Cell Cycle-Regulated Transcription of the \textit{CLB2} Gene Is Dependent on Mcm1 and a Ternary Complex Factor

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Clb2 is the major B-type mitotic cyclin required for entry into mitosis in the budding yeast \textit{Saccharomyces cerevisiae}. We showed that accumulation of \textit{CLB2} transcripts in G2 cells is controlled at the transcriptional level and identified a 55-bp upstream activating sequence (UAS) containing an Mcm1 binding site as being necessary and sufficient for cell cycle regulation. Sequences within the cell cycle-regulated UASs were shown to bind Mcm1 in vitro, and mutations which abolished Mcm1-dependent DNA binding activity eliminated cell cycle-regulated transcription in vivo. A second protein with no autonomous DNA binding activity was also recruited into Mcm1-UAS complexes, generating a ternary complex. A point mutation in the \textit{CLB2} UAS which blocked ternary complex formation, but still allowed Mcm1 to bind, resulted in loss of cell cycle regulation in vivo, suggesting that the ternary complex factor is also important in control of \textit{CLB2} transcription. We discuss the possibility that the \textit{CLB2} gene is coregulated with other genes known to be regulated with the same periodicity and suggest that Mcm1 and the ternary complex factor may coordinate regulate several other G2-regulated transcripts.

In the budding yeast \textit{Saccharomyces cerevisiae}, the \textit{CDC28} gene encodes a 34-kDa protein (p34$_{\text{CDC28}}$) which serves as the catalytic subunit for a cell cycle-regulated protein kinase. This kinase regulates the G$_2$-M transition, and passage through a control point in G$_1$ known as Start, where cells prepare for DNA replication and become irreversibly committed to a further round of cell division. Changes in p34$_{\text{CDC28}}$-dependent kinase activity during the cell cycle are controlled by posttranslational modifications and by its assembly into a protein complex with regulatory subunits known as cyclins. These proteins are so named after their cyclic accumulation and degradation during the cell cycle.

In the G$_1$ phase, Cdc28 complexes with one of three functionally redundant G$_1$ cyclins (Cln1, Cln2, or Cln3), which together are required for execution of Start and for the G$_1$-to-S transition. Regulation of the G$_2$-M transition by p34$_{\text{CDC28}}$ requires its assembly into a complex with a separate group of cyclins known as mitotic B-type cyclins. Four mitotic B-type cyclins have been identified in \textit{S. cerevisiae}: \textit{CLB1}, \textit{CLB2}, \textit{CLB3} and \textit{CLB4} (10, 11, 23, 28). Clb1 and Clb2 are closely related to one another and to Cdc13, a cyclin B protein essential for mitosis in \textit{Schizosaccharomyces pombe}, whereas Clb3 and Clb4 more closely resemble the \textit{S. pombe} B-type cyclin homolog Cig1 (23). None of the mitotic cyclins in \textit{S. cerevisiae} by themselves are essential for viability as they are, at least to some extent, functionally redundant (10, 11, 23, 28). Clb2, however, appears to be the most important B-type cyclin for initiation and completion of mitosis (10, 11, 28) and appears to be important in processes such as spindle elongation (17, 28) and negative regulation of bud emergence (5, 17).

In contrast to \textit{CLB3} and \textit{CLB4} transcripts, which increase early in the S phase, \textit{CLB1} and \textit{CLB2} transcripts begin to accumulate late in S phase and remain elevated until late in mitosis (10, 11, 23, 28). Increased levels of \textit{CLB2} transcripts at this time correlate with Clb2-associated kinase activity, which peaks just before and disappears immediately following anaphase (27). These observations suggest that \textit{CLB2} mRNA levels play an important role in control of Clb2 activity. It is not known, however, if levels of mitotic cyclins are controlled at the transcriptional or posttranscriptional level. Several other genes, such as \textit{CLB1}, \textit{CDC5}, \textit{ACE2}, and \textit{SWI5}, are expressed at the same time in the cell cycle as \textit{CLB2} (8, 16, 28), raising the possibility that these genes are coregulated. Our understanding of G$_2$-specific transcription, however, is based exclusively on studies of the \textit{SWI5} gene. Regulation of \textit{SWI5} transcription is dependent on a cell cycle-regulated upstream activation sequence (UAS) which binds the Mcm1 transcription factor and a second protein, the \textit{SWI5} factor (SFF), which binds only as part of a ternary complex with Mcm1 (18). This is similar to the recruitment of ternary complex factors by the mammalian counterpart of Mcm1, the serum response factor SRF (7, 29). Through its interactions with cell type-specific coactivators and corepressors, Mcm1 also regulates genes not under cell cycle control, such as cell type-specific pheromone and receptor genes. It is therefore likely that Mcm1 has no intrinsic cell cycle-regulated activity and that the regulatory component of the \textit{SWI5} transcription complex is provided by SFF.

This report concerns the mechanism which controls \textit{CLB2} expression during the cell cycle. We show that \textit{CLB2} transcription plays a major part in the control of \textit{CLB2} mRNA levels in the cell cycle. Furthermore, we identified a UAS from the \textit{CLB2} promoter that is necessary and sufficient for cell cycle control and requires the Mcm1 transcription factor together with an associated ternary complex factor for activity. We discuss the possibility that this transcription factor complex functions in the coregulation of other genes expressed late in the cell cycle.
MATERIALS AND METHODS

Yeast strains and media. Cells were routinely grown at 30°C in YEPD medium (1% yeast extract, 2% Bacto Peptone, 0.1 mg of adenine per ml, 2% glucose). All other yeast manipulations were done as described previously (7). The yeast strains used in this study are listed in Table 1.

Deletion analysis of the CLB2 promoter. A CLB2 promoter fragment beginning at the ATG initiator methionine and extending to position −2,992 was generated by PCR and subcloned into pGLa-178 (13) as a SalI-BamHI fragment (sites introduced by PCR); this generated a fusion between the CLB2 5′-flanking region and the lacZ gene, which reads 5′-ATGATAGACAACGGAGTCGGAGCTTG-3′ at the junction (initiator ATG in boldface, BamHI site underlined). The PCR-generated CLB2 portion of this construct was replaced with an internal Xhol-Xhol CLB2 restriction fragment from pCLB2/5.5 (pBSK+ with a 5.5-kb BamHI genomic insert containing the CLB2 gene) to eliminate possible PCR errors (P330); regions at each end not replaced by this step were sequenced to confirm the absence of mutations. The CLB2 promoter-lacZ fusion gene was inserted into pRS316 (25) by transferring the BamHI fragment from pCLB2/5.5-generated from the major start site), no introns were found in this region and the ATG defining the initiator methionine is the first in the CLB2 promoter. Long promoter leader sequences (>200 nucleotides) have been previously reported for other cell cycle-regulated transcripts (20). The first 889 bases of this sequence are shown.

5′-flanking sequences are required for cyclic accumulation of CLB2 transcripts. CLB2 mRNA levels increase late in the S phase, reach maximum levels late in G2, and decline rapidly as cells complete mitosis (10, 23, 28). To determine if 5′-flanking sequences are required for periodic accumulation of CLB2 transcripts, we constructed a CLB2 promoter-lacZ fusion gene and integrated it at the URA3 locus. Cells were synchronized with α-factor, RNA levels were analyzed by Northern blot analysis, and progression through the cell cycle was monitored by assessing mitotic spindle formation. This analysis showed that lacZ transcripts from the CLB2-lacZ gene were regulated with kinetics almost indistinguishable from those of the endogenous CLB2 gene (Fig. 2), indicating that cyclic regulation requires a promoter-5′ untranslated region. Transcription start sites used in the CLB2-lacZ fusion were shown to be the same as in the natural CLB2 gene (data not shown). This analysis does not distinguish between transcriptional control and the possibility that the 5′ untranslated region plays a role in influencing transcript stability.

A 55-bp UAS is sufficient for cell cycle-regulated transcription. To identify regulatory sequences within the CLB2 promoter, we constructed a series of deletions and assayed pro-
moter function by measuring β-gal activities from CLB2 promoter-lacZ fusion genes. This analysis defined a region from positions −362 to −131 as being important for CLB2 promoter activity (Fig. 3). Deletion of these sequences resulted in reduction of β-gal activity to 11% of the wild-type level (Fig. 3) and loss of cell cycle regulation (data not shown). Sequences essential for cell cycle regulation therefore lie within the −2362 to −2131 region. Smaller deletions within this region also resulted in substantial decreases in promoter activity but to a lesser extent than the −2362 to −131 deletion (Fig. 3), suggesting that multiple elements within this region contribute to the overall control of CLB2 transcription.

To test if sequences defined in the previous experiment are sufficient and necessary for cell cycle regulation of CLB2 transcription, we inserted a 232-bp fragment corresponding to the −2362 to −2131 region of the CLB2 promoter upstream of a ubiYlacZ gene (Materials and Methods) and chromosomally integrated at the URA3 locus of S131 (MATα bar1 ura3). (A) CLB2, lacZ, H2A, and Prt1 transcript levels in a synchronous culture. Numbers across the top are the times in minutes of release after the α-factor block. (B) Quantitative analysis of CLB2 and lacZ transcripts. Levels of lacZ (○) and CLB2 (●) transcripts were determined after Phosphorimager analysis and standardization against the non-cell cycle-regulated control transcript, Prt1. (C) Culture synchrony. Cell synchrony after release from the α-factor block was judged by staining cells with anti-tubulin antibodies and scoring spindle formation at the time points indicated (see Materials and Methods).
sequences necessary and sufficient for cell cycle regulation localized to a 164-bp region between −362 and −199. These results are summarized in Fig. 4A. Although this excluded Mcm1 sites at positions −175 and −194 as being necessary for regulation, it was still possible that the −322 Mcm1 site was involved. It should be emphasized that of these sites, at least the −194 and −322 sites are capable of binding Mcm1 in vitro (30).

To test if the −322 Mcm1 site (CCGAATCAGG) and surrounding sequences are sufficient for cell cycle regulation, a 55-bp synthetic oligonucleotide (positions −336 to −282; Fig. 5A) was inserted upstream of a ubiYlacZ gene, which was then integrated at the URA3 locus. Figure 5 shows that these sequences are sufficient to drive cell cycle-regulated transcription in a pattern indistinguishable from that of endogenous CLB2 transcripts. This element therefore functions as a cell cycle-regulated UAS in vivo.

We specifically mutated sequences within the −322 Mcm1 site to determine its role in the CLB2 UAS. Both the T mutation at −317 (−317T; a G-to-T change) and −318C (a G-to-C change) in this UAS, which would be expected to significantly impair the ability of Mcm1 to bind, completely abolished cell cycle control of ubiYlacZ transcripts. Although other Mcm1 sites (positions −175 and −194) were identified in the −362 to −199 UAS (Fig. 4A), they were dispensable for correct regulation (Fig. 5). However, in the context of the entire promoter, these Mcm1 binding sites appear to be functionally redundant (our unpublished observations; see Discussion).

Mcm1 also plays an important role in control of SWI5 transcription (18). Promoter sequences necessary and sufficient for cell cycle control of SWI5 consist of an Mcm1 binding site and juxtaposing sequences that are required for the recruitment of a second transcription factor, SFF (18). As SWI5 is transcribed at the same time in the cell cycle as CLB2, and as Mcm1 is implicated in the control of both genes, this raised the possibility that both genes are under control of the same regulators. To determine if these genes share other cis-regulatory elements besides an Mcm1 binding site, we compared the SWI5 and CLB2 UAS elements to determine if any other similarities exist. Alignment of the two UAS elements revealed significant sequence similarities to the 3′ side of the Mcm1 site which coincide with sequences known to be important in the SWI5 promoter (Fig. 5A). A C residue at position −296 of the SWI5 promoter has previously been shown to be important in the regulation of this gene by making base-specific contacts with the SFF transcription factor (18). As this residue and surrounding sequences are conserved in the CLB2 UAS, we decided to test the effect of introducing multiple- and single-point mutations at these positions (Fig. 5A). Northern blot analysis revealed that the −306A mutation and the −307G/−306T/−305G triple UAS mutation severely reduced transcription of the ubiYlacZ reporter gene and appeared to result in loss of cell cycle control (Fig. 5B and C). At least two separate cis-
regulatory sequences in the CLB2 UAS are therefore required for cell cycle regulation, one which resembles an Mcm1 binding site and adjacent sequences which are conserved between the SWI5 and CLB2 UAS elements. An Mcm1-Vp16 fusion protein activates the CLB2 promoter in vivo. To test the idea that Mcm1 binds the CLB2 UAS in vivo, we expressed an Mcm1-Vp16 fusion protein from a galactose-inducible promoter and determined if this could activate the CLB2 UAS-ubiYlacZ gene when cells were blocked at stages in the cell cycle when the CLB2 UAS is usually silent. This experiment took advantage of the unstable lacZ derivative (ubiYlacZ; half-life, 10 min [18]) and the strong, constitutive transcriptional activation domain of the herpes simplex virus Vp16 protein, which when fused to a heterologous DNA binding domain, activates transcription if recruited to a binding site (7). Cells were arrested by the cdc34 temperature-sensitive mutant (late G1) or by the cell cycle inhibitor α-factor (G1) or nocodazole (metaphase), and one-half of the culture was induced with galactose to promote expression of the Mcm1-Vp16 fusion. In cycling cells and nocodazole-blocked cells, where CLB2 transcription is on, β-gal activity was considerably higher than in α-factor- or cdc34-blocked cells, where CLB2 transcription is normally off (Fig. 6). Expression of Mcm1-Vp16 significantly increased β-gal activity in all cultures but was most pronounced in α-factor- and cdc34-blocked cells,

FIG. 6. An Mcm1-Vp16 fusion protein activates a CLB2-ubiYlacZ reporter gene in vivo. A fusion gene consisting of the first 98 amino acids of Mcm1 was fused to the acidic activation domain of Vp16 (amino acids 412 to 490) under control of the inducible GAL1-10 promoter and integrated at the TRP1 locus in S00 (CLB2 UAS-WT-ubiYlacZ::ura3), S01 (CLB2 UAS-318C-ubiYlacZ::ura3), S02 (CLB2 UAS-WT-ubiYlacZ::ura3), S03 (RP39 UAS-ubiYlacZ::ura3), or S04 (CLB2 UAS-306A-ubiYlacZ::ura3). Cells were originally grown in YEPD medium plus 2% raffinose (Raff) and, when appropriate, arrested under restrictive conditions (at 37°C or in the presence of a cell cycle inhibitor [0.2 μg of α-factor per ml or 150 μg of nocodazole per ml]) and split in two, at which time expression of the Mcm1-Vp16 fusion was induced in one of the cultures by addition of galactose (Gal) to 2%. Extracts were prepared 120 min after the time of galactose addition. Liquid culture β-gal assays were performed as described in Materials and Methods.

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sites (Fig. 5A) were endlabeled with $^{32}$P and incubated with various amounts of h, and k), $^{313}$C (c, i, and l), and $^{306}$A (d, g, j, and m) CLB2 UAS binding sites (Fig. 5A) were end labeled with $^{32}$P and incubated with various amounts of where activity of the uninduced (cells grown in raffinose medium) culture was very low. A point mutation in the CLB2 UAS shown to severely reduce Mcm1 binding (see Fig. 7) also showed severely reduced ubiYlacZ activity compared with the wild-type control, showing that trans-activation by Mcm1-Vp16 is dependent on its ability to bind the UAS. In contrast, activity of the $^{306}$A mutant UAS was similar to that of the wild type. To show that this is specific for the CLB2 UAS, the same experiment was performed with a strain carrying a ubiYlacZ gene driven by the non-cell cycle-regulated RP39 UAS. In this strain, Mcm1-Vp16 failed to increase reporter gene activity, confirming that trans activation requires an intact Mcm1 binding site.

Mcm1 recruits a ternary complex factor to the CLB2 UAS in vitro. To determine if we could detect binding of Mcm1 to the cell cycle-regulated CLB2 UAS from crude whole-cell extracts, we performed band shift experiments using the wild-type CLB2 UAS as a probe. In this assay, we detected several specific DNA-protein complexes (Fig. 7A), designated here T, M, and B complexes (lanes b to m), which did not appear to vary with the cell cycle (data not shown). These protein-DNA complexes are all sensitive to mutations in the predicted Mcm1 binding site which would be expected to abolish or significantly reduce Mcm1 binding. For example, the $^{318}$C and $^{317}$T mutations which change essential DNA contact points for Mcm1 and abolish cell cycle-regulated UAS activity in vivo severely reduced or abolished formation of T, M, and B complexes in vitro (Fig. 7A, lanes c, f, i, and l, and our unpublished results). UAS mutations outside of the predicted Mcm1 binding site ($^{306}$A and $^{307}$G/$^{306}$I) which abolished cell cycle control in vivo (Fig. 5) also blocked the formation of ternary complexes in vitro (Fig. 7A, lanes d, g, j, and m, and 7B, lanes 4 and 5). Hence, a strong correlation exists between the ability of the CLB2 UAS to bind factors from crude cell extracts in vitro and its ability to function as a cell cycle-regulated UAS in vivo.

Fig. 7. Formation of ternary complexes on the CLB2 UAS in vitro correlates with activity and cell cycle regulation in vivo. (A) Wild-type (WT; lanes a, b, c, h, and k), $^{313}$C (c, i, and l), and $^{306}$A (d, g, j, and m) CLB2 UAS binding sites (Fig. 5A) were end labeled with $^{32}$P and incubated with various amounts of crude extract from strain S130 (MATa). Lanes: a, no extract; b, c, and d, 5 μg of extract; e, f, and g, 10 μg of extract; h, i, and j, 15 μg of extract; k, l, and m, 5 μg of extract from MATa S1 cells. Positions of free probe (F), nonspecific complexes (NS), and T, M, and B complexes are indicated. (B) T, M, and B complexes are dependent on Mcm1. Wild-type (lanes 1, 2, 6, 7, 11, and 12), $^{318}$C (lanes 3, 8, and 13), $^{307}$G/$^{306}$I/−305G (lanes 4, 9, and 14), and $^{306}$A (lanes 5, 10, and 15) CLB2 UAS binding sites (Fig. 5A) were end labeled with $^{32}$P and incubated with or without crude extract, 10 μg of S130 extract (lanes 2 to 5), 5 μg of S226 extract (lanes 7 to 10), 5 μl of insect cell rBV extract (lanes 12 to 15), or 5 μl of mock-infected insect cell extract (lane 11). No extract was added to the reactions in lanes 1 and 6. Positions of Mcm1-TCF (lanes 7 to 10), specific complexes (T, M, and B, lanes 2 to 5), Mcm1,−TCF complexes (lane 7), and free probe (F) are shown. Note that the mobilities of the UAS-Mcm1-alone B complexes (lanes 2, 4, and 5) and UAS-rMcm1 complexes (lanes 12 to 15) are slightly different. The right-hand panel (lanes 16 to 23) shows that antibodies raised against an Mcm1 synthetic peptide recognize CLB2 UAS-associated complexes from crude yeast cell extracts. A wild-type CLB2 UAS probe was incubated with 5 μg of crude yeast cell extract (lane 16), a 1:20 (lane 17) dilution of preimmune serum, or a 1:2,000 (lane 18) or 1:20 (lane 19) dilution of an anti-Mcm1 peptide antibody. Nonspecific or specific Mcm1 competitor peptides were added to band shift reactions as follows: 1 (lane 20) or 100 (lane 21) ng of a nonspecific peptide and 1 (lane 22) or 100 (lane 23) ng of a specific competitor peptide. The spread of complexes resulting from these experiments is indicated by the vertical bar. (C) A second protein, along with Mcm1, is recruited into a ternary complex at the cell cycle-regulated CLB2 UAS. A wild-type CLB2 UAS probe was incubated with 10 μg of S130 total crude extract (lane a), 5 μl of rBV Mcm1 extract, and/or 2.5-μl fractions of crude extract after chromatography over a Q-Sepharose column, the flowthrough at 50 mM KCl fraction, the 0.3 M KCl fraction, or the 0.1 M KCl fraction, or the 0.1 M KCl fraction, or the 0.1 M KCl fraction, or the 0.1 M KCl fraction, or the MCm1 binding site.
To show that Mcm1 is a component of the complexes forming on the CLB2 UAS, an extract from a strain expressing only a truncated form of Mcm1 (Mcm1\textsubscript{305G\_306A\_307G}) was incubated with a probe. Under these conditions, the T, M, and B complexes disappeared on a wild-type UAS and were replaced by a faster-migrating complex, corresponding to truncated Mcm1, and a more slowly migrating ternary complex (Fig. 7B, lane 7). Truncated Mcm1 showed decreased binding to the −318C mutant (Fig. 7B, lane 8) and was unable to form ternary complexes on the −306A and −307G/−306T/−305G mutant forms (Fig. 7B, lanes 9 and 10), which we have shown to cause loss of cell cycle control in vivo (Fig. 5); these results are therefore consistent with those obtained with full-length Mcm1 (Fig. 7A and B, lanes 2 to 5).

To characterize the binding of Mcm1 to the CLB2 UAS further, we used an rBV expression vector to produce high levels of Mcm1 (rMcm1) in insect cells. rMcm1 binding activity in crude insect cell extracts was detected by band shift analysis (Fig. 7B, lanes 11 to 15) and was similar to the DNA binding specificity of Mcm1 from crude yeast extracts (Fig. 7B, compare lanes 2 to 5 to lanes 12 to 15) but generated a protein-DNA complex with slightly higher mobility (Fig. 7B). The same UAS point mutations which severely reduced yeast Mcm1 binding had similar effects on Mcm1 produced in insect cells. Extracts prepared from mock-infected insect cells showed no Mcm1-like binding activity in this assay (Fig. 7B, lane 11). Importantly, we showed that binding of Mcm1 from crude cell extracts and from a recombinant source is not affected by the −306A and −307G/−306T/−305G mutations. Thus, loss of cell cycle control caused by these mutations in vivo cannot be explained by decreased Mcm1 binding.

We also showed that a protein antigenically related to Mcm1 is present in the B complex by antibody supershift experiments (Fig. 7B, lanes 16 to 23). Antibodies raised against a peptide epitope of Mcm1 (14) specifically shifted Mcm1-dependent B complexes in band shift reactions (Fig. 7B, lanes 16 to 23); no shift was seen when preimmune serum was included instead of immune serum. The specificity of this antibody was shown by preincubation of the immune serum with peptides. The peptide used as an antigen to raise antibodies (14) blocked the supershift of B complexes (Fig. 7B, lane 23), while a second peptide, which corresponds to an epitope from the Cdc45 protein (13a), failed to block the super shifting of B complexes (lane 21) under equivalent conditions. Together, these results show that the B complex is composed of an Mcm1-UAS complex and that M and T complexes must be generated by the recruitment of other proteins, in addition to Mcm1, by protein-protein and/or protein-DNA interactions.

To formally show that factors other than Mcm1 are components of the M and T bandshift complexes, rMcm1 was incubated with protein fractions from yeast extracts depleted of Mcm1 by chromatography on a Q-Sepharose column. Mcm1-depleted extracts were then used to supplement rMcm1 in bandshift reactions as an assay for ternary complex formation. An activity collected in the low-salt flowthrough fraction (and to a lesser extent in the 0.3 M wash fraction) was found to form a ternary complex with rMcm1-DNA complexes. This activity, which we refer to as TCF\textsubscript{CLB2}, did not bind DNA in the absence of rMcm1 (Fig. 7C, lane b) and could only be recruited to the CLB2 UAS as part of a ternary complex (Fig. 7C, compare lanes b and g). The mobility of the ternary complex generated by addition of the Mcm1-depleted flowthrough fraction to rMcm1 was identical to that of the T complex from crude extracts and was abolished when the −306A mutant probe was used (data not shown). We judge this activity to be the same as that detected in crude yeast extracts on the basis of these criteria. Although rMcm1-DNA complexes display greater mobility than yeast Mcm1-DNA complexes, addition of the flowthrough fraction from the Q-Sepharose column shifted rMcm1-DNA complexes to a mobility similar to that of yeast Mcm1-DNA complexes. This is probably due to association with an unidentified protein in yeast cell extracts or to extract-dependent posttranslational modifications of rMcm1 (1).

Our results suggest that the CLB2 and SWI5 UAS elements are not only functionally equivalent but are controlled by the same pair of trans-acting regulators, Mcm1 and ternary complex factor (TCF)/SFF. To determine if the DNA sequence specificities for TCF-Mcm1 and SFF-Mcm1 complexes are the same or related, we performed competition binding experiments in which complexes formed on a radiolabeled CLB2 UAS were challenged by various excess amounts of the wild-type or mutant unlabeled SWI5 UAS binding site (Fig. 8). The addition of the wild-type SWI5 binding site at a 5-fold molar excess markedly reduced complex formation on the CLB2 probe (lane g); addition of more of the SWI5 UAS competitor, up to a 200-fold molar excess (lane d), almost completely eliminated T, M, and B complex formation on the probe. As
another competitor, we used a mutant SW15 UAS (T308) previously reported to block Mcm1- and SFF-dependent complexes through its inability to form specific base contacts with Mcm1 (18). Little or no effect was seen when an unlabeled mutant T308 SW15 competitor was used over a 5- to 50-fold molar excess (lanes i to k), and only partial elimination of complexes was seen at a 200-fold molar excess (lane h). The inability of this mutant binding site to compete successfully for TCF\textsubscript{CLB2} against the CLB2 probe is reminiscent of that of the SFF factor, which also cannot bind the SW15 site in the absence of Mcm1. A SW15 UAS unable to bind SFF (A296; see reference 18) is predicted to be a good competitor of T, M, and B complexes which form on a CLB2 probe under these conditions as an intact Mcm1 site in such a competitor DNA would still effectively compete for Mcm1, thereby removing Mcm1-dependent ternary complexes from the CLB2 probe. These observations are consistent with the possibility that the same, or similar, proteins in cell extracts bind the CLB2 and SW15 cell cycle-regulated UAS elements.

**DISCUSSION**

A role for Mcm1 and a ternary complex factor in CLB2 transcription. We have shown that cell cycle-regulated changes in levels of CLB2 transcripts are controlled at the level of transcription by demonstrating that a CLB2-lacZ fusion gene is regulated in a manner indistinguishable from that of the endogenous CLB2 gene. This periodicity is very similar to that of SW15, CDC5, ACE2, and CLB1, which are also expressed from the late S phase through to late mitosis (8, 16, 21, 27). A 55-bp CLB2 UAS found to be sufficient for cell cycle regulation in vivo also binds two proteins in vitro, of which we believe one is Mcm1 and the other is a ternary complex factor resembling SFF (18). The ternary complex factor (TCF\textsubscript{CLB2}) identified here exhibits no autonomous DNA binding activity but is recruited into a ternary complex by DNA-bound Mcm1. Several pieces of evidence suggests that Mcm1 binds the cell cycle-regulated UAS and regulates CLB2 transcription. First, a protein that displays DNA binding characteristics similar to those of Mcm1 and is antigenically related to Mcm1 binds the CLB2 UAS in vitro. Second, a strain expressing only a truncated derivative of Mcm1 forms a faster-migrating complex on gel shifts in the absence of a more slowly migrating complex seen in Mcm1 wild-type strains. Third, an Mcm1-Vp16 fusion protein was capable of ectopically activating a did not abolish cell cycle control in the intact CLB2 promoter, but mutagenesis of all three Mcm1 sites in the CLB2 UAS resulted in complete loss of activity (our unpublished results). We interpreted this to mean that clustered Mcm1 sites in the CLB2 promoter are functionally redundant. It is unclear, however, if other promoter elements, such as those required for TCF\textsubscript{CLB2} recruitment, are also functionally redundant. It is clear, though, that not all Mcm1 sites (those which bind Mcm1 in vitro) in the CLB2 UAS have closely associated sequences which are sufficient for cell cycle control (Fig. 4A).

Evidence that Mcm1-TCF\textsubscript{CLB2} complexes regulate multiple genes in G\textsubscript{2}. Mcm1 is a member of an evolutionarily conserved class of transcription factors (the MADS family [24]) which have related DNA binding and dimerization domains. Other members of this family include a regulator of arginine biosynthesis in S. cerevisiae, Arg80 (9); the human serum response factor SRF (22); and the products of the plant homeotic genes deficiens (26) and agamous (31). Mcm1 controls the expression of a group of diversely regulated genes. In MAT\textalpha{} cells, Mcm1 forms a ternary complex with the coactivator α1 to activate transcription of a-specific genes involved in determining cell identity, such as STE3 (4), and with the corepressor α2 to silence α-specific genes, such as STE6 (15). In MAT\textalpha{} cells, Mcm1 binds to the promoters of α-specific genes involved with α-factor responses with the Ste12 transcription factor. In each case, sequences flanking the Mcm1 binding site have an important role in recruitment of accessory factors to DNA, thus facilitating the formation of promoter-specific transcription complexes. We believe that the ternary complex factors (M and T) assembling on the CLB2 UAS are distinct from α1, α2, and STE12, as the TCF activity was detected in MAT\textalpha{} cells (in which α1 and α2 are absent) and MAT\textalpha{} cells (Fig. 7A) and is unlikely to be Ste12, as no sequence resembling a PRTE (Ste12 binding site) was identified in the CLB2 UAS. It is likely that the M and T complexes are composed of Mcm1 and distinctly different ternary complex factors, both of which are dependent on Mcm1 for recruitment to the CLB2 UAS. The possibility that the M TCF is a breakdown product of the T TCF is unlikely, as both complexes do not appear to have the same sequence requirements (−306A mutation abolishes the T complex but not the M complex). Although mutations which abolish the T complex result in loss of cell cycle control, we know little about the role of M complexes in CLB2 control. The identification of mutants defective in M complex formation but competent to form T complexes may help address this question.

We favor the possibility that the ternary complex factor binding the CLB2 UAS in conjunction with Mcm1 is the same factor previously implicated in SW15 regulation, SFF (18). Several lines of evidence suggest that CLB2 transcription and SW15 transcription are controlled by the same (or similar) regulators and that the respective UAS elements are functionally equivalent. First, both UAS elements confer cell cycle regulation with the same periodicity; second, Mcm1 is required for regulation in both cases; third, the cis regulatory sequences flanking the Mcm1 binding site in both UAS elements are very similar; and finally, both UAS elements bind at least one ternary complex factor, in addition to Mcm1, which has no detectable autonomous DNA binding activity. It is unclear if other genes expressed at the same time as CLB2 and SW15 (such as CDC5, CLB1, and ACE2) are also under Mcm1-TCF\textsubscript{CLB2}–SFF control. We have identified putative Mcm1 binding sites in the CLB1 promoter, but their functional role has not been established (our unpublished observation).

We have presented evidence which suggests that the SW15 and CLB2 genes are coregulated by a transcription factor complex consisting of Mcm1 and a ternary complex factor, SFF-TCF\textsubscript{CLB2}. Although molecular and biochemical characterization of SFF-TCF\textsubscript{CLB2} has not been performed, we believe it most likely that SFF-TCF\textsubscript{CLB2} is the target of regulation for this cell cycle-regulated transcription complex, as Mcm1 has not been previously shown to exhibit any intrinsic cell cycle regulation. We cannot, however, rule out the possibility that
some other, unidentified factor is involved in periodic activation of these genes. Given the well-characterized precedent that Mcm1-regulated genes are controlled in conjunction with corepressors and coactivators, it is likely that TCF_{CLB2} is the regulatory subunit of the complex. This would also be similar to regulation of the SRF-elk1–TCF1 transcription complex which binds the serum response element in the human c-fos promoter (29).

It has been previously suggested that the B-type mitotic cyclins are required for their own synthesis involving a positive autoregulatory loop (2). In this report, we have demonstrated that cell cycle regulation of \( CLB2 \) mRNAs occurs at the level of transcription. Although \( CLB2 \) mRNA is unstable, its stabi-

Concerning control of G1 cyclin transcription (SCB-dependent response has been ruled out (18). Moreover, a direct role for B-type and G2 phases. We are currently investigating this possibility.

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### REFERENCES


24. Schwarz-Sommer, Z., P. Huijser, W. Nacken, H. Saedler, and H. Sommer. 1990. Genetic control of flower development by homeotic genes in Antirhini-


