *RP39-SWI5* promoter fusion also causes switching in daughter cells (0.26 switches per division) and germinating spores (0.44 switches per division). This result confirms the importance of cell cycle regulation of the *SWI5* promoter in restricting mating-type switching to mother cells (Nasmyth et al. 1987); spores and daughter cells usually never switch mating type (Strathern and Herskowitz 1979).

A small promoter sequence is essential for SWI5 transcription

To identify regulatory sequences within the *SWI5* pro-

moter, a series of deletions created in front of a *swi5::lacZ* reporter gene were transplanted to the *SWI5* locus and tested for their β -galactosidase activities (Fig. 1). The most distal, essential element of the promoter was identified by a series of deletions that started 1014 bp upstream of the AUG codon and removed progressively more DNA 3' of this position (Fig. 1). Deletion 31 ($\Delta 31$), which removes all of the sequences between 1014 and 349 bp upstream of the AUG, still possesses >60% of the activity of the full-length promoter; and $\Delta 32$ (1014–318 bp), which removes slightly more DNA, has >40% activity. However, deletions that go slightly further 3' such as $\Delta 1$ (1014–304 bp) and $\Delta 33$ (1014–289 bp), reduce promoter activity to <3% of the wild-type levels. These findings suggest that there is an essential activation region somewhere 3' of –318. A complementary set of deletions was designed to identify the site of essential sequences most proximal to the TATA box. $\Delta 42$ (270–295 bp) has >60% activity, yet $\Delta 40$ (270–320 bp) and other larger deletions show that sequences 5' of –295 are important for full promoter activity. Taken together,

Figure 1. Deletion analysis of the *SWI5* promoter: A series of deletions were generated in front of a *swi5::lacZ* gene fusion and transplanted to the *SWI5* locus of strain D1449. The left-most column indicates the number of each deletion, the next column indicates their β -galactosidase activities, and the size and location of each deletion is indicated by the absence of a solid line. The numbers on the top line and above the deletion end points refer to the positions upstream of the AUG codon. We have shown previously that the majority of the RNA start sites cluster 160 bp upstream of the AUG and that there is a putative TATA box (indicated on the top line), 70 bp farther upstream (Nasmyth et al. 1987).

these results imply that there is a single essential activation region in the promoter between 295 and 318 bp upstream of the AUG codon. A series of smaller deletions centered on this region, such as $\Delta 21$ (304–320 bp) and others that remove the overlapping sequences, shows that a sequence between 295 bp and 318 bp upstream of the AUG is essential for transcription. Other small deletions elsewhere in the promoter such as $\Delta 41$ (270–283 bp), $\Delta 42$ (270–295 bp), and $\Delta 25$ (370–418 bp) have minimal effects on transcription.

Two simple mechanisms could explain the lack of *SWI5* transcription during G_1 . The promoter might contain the binding site for either a periodic activator or a periodic repressor of transcription. To identify the binding site for a putative repressor, all deletion-carrying strains were tested for their β -galactosidase activities when arrested in G_1 by α -factor. The removal of a repressor-binding site should result in high levels of transcription in both cycling and in G_1 -arrested cells. However, none of the deletions showed this phenotype (data not shown; for details, see Materials and methods). It seems unlikely therefore that the *SWI5* promoter is regulated by a G_1 -specific repressor binding to sequences that are separated from the essential activating sequences of the promoter.

55 bp is sufficient for cell cycle-regulated transcription

To test whether sequences encompassing the essential region of the *SWI5* promoter are capable of activating transcription without the aid of other promoter sequences, these sequences were placed in front of the *CYC1* TATA box linked to a reporter gene (*ubiYlacZ*; see the legend to Fig. 3, below). Transcription of the reporter gene is dependent on the insertion of a UAS (Guarente et al. 1982). We find that a single copy of a synthetic oligonucleotide corresponding to promoter sequences 272–326 bp upstream of the AUG has strong UAS activity that is orientation independent ($\sim 30\%$ as much activity as the *RP39* UAS or 40% the *CYC1* UAS; data not shown).

If the *SWI5* UAS is responsible for the cell cycle regulation of the complete promoter, it should be able to cause cell cycle-regulated transcription in isolation of other *SWI5* sequences. To test whether this was the case, the reporter construct described above containing just the 55-bp *SWI5* UAS was integrated at the *URA3* locus of a strain that also carried the wild-type *SWI5* gene. This strain was synchronized in G_1 by α -factor treatment and released from the G_1 arrest by washing away the pheromone, and the *SWI5* and *ubiYlacZ* RNA levels were measured. The two RNA species accumulated and declined with very similar kinetics during two synchronous cell cycles following α -factor release (Fig. 2A,B). There is a slight difference in the time at which the two RNAs reach maximal levels during the first cycle but none during the second. During both cycles the *SWI5* RNA and *ubiYlacZ* levels peak at ~ 20 min before anaphase (Fig. 2B,C). We conclude that just 55 bp of the

SWI5 promoter is sufficient to cause cell cycle-regulated transcription.

Identification of the sequences important for activating transcription

To identify which sequences within the 55-bp *SWI5* UAS are required for its activity, we have tested a series of mutated versions in which clusters of 6 bp are replaced by *EcoRI* sites. We find that mutations in a 20-bp interval destroy UAS activity (mutants 15, 16, 17, and 18; Fig. 3B), whereas mutations outside this region have little or no effect. The 20-bp sequence important for UAS activity overlaps with the region of the *SWI5* promoter removed by $\Delta 21$, the smallest crippling promoter deletion (Fig. 3A).

Proteins bind to the *SWI5* UAS in vitro

Gel-retardation assays were used to identify potential regulators of *SWI5* transcription (Fried and Crothers 1981; Garner and Revzin 1981). Four DNA–protein complexes, labeled T, M, B, and N, are formed when a radioactively labeled *SWI5* UAS is mixed with a crude yeast extract. The T and M complexes are only formed at high protein concentrations (data not shown). Furthermore the T, M, and B complexes do not form on a mutant oligonucleotide that is devoid of activity in vivo, and they are specifically competed by the addition of nonradioactive wild-type UAS DNA but not by transcriptionally inactive mutated DNA (Fig. 4). Therefore, there is a correlation between T-, M-, and B-complex formation in vitro and transcription in vivo, which suggests that these complexes might contain proteins required for activation in vivo.

Dimethylsulfate (DMS) methylation interference experiments have been used to determine which bases in the *SWI5* UAS are important for T- and B-complex formation in vitro (Siebenlist and Gilbert 1980). If methylation of a guanine in the *SWI5* UAS were to interfere with the formation of either the B or the T complex, these complexes should contain only unmethylated DNA at these critical positions. Figure 5 shows that the B complex is not formed if methylation occurs at any of four different guanine residues (G residues 308, 309, 316, and 317 bp upstream of the AUG, indicated by solid circles). These four residues form two pairs, on opposite strands of DNA, separated by 6 bp. The methylation interference of the T complex is essentially the same as that of the B complex except that one additional guanine (G296, solid square) is important for its formation.

The most likely explanation for the findings that the B and T complexes produce similar methylation interference patterns and that the T complex forms only at high protein concentrations is that the T complex is built upon the B complex. According to this model the B complex is a binary complex formed when one protein binds to the DNA (the square) and that the T complex is a ternary complex formed by the simultaneous binding of another protein (oval, Fig. 5B).

Figure 2. The *SWI5* UAS imposes cell cycle-regulated transcription on a reporter gene. **(A)** *SWI5*, *ubiYlacZ*, and *MATa1* RNA levels in a synchronous culture. RNA levels were measured in a synchronously growing culture by the S1 nuclease protection technique. Each RNA is indicated, by arrows, at right (*MATa1* contains an intron and therefore produces two bands). The numbers at top indicate the time in minutes after α -factor release that the culture was harvested. (FP) Free probe; the signal represents one hundredth the total amount of probe that was mixed with each RNA sample. (CYC) Cycling control culture. An exponentially growing culture of D1840 was arrested in G_1 by α -factor treatment (2.5 hr at 24°C, 1 μ g/ml α -factor) and released from the arrest by filtering the culture, rinsing in medium, and resuspending the culture in BAR1 “conditioned” medium (Nasmyth et al. 1990). Thereafter, aliquots of the culture were harvested every 10 min: One milliliter was fixed, by the addition of formaldehyde to 3.7% (one-tenth volume), and later processed for in situ immunofluorescence analysis. The remainder of each aliquot was harvested, washed in ice-cold RNA buffer, and stored at -80°C ; subsequently, RNA was isolated (Nasmyth 1983). **(B)** Quantitative analysis. The *SWI5* and *ubiYlacZ* bands were cut out of the gel (A), and the amount of radioactivity in each band was determined by scintillation counting. **(C)** Culture synchrony. The proportion of cells in the culture that had budded or that had anaphase spindles (scored by in situ immunofluorescence with antibodies against tubulin; Nasmyth et al. 1990) is shown. To ensure that there was no bias when counting the number of budded cells or the number with mitotic spindles, the samples from each time point were coded and mixed by a third person before they were examined.

MCM1 is a component of the T, M, and B complexes

The methylation interference pattern of the B complex is strikingly similar to that observed when the MCM1 protein binds to the *STE2* UAS [*STE2* encodes the α -factor

receptor and MCM1 is a transcription factor (see Sprague 1990; for other MCM1 binding sites, see Passmore et al. 1989; Dolan and Fields 1991). Four lines of evidence indicate that MCM1 is a component of the T, M, and B complexes.

Figure 3. Mutational analysis of the *SWI5* UAS. (A) The site of Δ21. The *SWI5* promoter sequences between 272 and 326 bp upstream of the AUG are illustrated in the top line. The site of Δ21, which reduces *SWI5* promoter activity severely, is shown below, as are the end points of two other deletions that have minimal effects on promoter activity. Δ53 removes sequences between 318 and 348 bp upstream of the *SWI5* AUG. (B) The effects of mutations on *SWI5* UAS function. Oligonucleotides corresponding to *SWI5* promoter sequences flanked by restriction enzyme sites (indicated by lowercase letters) were synthesized and cloned in front of the *ubiYlacZ* reporter gene (into pDL1 498). The left-most column gives each UAS a number or name; the next column shows their β-galactosidase activities when cloned in front of the reporter gene and transformed into yeast strain Y1534. UAS9 comprises DNA corresponding to wild-type *SWI5* promoter sequences flanked by an *XhoI* site at the 5' end and a *BglII* site at the 3' end. All other sequences are the same as the wild-type UAS9, except where mutations are indicated by uppercase letters. UAS16 also carried a single-base-pair deletion, the site of which is indicated by the star. UAS numbers 3 (mutant) and 4 (wild type) are flanked by two *XhoI* sites and were cloned into pDL1460. The PPAL sequence is based on the MFα-1C' UAS described in Ammerer (1990); its activity is independent of α1. β-Galactosidase activities were determined after cells had been growing exponentially

or had been treated with α-factor (500 ng/ml for 4 hr in Ura⁻ medium, only illustrated for UASs 9 and PPAL). The β-galactosidase activities of strains carrying plasmids without UASs (i.e., pDL1460 or pDL1498) were subtracted from the values obtained for other plasmids to control for background levels of activity (the wild-type UAS had an activity of >2000 units; the vector, <3 units). Activities were determined as in Breeden and Nasmyth (1987).

First, purified MCM1 produces a complex on the *SWI5* UAS that is of similar mobility to the B complex (Fig. 6A, lanes 12,13). Second, MCM1 antiserum contains antibodies that recognize a protein that binds *SWI5* UAS DNA (Fig. 6A, lanes 3,4). When MCM1 antiserum is added to a gel-retardation assay a small proportion of the radioactive probe does not enter the gel, presumably because DNA-protein complexes are cross-linked by the antibodies to form large complexes that cannot leave the well. The antiserum also results in the generation of a new, slower-migrating complex (labeled AB), which is presumably due to antibodies binding MCM1 on DNA, and a slight reduction in the levels of the T, M, and B complexes. These effects are specific because they are not caused by preimmune serum (Fig. 6A, lane 5). Third, plasmids that contain strong MCM1-binding sites are efficient competitors of the T, M, and B complexes (Fig. 6A, lanes 6–11). Fourth, by using a yeast extract prepared from a strain containing a truncated yet functional version of the *MCM1* gene, called *MCM1*-98 (containing

just the first 98 amino acids of MCM1), we are able to affect the size of the T, M, and B complexes. Three novel complexes are formed by an extract prepared from such a strain, and we have named them M98T, M98M, and M98B (Fig. 6B). Because both T and M98T complex formation is inhibited by the same mutation in the *SWI5* UAS (A296; Fig. 8A, below) it seems likely that these complexes are analogous, differing only in the size of the MCM1 protein that they contain. MCM1 is also probably a component of the M complex, because both the M and M98M complexes are reduced by the C309 mutation (Fig. 8A), and a component of the B complex, because both the B and M98B complexes are reduced by the A309 mutation (Fig. 8A).

The T complex includes MCM1 and another protein, SFF

To identify factors other than MCM1 that are part of the T and M complexes we have fractionated a yeast extract

Figure 4. T-, M-, and B-complex formation in vitro correlates with activity in vivo. Radioactively labeled oligonucleotides mUAS3 [devoid of UAS activity; Fig. 3] and wUAS4 [an active UAS; Fig. 3] were used for gel-retardation assays with 20 μ g of Y1268 extract. Letters at left (T, M, B, N, F) refer to the complexes formed on the DNA. (Top) FP is a lane on the gel that contained no protein extract; mUAS3 shows the complexes that formed on this mutant UAS DNA; the remaining lanes all show the complexes formed on the active, wild-type wUAS4. The labeling indicates the molar excess of nonradioactive, competitor oligonucleotide that was added to each reaction before the protein extract.

by applying it to a heparin-agarose column and eluting proteins with an ammonium sulfate gradient. The eluted fractions are depleted in MCM1 because MCM1 binds tightly to the heparin-agarose column, only eluting when the column is washed at high concentrations of NaCl (fractions 130–150; Ammerer 1990). Fractions were assayed by the gel-retardation assay with or without the addition of purified MCM1 (Fig. 7). No protein eluting at low concentrations of ammonium sulfate can bind tightly to the *SWI5* UAS. However, there is an activity peaking in fractions 38–42 that can interact cooperatively with MCM1 to form the T and M complexes.

Three yeast proteins, $\alpha 1$ (Bender and Sprague 1987), $\alpha 2$ (Keleher et al. 1988), and STE12 (Errede and Ammerer 1989; M. Primig and G. Ammerer, in prep.) are already known to bind cooperatively with MCM1 to DNA. The activity peaking in fractions 38–42 cannot be any of these because it is present in *MATa* cells (which contain neither $\alpha 1$ nor $\alpha 2$) and in cells carrying a deletion of the *STE12* gene (data not shown). Therefore, we have named it SFF. SFF is a factor that binds with MCM1 to the *SWI5* UAS to produce the T complex. We do not know the nature of the M complex; it might be a degradation product of the T complex or be a complex between MCM1 and another DNA-binding protein.

SFF binding is essential for transcription but not for recruiting MCM1

To determine whether SFF might be an activator or a repressor of *SWI5* transcription, its binding site in the *SWI5* UAS was mutated. Methylation interference experiments had identified a guanine in the *SWI5* UAS (G296) that is important for T- but not for B-complex production and presumably, therefore, particularly important for SFF binding. A transversion of this base pair greatly reduced *SWI5* UAS activity in vivo (600-fold, A296 mutation, Fig. 8A) and its ability to form the T complex in vitro (Fig. 8C); therefore, we have concluded that the *SWI5* UAS is inactive unless it can recruit SFF.

Figure 8, B and C, also shows that the mutant A296 can still bind MCM1 as well as or even better than the wild-type UAS, suggesting that SFF binding is not required to recruit MCM1 onto DNA. To further exclude this possibility, the MCM1-binding site in the *SWI5* UAS was replaced by a “perfect” binding site [(PPAL) perfect palindrome]; this artificial site possesses a high affinity for MCM1 and is independent of the coactivator $\alpha 1$ (Bender and Sprague 1987; Jarvis et al. 1988; Ammerer 1990). The PPAL *SWI5* UAS behaves indistinguishably from the wild type because it activates similar levels of transcription in cycling cells, and it is inactive during α -factor-induced G_1 arrest (Fig. 3B). Moreover, PPAL UAS activity is still highly dependent on SFF binding because the A296 mutation reduces activity severely (25-fold, Fig. 3B). These data suggest that the role of SFF in *SWI5* activation is not primarily one of recruiting MCM1 onto DNA.

MCM1 is important for recruiting SFF and for activating transcription

To test the role of MCM1 in *SWI5* transcription, several mutations were introduced into its binding site. Figure 8A shows that some, but not all, mutations have severe effects on transcription. Whereas the single- and double-base-pair transversions C309 (0.3%) and C308 and C309 (0.1%) reduce UAS activity drastically, other mutations such as A309 (66%) have little effect. Similarly, the multiple mutations present in UASs 3 (0.1%), 15 (0.3%), and 16 (0.3%) reduce transcription, whereas those of UAS 14 (41%) do not (Fig. 3). The different effects of the various mutations would be explained most simply if the inactive mutant UASs could not bind MCM1 and the active ones could. To our surprise, however, we find no correlation between the ability of a mutated UAS to bind purified MCM1 in vitro and to activate transcription in vivo (Fig. 8B). For example, the mutant UASs A309 and mUAS14 (Figs. 8A and 3B, respectively) are transcriptionally active in vivo yet do not bind MCM1 in vitro, whereas the mutant C309 is transcriptionally silent in vivo and clearly binds MCM1 in vitro (although less well than the wild-type UAS; Fig. 8B). These findings suggested that MCM1 binding was not important for activating *SWI5* transcription.

When we incubated the same mutant UASs with whole-cell extracts, however, we observed a good corre-

Figure 5. Identification of bases that are important for T- and B-complex formation. (A) Methylation interference of the T and B complexes. The methylation status of the DNA present in T and B complexes, as well as in the free probe, is shown. The top strand is shown in Fig. 3; the bottom strand is complementary. (GA) DNA that was cleaved at guanines and adenines; (F) the methylation pattern of the free probe; (B) the methylation pattern of the B complex; (T) the methylation pattern of the T complex. At left and right is the DNA sequence of the *SWI5* promoter. (●) Sites that interfere with B- and T-complex formation when methylated; (■) a G that, if methylated, only interferes with T-complex formation. (B) Hypothetical nature of the T complex. The bases that interfere with T-complex formation, when methylated, are indicated by ● and ■. The large open rectangle represents a protein that binds to the DNA to give the B complex; the large open oval represents a protein that binds with the square to give the top complex. The methylation interference pattern of MCM1 binding to the *STE2* UAS is also shown (from M. Primig and G. Ammerer, in prep.).

lation between T-complex formation in vitro and biological activity in vivo (Fig. 8A,C). For example the active mutant A309 can neither bind purified MCM1 nor MCM1 in a crude yeast extract to form the B complex, but it can clearly bind MCM1 in a ternary complex with SFF to form the T complex. Thus, A309 demonstrates cooperative binding between MCM1 and SFF in forming the T complex. In contrast, MCM1 can bind to the inactive mutant C309 to form the B complex [albeit at low efficiency, compared with the wild type], but it cannot cooperate with SFF to form the T complex. Thus, although initially confusing with respect to MCM1 binding, the UAS activities of all mutant UASs are consistent with a model in which transcription of *SWI5* requires the production of a ternary complex comprising the *SWI5* UAS, MCM1, and SFF.

Point mutations in the SWI5 promoter reduce gene expression and affect RNA start site choice

To determine whether the MCM1- and SFF-binding sites

in the complete *SWI5* promoter are important for transcription we have transplanted point mutations to the *SWI5* locus and measured their effect on *SWI5* RNA production and on *swi5::lacZ* expression. Both the MCM1 (C309)- and SFF (A296)-binding sites are important because point mutations cause >10-fold reductions in *swi5::lacZ* expression (β -galactosidase activity; Fig. 9A). Surprisingly, the mutations do not reduce transcription per se but, instead, affect the choice of RNA start site used, resulting in a shift from the wild-type RNA start sites to ones farther upstream (Fig. 9B). The upstream RNAs probably do not produce functional protein because the *SWI5* promoter contains other AUG codons, 183 and 229 bp upstream of the one that is used to make *SWI5*, and both are followed quickly by in-frame stop codons. It is plausible that there is another, cryptic UAS in the *SWI5* promoter that can only interact with the transcription machinery when the real *SWI5* UAS is inactivated (deletion analysis suggests that this might be the case; data not shown). Proteins binding this cryptic UAS perhaps interact with the transcription machinery

Figure 6. MCM1 binds to the *SWI5* UAS. (A) MCM1 binds to the *SWI5* UAS. Gel-retardation assays used 20 μ g of Y1268 protein extract and UAS9 probe. The letters at left (T, M, B, N, F) refer to the complexes formed on the DNA. (AB) A complex that was only seen when MCM1 antiserum was added to a gel-retardation assay. (Lane 1) The migration of a *SWI5* UAS probe in the absence of yeast cell protein extract; (lane 2) the complexes formed when protein extract was added; (lane 3) the pattern seen when rat MCM1 antiserum (1 : 400 final concentration; Ammerer 1990) was added to the gel-retardation assay 6 min after the addition of yeast extract; (lane 4) same as lane 3 except a 1 : 2000 dilution of serum was used; (lane 5) when a 1 : 400 dilution of preimmune serum was added to a gel-retardation assay; (lanes 6–8) the effects of adding 1 μ g of competitor plasmid DNA, containing 0, 1, or 3 perfect MCM1-binding sites (MF α 1C'-binding site; Ammerer 1990, cloned in pIC19R) [pBS⁺ was the competitor without an MCM1-binding site (lane 6)]; (lanes 9–11) analogous to lanes 6–8 except they contained only 0.2 μ g of competitor DNA; (lane 12) the pattern seen when 0.2 μ l of purified MCM1 was used; (lane 13) the pattern seen with 0.05 μ l of purified MCM1. (B) Complex formation on mutated *SWI5* UASs. A wild-type and three mutant UASs were used for gel-retardation assays with protein extracts from MCM1 (Y1268) or MCM1-98 (SL3-10B) yeast strains. The probes used in each assay are indicated at top. (WT) The wild-type probe, the other lane designations are described in Fig. 8A. The complexes are labeled analogously to Fig. 4. The M98 prefix indicates that the complex is MCM1-98 dependent. The M98M complex has resolved into two complexes on this gel, labeled M98M and M98M*.

differently from those binding the real UAS to generate a different set of transcriptional start sites.

The role of MCM1 in SWI5 transcription in vivo

It is difficult to test whether the MCM1 protein is important for activating *SWI5* transcription in vivo because *MCM1* is an essential gene and there are no conditionally lethal alleles available. However, there is an allele, *mcm1-1*, that is specifically defective in activating α -specific transcription and in plasmid maintenance but is not defective in α -specific transcription nor in essential functions (Passmore et al. 1988). Although the *mcm1-1* mutation has little overall effect on the level of *SWI5* RNAs with the correct 5' start site, it does cause a noticeable increase in the number of nonfunctional upstream transcripts (Fig. 9B). This phenotype is similar to the effect of a mutation in the MCM1-binding site in the *SWI5* promoter, and we take the appearance of

these upstream transcripts as evidence that the *mcm1-1*-encoded protein is partially defective in binding to the *SWI5* UAS.

A further indication that MCM1 binds to the *SWI5* UAS in vivo has come from the analysis of a strain whose growth is dependent on *MCM1-98* expressed from the *GAL1-10* promoter (strains expressing *MCM1-98* produce about half the amount of *SWI5* RNA as wild-type strains). Although there is only a slight reduction in *SWI5* transcription using the correct RNA start sites during a 26-hr period following repression of *MCM1-98* transcription (by glucose addition), there is a significant increase in the number of nonproductive upstream RNAs (Fig. 9C). We suggest that a reduced intracellular MCM1 concentration results in a lowered occupancy of the *SWI5* UAS, which causes an increase in the number of aberrant upstream transcripts produced. Perhaps normal transcripts are not reduced drastically because the essential function of MCM1 is more sensitive to MCM1 con-

MCM1 is a member of an evolutionarily conserved class of DNA-binding proteins, sharing a DNA-binding motif with the human serum response factor (SRF) protein [Norman et al. 1988], the plant homeotic gene products *agamous* [Yanofsky et al. 1990] and *deficiens* [Sommer et al. 1990], and the yeast regulator of arginine metabolism ARG80 (or ARG1; Dubois et al. 1987). MCM1 is involved in diverse forms of transcriptional regulation. In *MAT α* cells, MCM1 either binds with the coactivator α 1

Figure 8. Point mutations in the *SWI5* UAS compromise its activity severely. (A) The effect of point mutations on the *SWI5* UAS. (Left) The UAS sequences were identical to wUAS9 (Fig. 3), except where indicated; their activities were determined after they had been cloned into pDL1498. (Right) Their ability to bind proteins to form the T, M, and B complexes is indicated and was determined from C. (B) The affinity of mutant UASs for MCM1. Purified MCM1 (1 μ l) was used for gel-retardation assays with radioactively labeled mutant UASs (defined in Fig. 3 and A, above). A small polyacrylamide gel was used in this experiment. (C) The affinity of mutant UASs for proteins in crude yeast extracts. Radioactively labeled mutant UASs, as in B, were incubated with a crude yeast extract (20 μ g of Y1268) to determine the types of complexes formed on the DNAs.

to activate α -specific promoters such as *STE3* (encoding the α -factor receptor; Bender and Sprague 1987) or it combines with the corepressor $\alpha 2$ to repress α -specific promoters such as *STE6* (*STE6* is important for α -factor secretion; Kelleher et al. 1988). In *MATa* cells, MCM1 binds with STE12, a transcription factor involved in the induction of genes by mating pheromones, to activate the *STE2* UAS (*STE2* encodes the α -factor receptor; Errede and Ammerer 1989; M. Primig and G. Ammerer, unpubl.). Our analysis of the *SWI5* promoter suggests

that by binding with SFF, MCM1 brings about cell cycle-regulated transcription. The different types of regulation associated with MCM1 depend on the DNA sequences surrounding its binding sites. These sequences, in turn, determine which DNA-binding partner ($\alpha 1$, $\alpha 2$, STE12, or SFF) is recruited into a ternary complex on DNA. Because in each case it is the partner of MCM1 that specifies the type of transcriptional regulation, a question remains as to the role played by MCM1 in transcriptionally active complexes. Does MCM1 function primarily

MCM1 activates *SWI5* transcription

Figure 9. *SWI5* expression in the presence of promoter mutations and *mcm1* mutations. (A) Point mutations transplanted to the *SWI5* locus. Four different point mutations, as illustrated and named at left, were transplanted to the *SWI5* locus. Transcription from the mutant promoters was assessed by either measuring the β -galactosidase activities of mutated promoters fused to the *swi5::lacZ* fusion gene (Y1143) or by S1 mapping the RNA produced in strains carrying the point mutations in front of the wild-type *SWI5* gene (see B). (Wild-type RNA) Species that initiated at the usual start sites; (total RNA) species that initiate farther upstream and become particularly apparent in the presence of a mutation. (B) *SWI5* RNA transcription in strains carrying promoter point mutations or *MCM1* mutations. RNA levels were measured in exponentially growing yeast strains by S1 mapping. The left-most lane shows molecular mass markers (*MspI*-cut pUC18). The next four lanes show the effects of the *mcm1-1* mutation on *SWI5* transcription. (*MAT α MCM1*) Strain Y699; (*MAT α mcm1-1*) strain Y1856; (*MAT α MCM1*) strain Y700; (*MAT α mcm1-1*) strain Y1857. The lanes at right show the effect of point mutations in the *SWI5* promoter on transcription. The mutations and the wild-type gene were transplanted into D1449. Numbers at right show the distance upstream of the *SWI5* AUG, where transcription is beginning and the site of the two upstream AUG codons which, if translated, would lead to quickly terminating peptide sequences. The *MCM1*-binding site is around -315 (see Fig. 5). The S1 probe contained sequences up to but not beyond -242 . The bands at -245 are the result of full-length protection and are therefore the result of transcripts originating somewhere upstream of -242 . (C) Depletion of *MCM1*-98 from yeast cells affects *SWI5* transcription. *SWI5* RNA levels were determined, by S1 mapping, in a yeast strain in which *gal:MCM1-98* gene transcription had been stopped by the addition of glucose to a culture of SL2-2B. RNA was prepared from cells harvested at various times after the addition of glucose to a culture (indicated in hours).

to recruit other transcriptional activators onto DNA, or is MCM1 a transcriptional activator whose properties are regulated tightly by its DNA-binding partners?

It has been proposed that MCM1 is the primary activator of both the α - and the α -specific genes (Bender and Sprague 1987; Herskowitz 1989; for review, see Sprague 1990; Tan and Richmond 1990). This hypothesis is based on the finding that a synthetic high-affinity MCM1-binding site, which does not contain binding sites for either coactivators or corepressors, activates transcription in all cell types (PPAL sequence; Jarvis et al. 1988). This observation is interpreted to indicate that a PPAL sequence activates transcription universally because MCM1 binds this sequence tightly and in a transcriptionally active conformation. However, our experiments question this simple model because the conversion of the MCM1-binding site in the *SWI5* UAS to a PPAL sequence does not relieve the UAS of its dependence on an adjacent SFF-binding site (Fig. 3). This demonstrates that a high-affinity PPAL sequence is not always sufficient to activate high levels of transcription. Perhaps, at least in the case of the *SWI5* UAS, it is more appropriate to think of MCM1 as a protein whose major role is one of recruiting other transcriptional activators onto DNA rather than as the sole direct activator of transcription. Consistent with this interpretation is the observation that both the acidic and polyglutamine regions of MCM1 can be deleted from the protein with little effect on *SWI5* transcription (Fig. 9C).

During the course of our experiments we have identified mutant *SWI5* UASs, containing altered MCM1-binding sites, that allow MCM1 to bind to form the B complex but that fail to bind SFF to form the T complex. These mutants are transcriptionally silent in vivo (e.g., C309; Fig. 8) and have a lower affinity for MCM1 than the wild-type UAS does. However, their poor affinity for MCM1 cannot explain their failure to activate transcription because other mutants with even lower affinities for MCM1 are both able to recruit SFF onto DNA and to activate transcription in vivo (e.g., A309). The anomaly could be explained if these inactive mutant UASs bind MCM1 in a conformation that inhibits cooperative binding with SFF and would be consistent with the proposal that MCM1 can bind DNA in two conformations (Tan and Richmond 1990).

Similarities between the SWI5 UAS and the human c-fos serum response element

Human SRF (Norman et al. 1988) is the best characterized of the higher eukaryotic MCM1 homologs. It binds to the *c-fos* promoter, at the serum response element (SRE), along with a coactivator $p62^{TCF}$, and is important for the transient stimulation of *c-fos* transcription caused when serum is added to tissue cultures. There are several parallels between the human SRE and the *SWI5* UAS, between human SRF and MCM1, and between human $p62^{TCF}$ and SFF. First, both sequence elements cause transient activation (and perhaps, therefore, repression; for details of the SRE, see Rivera et al. 1990).

Second, the SRE binds SRF efficiently, but $p62^{TCF}$ only binds in a ternary complex with SRF (Shaw et al. 1989; Schröter et al. 1990). Thus, SRF behaves like its yeast homolog MCM1, and $p62^{TCF}$ behaves like SFF. Third, some mutations in the SRE at or close to the SRF-binding site cause high levels of transcription in vivo despite binding SRF poorly in vitro (e.g., a mutant SRE called LXma; Norman and Treisman 1988). Such mutant SREs behave analogously to some of the mutant *SWI5* UASs, which cannot bind MCM1 except as part of a ternary complex with SFF (e.g., A309, Fig. 8). On the basis of these properties SFF seems to be the best candidate yet for a functional yeast homolog of the human $p62^{TCF}$ protein. It is tempting to speculate that similarities will exist between them, perhaps with respect to the mechanisms by which they are activated by signals generated by the cell as it enters the cell cycle ($p62^{TCF}$) or traverses the cycle (SFF).

Materials and methods

DNA constructs

pDL1460 was used to test for UAS activity. It is identical to the "UAS trap" pLGΔ178 (Guarente et al. 1982), except that it contains the *ubiYlacZ* reporter gene instead of *lacZ* and therefore produces a β -galactosidase protein with a half-life of 10 min instead of >20 hr. It was made by swapping the *lacZ* gene of pLGΔ178 (Guarente et al. 1982) with the *ubiYlacZ* gene from pUB23Y (Bachmair et al. 1986) by the use of unique *XhoI* and *XbaI* sites in each plasmid. pDL1498 was created by inserting a linker so that the sequence CTGAGATatcAGACTC was inserted into the *XhoI* site, with the new *XhoI* site (CTGAG) being closest to the *URA3* gene of the plasmid and the *BglII* (AGATCT) site being closest to the reporter gene. pDL1517 contained the wild-type *SWI5* UAS 9 (Fig. 3) inserted into pDL1498.

The *RP39-SWI5* promoter fusion was created by blunt-ended ligation of a 128-bp *XhoI*-*BglII* fragment of the *RP39* UAS (from pBA147 obtained from Brenda Andrews, as described by Rotenberg and Woolford 1986) into the *EcoRI* site of *SWI5* promoter deletion 21 (see below). Oligonucleotide site-directed mutagenesis of the *SWI5* promoter was performed by the use of a kit purchased from Amersham International. Point mutations were introduced into a *SalI* fragment of the *SWI5* promoter (and gene) cloned into *SalI*-digested m13mp9, that is, into the same construct used for making S1 probes (Nasmyth et al. 1987). The mutagenesis was checked by sequencing, and mutated promoters were then cloned upstream of *SWI5* or *swi5::lacZ* as a *SalI*-generated DNA fragment. These constructs were then transplanted into the *SWI5* locus of strains D1449 (for *SWI5*) or Y1143 (for *swi5::lacZ*).

S1 probes

S1 probes were radioactive, single-stranded DNA probes prepared from M13 template DNA (Nasmyth 1983). The *SWI5* probes have been described previously: The short probe (used in Fig. 9B) is described in Figure 1 of Nasmyth et al. (1987); the longer probe used on all other occasions is from Figure 2 of the same paper. The short probe extends from -85 to -242 (from the AUG). The *MATa1* probe is described in Nasmyth (1985). The *ubiYlacZ* probe template was prepared by cloning an *XhoI* (upstream of the RNA start site) and *PvuII* (which cuts just inside the *lacZ*-coding sequence) fragment of pUB23 into *SmaI*- and *SalI*-digested m13mp9. This probe detects RNA spe-

cies that are a fusion between the *CYC1* leader sequences, the yeast *UBI4* gene, and the *Escherichia coli lacZ* gene.

Yeast growth, genetics, and strains

Standard media and methods were used. Yeast strains are as follows:

- Y699:** (W303 1A) *MATa*, *ade2-1*, *trp1-1*, *can1-100*, *leu2-3,112*, *his3-11,15*, *ura3*, *GAL*, *psi*⁺
Y700: (W303 1B) *MATa* genotype, otherwise identical to Y699
H990: *HO*, *swi5::SUP4-o*, *ura3*, *leu2*
Y1143: *MATa*, *HMLa*, *swi5::SUP4-o*, *ade2-1*, *ho*, *trp1-1*, *can1-100*, *leu2-3,112*
Y1268: (BJ2168) *MATa* *ura3*, *trp1*, *leu2*, *ade2-1*, *pep4-3*, *prb*, *prc*
D1449: *MATa*, *ade2-1*, *swi5::SUP4-o*, *bar1::LEU2*, *can1-100*, *ho*, *ura3*
Y1534: *MATa*, *bar1::hisg*, *ade2-1*, *trp1-1*, *can1-100*, *leu2-3,112*, *his3-11,15*, *ura3*, *GAL*, *psi*⁺ (from Y699)
D1840: *MATa*, *ura3::swi5uasubiYlacZ::URA3*, *bar1::hisg*, *ade2-1*, *trp1-1*, *can1-100*, *leu2-3,112*, *his3-11,15*, *GAL*, *psi*⁺ (Y1534 transformed with pDL1517)
Y1856: *MATa*, *mcm1-1*, *leu2-3,112*, *his4del34* (from G. Sprague, sy1433, rm9-2c)
Y1857: *MATa* *mcm1-1*, *leu2-3,112*, *his4del34* (from G. Sprague, sy1434, rm9-2d)
SL2-2B: *MATa*, *mcm1::LEU2* *ura3::gal1-10:MCM1-98:URA3* (Y699 background; G. Ammerer, in prep.).
SL3-10B: *MATa*, *mcm1::LEU2*, *pADH:MCM1-98:URA3* (Y699 background; G. Ammerer, in prep.)

Deletion analysis of the *SWI5* promoter

The complete *SWI5* gene was cloned in two orientations in the pUC19 vector. Unidirectional deletions were created, in each direction, and the deletion end points were determined by double-stranded DNA sequencing (Henikoff 1987). Deletions in opposite orientations were joined by the use of the *EcoRI* site, which was situated with the vector at the junction of each deletion, and the combined deletions were placed in front of a *swi5::lacZ* fusion gene, by use of a *PstI* site (2049) lying between the promoter and the *lacZ* insertion at 2978 (at *BclI*). The deletion-containing plasmids were digested with *HindIII* and transplanted to the *SWI5* locus of strain D1449 as described in Nasmyth (1985). Four putative transplacements (canavanine-resistant colonies) were tested qualitatively for β -galactosidase activity after growth on plates for 8 hr either with or without α -factor (1 μ g/ml). Two clones were checked further by Southern blotting, using the *EcoRI* site generated at the site of the deletion, to confirm that the transplacements were at the correct locus. One clone was analyzed quantitatively for β -galactosidase activity after resuspending a 4-cm² 2-day-old patch of cells in 5 ml of PBS. This cellular suspension (1 ml) was centrifuged briefly and resuspended in 25 ml of YEPD containing α -factor (300 ng/ml). In parallel, 100 μ l of cellular suspension was inoculated directly into 25 ml of YEPD. The two cultures were shaken at 30°C for 8 hr, and the cells pelleted and frozen at -70°C before their activities were determined.

Most of the strains behaved as though they contained the wild-type promoter and produced about fourfold less β -galactosidase activity when arrested in G₁ in comparison with when they were cycling. Some of the deletion-containing yeast strains produced more β -galactosidase activity when arrested in G₁ in comparison with when they were cycling. However, such strains always produced comparatively little activity when

growing exponentially; therefore, their phenotypes cannot be attributable to the removal of a G₁-specific repressor-binding site (i.e., separable from an activator-binding site). The α -factor resistant promoters probably represent weakly constitutive promoters.

Insertion of the RP39 UAS into the *SWI5* promoter

The RP39 UAS was cloned into the site of promoter deletion 21 (Fig. 1), and the promoter fusion was then transplanted to the *SWI5* locus of yeast strain D1449. As a control, the wild-type promoter was transplanted into the same strain. *SWI5* RNA levels were measured by S1 mapping, followed by quantification by scintillation counting. The wild-type promoter produces 100 arbitrary units of RNA in a cycling culture and 3.4 units when the culture is arrested with α -factor (1 μ g/ml, 2.5 hr), whereas the RP39 UAS produces 207 units of RNA in a cycling culture and 128 units in α -factor. To measure effects on mating-type switching the promoter fusion was also transplanted into a strain carrying the wild-type *HO* gene (H990), and switching was determined as described by Hicks and Herskowitz (1976). With the wild-type promoter, 87/132 (66%) mother cells, 0/132 daughter cells, and 0 germinating spores switched mating type. With the RP39 promoter fusion, 30/46 (65%) mother cells, 12/46 (26%) daughter cells, and 11/25 (44%) germinating spores switched mating type.

Gel-retardation assays

Protein extracts were prepared essentially as described by Company et al. (1988) except that protease inhibitors and β -mercaptoethanol were not present in the extraction buffers. MCM1 was purified as described previously (Fig. 6; Ammerer 1990). Radioactive probes generally were prepared by using the Klenow enzyme to fill in restriction enzyme site sticky ends in the presence of radioactive dATP. The labeled probe was purified from the remainder of the plasmid and/or from unincorporated nucleotides by polyacrylamide gel electrophoresis (as in Treisman 1986). Gel-retardation assays were performed in microtiter plates at room temperature. Generally 10,000 cpm (Cerenkov) of probe was used for each assay. Each incubation contained 4 μ l of 5 $\times$ BS buffer [5 $\times$ is 100 mM Tris-HCl (pH 7.5); 250 mM NaCl; 15 mM MgCl₂; 5 mM DTT; 25 mM spermidine; 250 μ g/ml of BSA; 100 mM EDTA], 2 μ l of 50% glycerol, 2.5 μ l of 1 mg/ml poly[d(I-C)], radioactive probe, water, and protein extract to give a final volume of 20 μ l. Protein extracts were always added last, and the mixtures were then incubated for 15 min before being loaded directly onto a polyacrylamide gel (4% gel, 20:1 acrylamide/bis-acrylamide in 0.5 $\times$ TBE; 30-min prerun at 200 V for 2 hr). After electrophoresis, the gels were fixed (15 min in 10% MeOH, 10% acetic acid), dried, and exposed to X-ray film.

Methylation interference

Methylation interference experiments were essentially scaled-up gel-retardation experiments except that methylated pDL1517 was the source of the probe. A 15- μ g aliquot of CsCl-purified pDL1517 was digested with *XhoI* in the presence of alkaline phosphatase, phenol-extracted, ethanol-precipitated, and end-labeled at the dephosphorylated *XhoI* site by the use of T4 polynucleotide kinase and radioactive ATP (the other strand was labeled by cutting with *BglII*). A 200- μ l aliquot of cacodylate-containing buffer (unless otherwise stated, all procedures and buffers are as described by Maxam and Gilbert 1980) was added to the radioactive DNA (10 μ l), followed by 5 μ l of DMS. Methylation was allowed to proceed for 3 min at room temper-

ature before the addition of stop buffer, and the methylated DNA was ethanol-precipitated. The probe was liberated from the rest of the plasmid by digesting with a second restriction enzyme that cut at the other side of the UAS (e.g., *Bgl*II if the DNA had been end labeled at the *Xho*I site). The methylated, end-labeled probe was then gel-purified and used in a gel-retardation assay. A total of between 500,000 and 1,000,000 Cerenkov cpm was used for each experiment. The DNA-protein complexes were separated by electrophoresis and visualized in the wet gel by autoradiography for 2 hr at room temperature. The gel fragments containing the free probe and T and B complexes were excised, and DNA present within them was eluted and further treated according to the protocol of Treisman (Treisman 1986). After the piperidine treatment, an appropriate amount from each tube (typically 1000 Cerenkov cpm) was lyophilized once more and loaded onto a 12% sequencing gel (20 : 1, acrylamide/bis-acrylamide). After electrophoresis the gel was fixed (10% MeOH, 10% acetic acid) and dried, and the bands were visualized after autoradiography (−80°C, with intensifying screen, without preflashing the film). The G and A ladder, used to orientate the sequencing gel, was prepared by treating an aliquot of the end-labeled and methylated DNA with formic acid (Ausubel et al. 1987).

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A new role for MCM1 in yeast: cell cycle regulation of SW15 transcription.

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