Cell Cycle-Regulated Transcription of the *CLB2* Gene Is Dependent on Mcm1 and a Ternary Complex Factor

MAUREEN MAHER,[1] FENG CONG,[1] DAVID KINDELBERGER,[1] KIM NASMYTH,[2]
AND STEPHEN DALTON[1]*

*Roche Institute of Molecular Biology, Nutley, New Jersey 07110-1199,[1] and Research Institute
of Molecular Pathology, Vienna, Austria[2]*

Clb2 is the major B-type mitotic cyclin required for entry into mitosis in the budding yeast *Saccharomyces cerevisiae*. We showed that accumulation of *CLB2* transcripts in G_2 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 UAS 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 *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 *CLB2* transcription. We discuss the possibility that the *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 coordinately regulate several other G_2-regulated transcripts.

In the budding yeast *Saccharomyces cerevisiae*, the *CDC28* gene encodes a 34-kDa protein (p34^{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^{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^{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 *S. cerevisiae*: *CLB1*, *CLB2*, *CLB3* and *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 *Schizosaccharomyces pombe*, whereas Clb3 and Clb4 more closely resemble the *S. pombe* B-type cyclin homolog cig1 (23). None of the mitotic cyclins in *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 *CLB3* and *CLB4* transcripts, which increase early in the S phase, *CLB1* and *CLB2* transcripts begin to accumulate late in S phase and remain elevated until late in mitosis (10, 11, 23, 28). Increased levels of *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 *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 *CLB1*, *CDC5*, *ACE2*, and *SWI5*, are expressed at the same time in the cell cycle as *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 *SWI5* gene. Regulation of *SWI5* transcription is dependent on a cell cycle-regulated upstream activation sequence (UAS) which binds the Mcm1 transcription factor and a second protein, the *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 *SWI5* transcription complex is provided by SFF.

This report concerns the mechanism which controls *CLB2* expression during the cell cycle. We show that *CLB2* transcription plays a major part in the control of *CLB2* mRNA levels in the cell cycle. Furthermore, we identified a UAS from the *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.

* Corresponding author. Mailing address: Roche Institute of Molecular Biology, 340 Kingsland St., Nutley, NJ 07110-1199. Phone: (201) 235-2315. Fax: (201) 235-5848.

TABLE 1. Genotypes and sources of yeast strains used in this study

Strain	Genotype	Source
S1	*MATα his3-11,15 trp1-1 ade2-1 leu2-3,112 ura3 ho can1-100*; W303 1b	R. Rothstein
S129	*MAT**a** his3-11,15 trp1-1 ade2-1 leu2-3,112 ura3 ho can1-100*; W303 1a	R. Rothstein
S130	*MAT**a** ura3 trp1 leu2 GAL⁺ ade2-1 pep4-3 prb1-1-1122 prc1-407*	U. Surana
S131	*MAT**a** ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 GAL psi⁺ ura3 bar1::hisg*; from W303 1a	U. Surana
S226	*MAT**a** mcm1::LEU2 ADH-mcm1-98::URA3*; from W303 1a	G. Ammerer
S270	*MAT**a** ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 GAL psi⁺ URA3 bar1::hisg*; from S131	This study
S500	*MAT**a** bar1 CLB2$_{UAS-WT}$-lacZ::URA3*; from S270	This study
S501	*MAT**a** bar1 CLB2$_{UAS-318C}$-lacZ::URA3*; from S270	This study
S502	*MAT**a** cdc34-2 CLB2$_{UAS-WT}$-lacZ::URA3*	This study
S503	*MAT**a** bar1 RP39$_{UAS}$-lacZ::URA3*; from S270	This study
S504	*MAT**a** bar1 CLB2$_{UAS-306A}$-lacZ::URA3*; from S270	This study

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 pLGΔ-178 (13) as a *Sal*I-*Bam*HI fragment (sites introduced by PCR); this generated a fusion between the *CLB2* 5′-flanking region and the *lacZ* gene, which reads 5′-ATC.TTATAG.**ATG**.ACC.<u>GGA.TC</u><u>C</u>.GGA.GCT.TGG-*lacZ*-3′ at the junction (initiator ATG in boldface, *Bam*HI site underlined). The PCR-generated *CLB2* portion of this construct was replaced with an internal *Xba*I-*Nhe*I *CLB2* restriction fragment from pCLB2/5.5 (pBS.KS+ with a 5.5-kb *Bam*HI 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 −2,992 *CLB2-lacZ* fusion gene across from P330 as a *Hin*dIII-*Nco*I fragment blunted with T4 DNA polymerase; this construct (P523) then served as a template to generate all of the other promoter truncations described in this report (for a summary, see Fig. 3). Internal deletions of the *CLB2* promoter were generated by linearization of P523 with *Nhe*I, digestion with *Bal* 31 exonuclease (BioLabs), and recircularization. All deletions were sequenced on both strands to determine endpoints. Transcription start sites in the *CLB2* promoter were determined by primer extension analysis as described previously (6), with poly(A)⁺ RNA from S129.

Yeast reporter genes and indicator strains. For analysis of UAS sequences defined from deletion analysis of the *CLB2* promoter, fragments were cloned upstream of a *ubiYlacZ* reporter which expresses β-galactosidase (β-gal) activity with a half-life of 10 min instead of >20 h (3). All CLB2$_{UAS}$-*ubiYlacZ* reporter genes were initially constructed in pDL1460 as previously described (18). Reporter genes were excised with *Xho*I-*Nco*I, blunted with T4 DNA polymerase, inserted into the *Stu*I site of pUC.Ura3, and integrated at the *URA3* locus by homologous recombination as described previously (7). All integration events were confirmed by Southern blot analysis. Liquid culture determination of yeast β-gal has been described previously (7).

Cell synchrony experiments and Northern (RNA) analysis. α-Factor synchronization was performed by growing 1 liter of the appropriate *bar⁻* strain in YEPD at 30°C to 10⁷ cells per ml. α-Factor (from Ed Heimer, Hoffmann-La Roche Inc., Nutley, N.J.) was added to 0.1 μg/ml (3 to 5 μg/ml for *BAR⁺* cells), and 2 h later the cells were washed twice with 200 ml of fresh YEPD and resuspended in 1 part fresh YEPD to 1 part conditioned YEPD medium (made by growing W303 1a [*BAR⁺* strain] to 2 × 10⁷ cells per ml and using a filtrate as conditioned medium). Cell synchrony and release from α-factor arrest were routinely monitored by β-tubulin staining of mitotic spindles. Total yeast RNA was prepared by a modification of the bead-beat method (10). RNA samples (20 μg) were electrophoresed in 1% agarose-formaldehyde gels, transferred by capillary action onto Amersham Hybond hybridization filter membranes, and fixed onto filters with UV light by using a Stratalinker (Stratagene). Filters were prehybridized for at least 12 h at 42°C in 50% formamide–5× Denhardt's solution–6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH₂PO₄, and 1 mM EDTA [pH 7.7])–1% sodium dodecyl sulfate–100 μg of boiled, sonicated salmon sperm DNA per ml and probed with a radioactive probe (random primed) at 10 ng/ml (specific activity, >10⁹ cpm/μg of DNA). The gel-isolated DNA fragments used to generate probes were *CLB2* (1.3-kb internal fragment of the *CLB2* gene generated by PCR), *lacZ* (first 350 bp of the *lacZ* gene in pLGΔ-178 generated by PCR), *H2A/Prt*l (2.3-kb *Sac*I fragment from YpTRT1 [20]), and *URA3* (1.1-kb *Hin*dIII fragment from pUC.Ura3 [7]). Filters were washed to final stringency with 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 60°C for 60 min. Quantitation of signals on filters after probing was performed with a Molecular Dynamics Phosphorimager.

Other techniques. Protein extracts, mobility shift gels, and synthesis of radioactive DNA probes were prepared as described previously (18). Full-length Mcm1 (amino acids 1 to 286) was produced in Sf9 cells (19) by expression from a recombinant baculovirus vector (rBV; details to be described elsewhere). Q-Sepharose (Pharmacia) chromatography was performed by loading of crude S130 extract in PB50 (50 mM KCl, 20% glycerol, 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 8.0], 5 mM EDTA, 1 mM dithiothreitol [DTT], protease inhibitors) and then stepwise elution of bound proteins with PB300 and PB1000. A protocol described previously (14) was used to raise polyclonal antibodies in rabbits against a synthetic peptide corresponding to amino acids 139 to 155 of Mcm1. Gel mobility supershift and peptide competition experiments were performed essentially as previously described (14).

Nucleotide sequence accession number. The sequence extending to position −2,992 has been deposited in the GenBank database under accession number U14728.

RESULTS

The *CLB2* 5′-flanking region and mapping of 5′ ends of *CLB2* transcripts. We sequenced the *CLB2* promoter region (Fig. 1A) and determined the major transcription start sites by primer extension analysis (Fig. 1B). The major transcription start sites mapped by this method were the same regardless of the primer used for extensions. Although the 5′ untranslated region is unusually long (362 nucleotides for transcripts 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* transcript. Long 5′ untranslated 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 long 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-
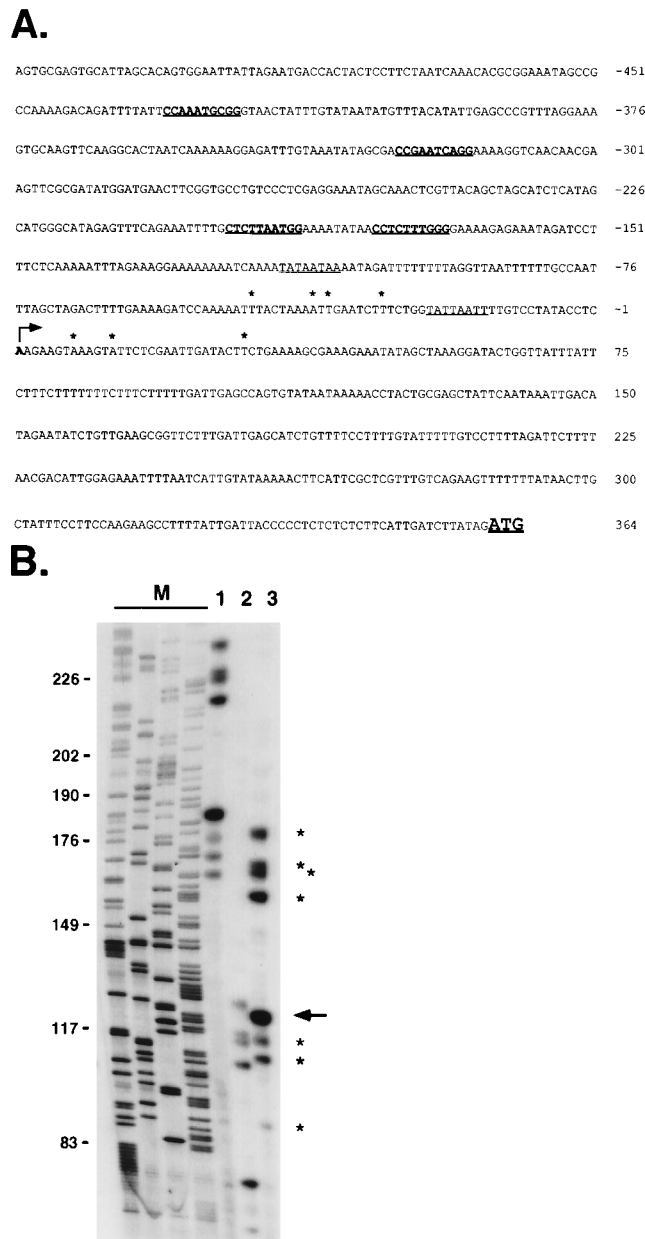
A.

```
AGTGCGAGTGCATTAGCACAGTGGAATTATTAGAATGACCACTACTCCTTCTAATCAAACACGCGGAAATAGCCG     -451

CCAAAAGACAGATTTTATTCCAAATGCGGGTAACTATTTGTATAATATGTTTACATATTGAGCCCGTTTAGGAAA     -376

GTGCAAGTTCAAGGCACTAATCAAAAAAGGAGATTTGTAAATATAGCGACCGAATCAGGAAAAGGTCAACAACGA     -301

AGTTCGCGATATGGATGAACTTCGGTGCCTGTCCCTCGAGGAAATAGCAAACTCGTTACAGCTAGCATCTCATAG     -226

CATGGGCATAGAGTTTCAGAAATTTTGCTCTTAATGGAAAATATAACCTCTTTGGGGAAAAGAGAAATAGATCCT     -151

TTCTCAAAAATTTAGAAAGGAAAAAAAATCAAAATATAATAAAATAGATTTTTTTTAGGTTAATTTTTTGCCAAT      -76
                                             *        *  *       *
TTAGCTAGACTTTTGAAAAGATCCAAAAATTTACTAAAATTGAATCTTTCTGGTATTAATTTTGTCCTATACCTC      -1
 ┌─►      *    *                          *
AAGAAGTAAAGTATTCTCGAATTGATACTTCTGAAAAGCGAAAGAAATATAGCTAAAGGATACTGGTTATTTATT       75

CTTTCTTTTTTTCTTTCTTTTTGATTGAGCCAGTGTATAATAAAAACCTACTGCGAGCTATTCAATAAATTGACA      150

TAGAATATCTGTTGAAGCGGTTCTTTGATTGAGCATCTGTTTTCCTTTTGTATTTTTGTCCTTTTAGATTCTTTT      225

AACGACATTGGAGAAATTTTAATCATTGTATAAAAACTTCATTCGCTCGTTTGTCAGAAGTTTTTTTATAACTTG      300

CTATTTCCTTCCAAGAAGCCTTTTATTGATTACCCCCTCTCTCTCTTCATTGATCTTATAGATG                 364
```

B.

FIG. 1. Sequence and features of the *CLB2* promoter. (A) The 5′-flanking region of the *CLB2* gene from a 5.5-kb *Hin*dIII genomic fragment was sequenced, and the first 889 bp are shown. Transcription start sites were determined as described in Materials and Methods. The major start site at +1 is indicated by an arrow, and other start sites are indicated by asterisks. Other features shown are the ATG translation initiator codon at position 362, putative TATA boxes at positions −19 and −113 (underlined), and four sequences which represent possible Mcm1 binding sites (28; in boldface type and underlined). (B) Mapping of the 5′ termini of *CLB2* transcripts. Primer extension analysis using three primers on 2 μg of poly(A)$^+$ RNA prepared from S129. Lanes: M, sequencing ladder markers; 1, 2, and 3, primer extension products generated by using primers which anneal on the *CLB2* transcript from positions −182, −76, and −125, respectively. Several other primers which primed closer to the ATG were used in separate reactions (data not shown). In all cases, the same 5′ *CLB2* transcript termini were identified. For primer extension products in lane 3, the major transcription start site (+1) is indicated by an arrow; other start sites are indicated by asterisks. The numbers on the left are marker sizes (in bases).

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
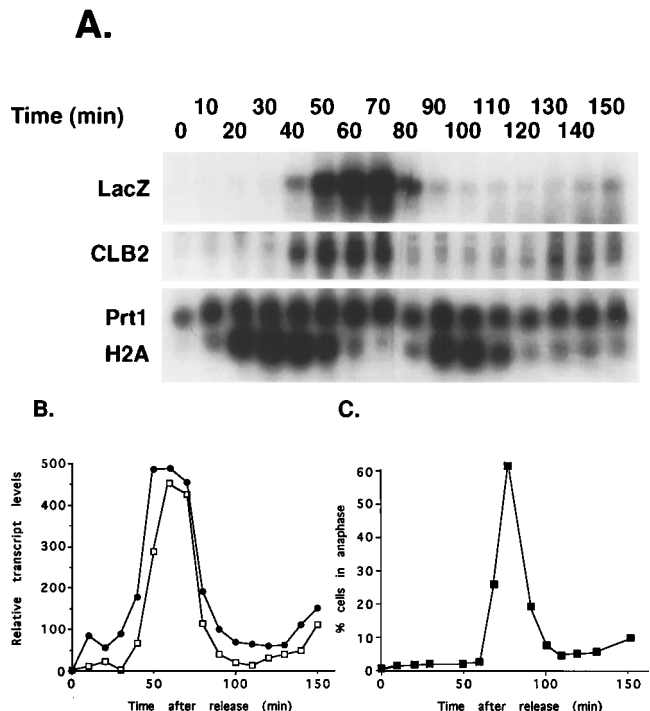
A.

FIG. 2. 5′-flanking sequences are required for cyclic regulation of *CLB2* transcripts. A *CLB2* promoter fragment extending from the initiator ATG to position −2,309 was fused to a *lacZ* gene (Materials and Methods) and chromosomally integrated at the *URA3* locus of S131 (*MAT*a *bar1 ura3*). (A) *CLB2*, *lacZ*, *H2A*, and Prt1 transcript levels in a synchronous culture. Cells were synchronized with α-factor, and RNA levels were determined by Northern blot analysis. 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).

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 −362 to −131 region. Smaller deletions within this region also resulted in substantial decreases in promoter activity but to a lesser extent than the −362 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 −362 to −131 region of the *CLB2* promoter upstream of a *ubiYlacZ* gene (half-life of β-gal, <10 min [3]) and integrated the gene fusion at the *URA3* locus. If the *CLB2* UAS is involved in cell cycle regulation of the *CLB2* promoter, it should be capable of conferring such regulation on a reporter gene in a heterologous promoter environment. To address this question, cells were synchronized with α-factor and transcript levels were determined by Northern blot analysis. This analysis showed that *ubiYlacZ* transcript levels were cell cycle regulated in a pattern indistinguishable from that of endogenous *CLB2* transcripts (Fig. 4B), indicating that the −362 to −131 promoter fragment could function as a cell cycle-regulated UAS. This corresponds to a region in the *CLB2* promoter which contains three sequences predicted to bind Mcm1 (Fig. 1). Testing of further deletions within this region showed that
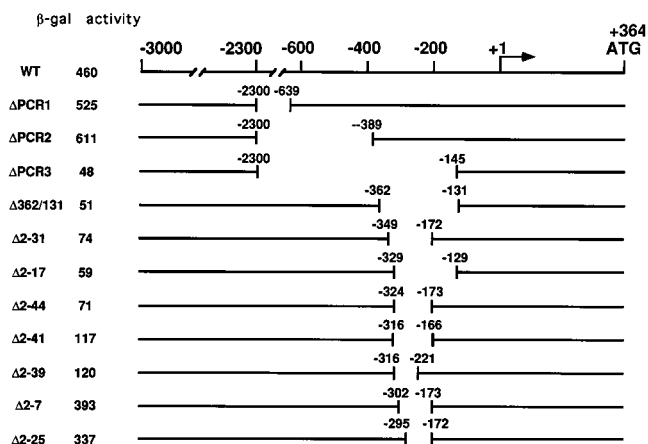
FIG. 3. Deletion analysis of the *CLB2* promoter. A series of promoter deletions were generated in the *CLB2-lacZ* fusion gene by PCR or by *Bal* 31 exonuclease digestion. Endpoints for each deletion are shown with respect to the major transcription start site at +1 (indicated by the arrow). Numbers along the top row indicate positions along the promoter. The left column indicates positions, the deletion name, and the next column shows the relative levels of β-gal activity generated from promoter-*lacZ* fusion genes. Assays were performed in duplicate. WT, wild type.

FIG. 4. The *CLB2* −362 to −131 (232-bp) UAS is necessary and sufficient for cell cycle-regulated transcription. (A) Mapping of sequences in the *CLB2* −362 to −131 UAS necessary for cell cycle-regulated transcription. Indicated are the endpoints of the UAS tested and the locations of predicted Mcm1 binding sites (shaded boxes). (B) Ability of UAS deletions to support cell cycle-regulated transcription. UAS sequences mapped by deletion analysis as for panel A were inserted upstream of a *ubiYlacZ* reporter gene and integrated into the *URA3* locus in strain S270. Cells were synchronized with α-factor, and *ubiYlacZ*, *CLB2*, *H2A*, and Prt1 transcript levels were assessed by Northern analysis in a synchronous population of cells. RNA samples from the culture were made at the times indicated before and after release from the α-factor block.

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
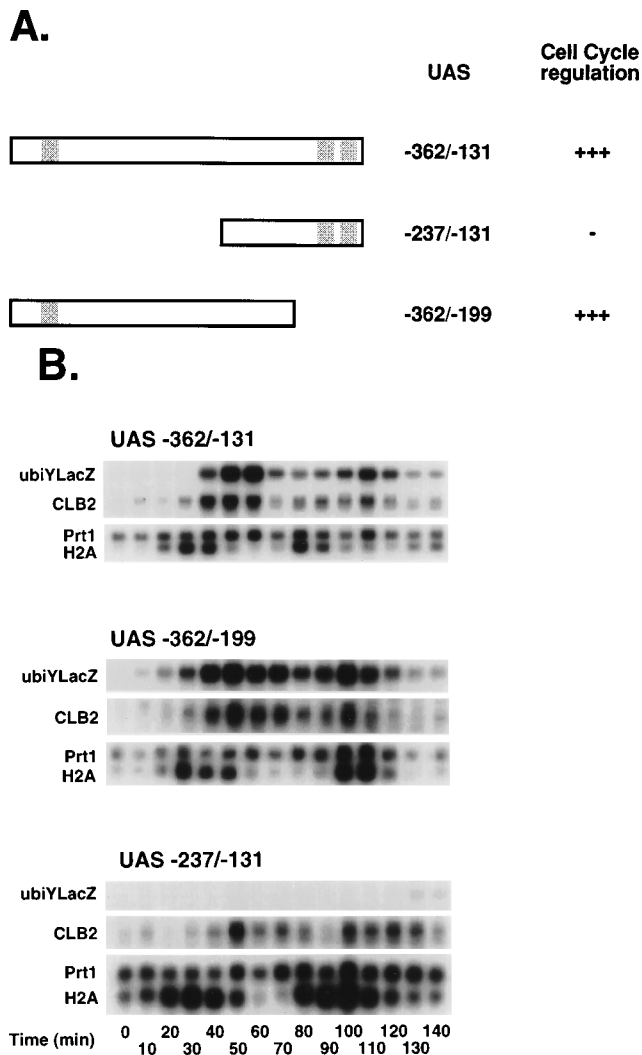
exist. Alignment of the two UAS elements revealed significant sequence similarities to the 3′ side of the Mcm1 site which coincide with sequences known to be important in the *SWI5* promoter (Fig. 5A). A C residue at position −296 of the *SWI5* promoter has previously been shown to be important in the 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*-
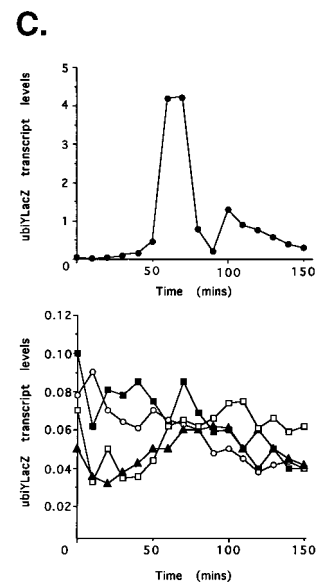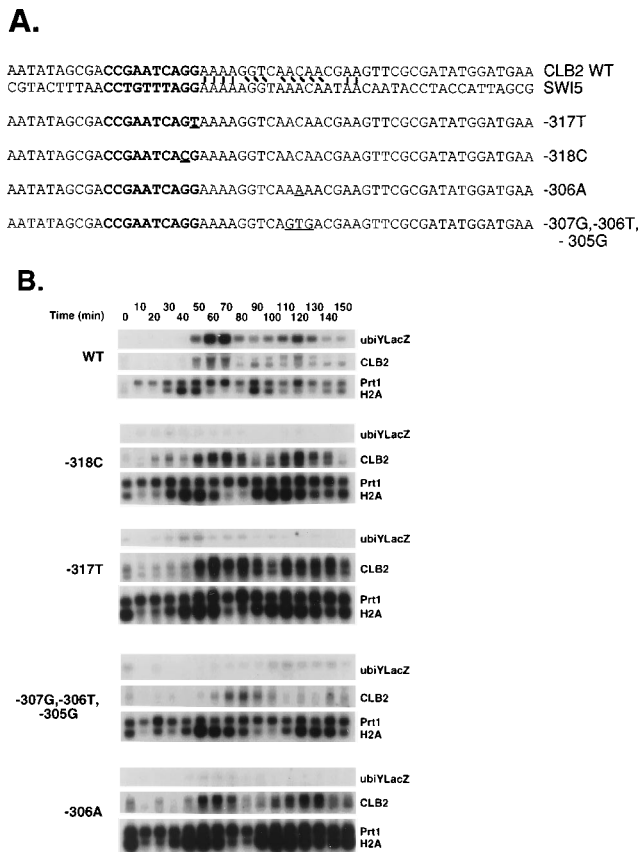
A.

```
AATATAGCGACCGAATCAGGAAAAAGGTCAACAACGAAGTTCGCGATATGGATGAA  CLB2 WT
CGTACTTTAACCTGTTTAGGAAAAAGGTAAACAATAACAATACCTACCATTAGCG  SWI5

AATATAGCGACCGAATCAGTAAAAGGTCAACAACGAAGTTCGCGATATGGATGAA  -317T

AATATAGCGACCGAATCACGAAAAGGTCAACAACGAAGTTCGCGATATGGATGAA  -318C

AATATAGCGACCGAATCAGGAAAAGGTCAAAAACGAAGTTCGCGATATGGATGAA  -306A

AATATAGCGACCGAATCAGGAAAAGGTCAGTGACGAAGTTCGCGATATGGATGAA  -307G,-306T,
                                                          - 305G
```

B.

C.

FIG. 5. Mutational analysis of a 55-bp *CLB2* UAS which is sufficient for cell cycle regulation of transcription. (A) The wild-type (WT) UAS (−336 to −282) and multiple or point mutant UAS sequences were tested for the ability to confer cell cycle regulation on a *ubiYlacZ* reporter gene (see panel B). The predicted Mcm1 binding site is in boldface type, mutations are underlined, and the specific mutant version of the UAS is noted in the right-hand column. An alignment of the *SWI5* UAS (positions −327 to −273 [18]) and the *CLB2* UAS (positions −336 to −282) is shown with regions of high sequence similarity outside of the Mcm1 site indicated by lines. (B) The 55-bp sequence shown in panel A was tested to determine if it could confer cell cycle-regulated transcription on a *ubiYlacZ* reporter gene together with point mutations (−318C and −317T; panel A) in a predicted Mcm1 binding site. Mutations were also introduced at other positions outside of the putative Mcm1 binding site predicted to be functionally important on the basis of similarities between the *CLB2* and *SWI5* UAS elements
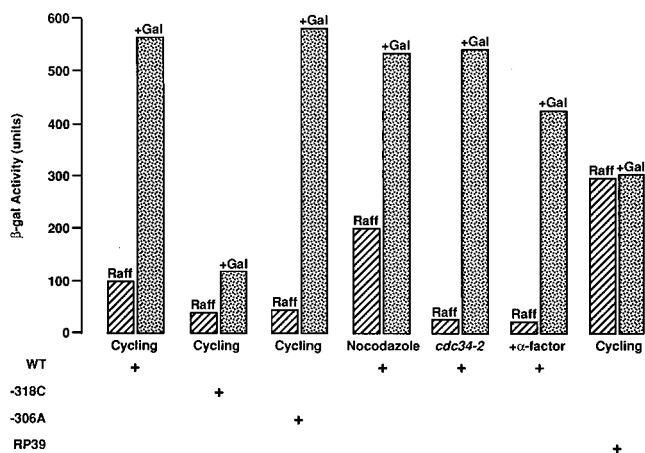

FIG. 6. An Mcm1-Vp16 fusion protein activates a *CLB2-ubiYlacZ* reporter gene in vivo. A fusion gene consisting of the first 98 amino acids of Mcm1 was fused to the acidic activation domain of Vp16 (amino acids 412 to 490) under control of the inducible GAL1-10 promoter and integrated at the *TRP1* locus in S500 (*CLB2$_{UAS-WT}$-ubiYlacZ::ura3*), S501 (*CLB2$_{UAS-318C}$-ubiYlacZ::ura3*), S502 (*CLB2$_{UAS-WT}$-ubiYlacZ::ura3 cdc34-2*), S503 (*RP39$_{UAS}$-ubiYlacZ::ura3*), or S504 (*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.

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 [3]) 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 G$_1$) or by the cell cycle inhibitor α-factor (G$_1$) 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,

<hr>

(18; see above): the −306A point mutation and the −307G/−306T/−305G triple mutation. (C) Northern blots were tested with a Phosphorimager to quantitatively determine levels of *ubiYlacZ* transcripts, which are shown relative to that of the non-cell cycle control transcript, Prt1. Symbols: ●, wild type; ○, −318C; ■, −317T; □, −307G/−306T/−305GC; ▲, −306A. UAS elements were inserted upstream of a *ubiYlacZ* reporter gene and integrated into the *URA3* locus of S131 for cell cycle analysis. Cell synchrony and analysis of transcripts were done as described in the legend to Fig. 4.
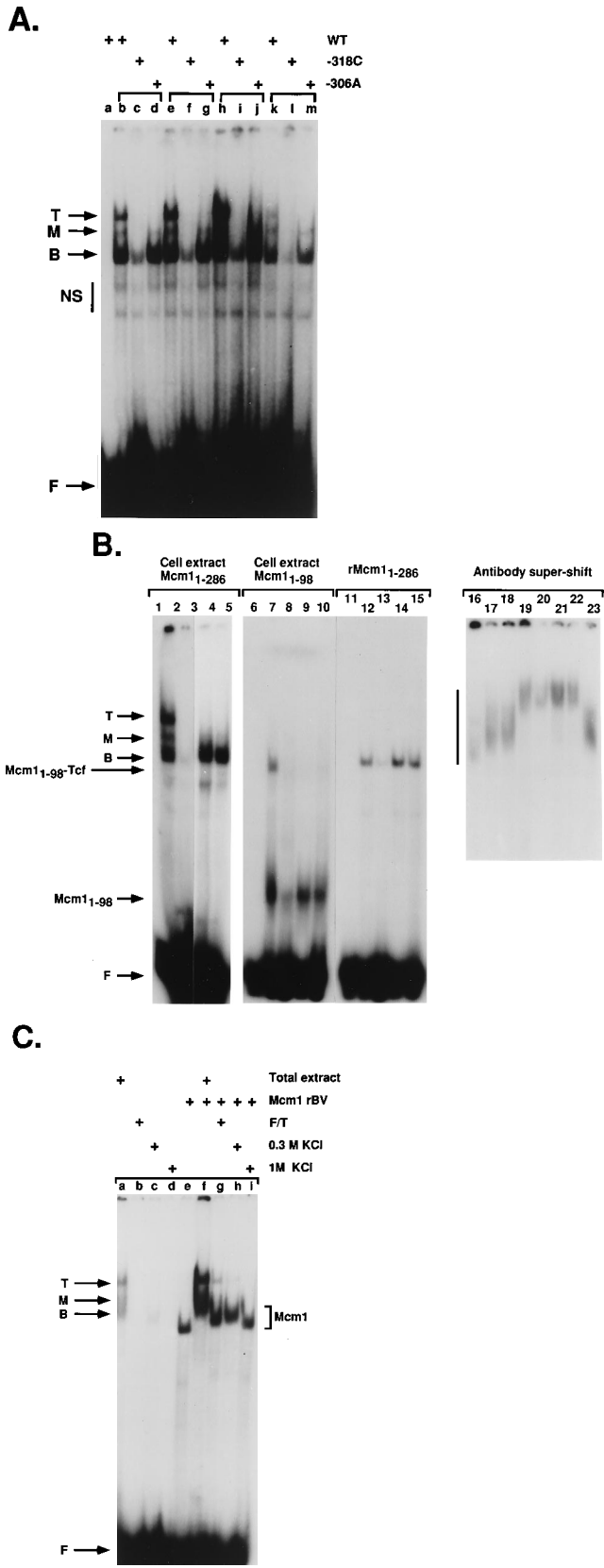
A.

B.

C.

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, e, h, and k), −318C (c, f, i, and l), and −306A (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 −306A 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 by 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 (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 −318C and −317T 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 (−306A and −307G/−306T/−305G) 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.

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 *MAT*α 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), −318C (lanes 3, 8, and 13), −307G/−306T/−305G (lanes 4, 9, and 14), and −306A (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_{1-98} (lanes 7 to 10), specific complexes (T, M, and B, lanes 2 to 5), Mcm1_{1-98}–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 S130 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 (F/T), the 0.3 M KCl fraction, or the 1 M KCl fraction. Positions of T, M, and B complexes from the S130 crude extract are indicated (lane a) for comparison with mobility shifts in lanes b to i. B-form (Mcm1-alone) complexes which show different mobilities are indicated by the bracket (lanes e to i). Addition of crude extract or extract fractions was sufficient to shift the mobility of rBV Mcm1 to that of the endogenous Mcm1 detected in crude extracts (lane f).

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 (Mcm$_{1-98}$) 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/−306G/−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 supershifting 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$_{CLB2}$, 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
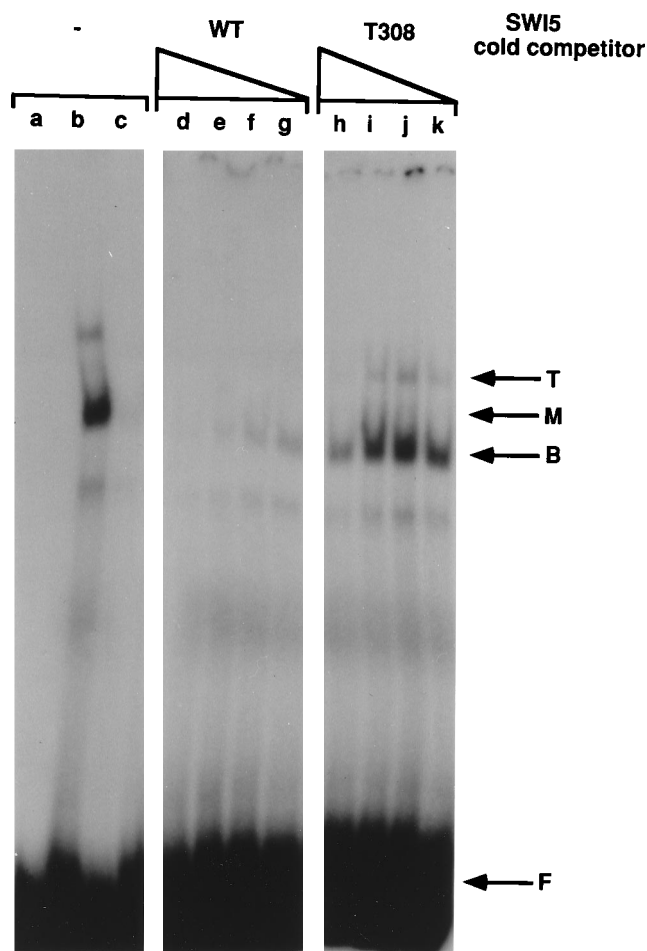

FIG. 8. *SWI5* UAS binding sites effectively compete for *CLB2* UAS-associated factors from crude cell extracts. A mutant (−318C; lane c) or wild-type (WT; lanes a, b, and d to k) *CLB2* probe was incubated without (lane a) or with (lanes b to k) 10 μg of total cell extract from S130. A 200-, 50-, 20-, or 5-fold molar excess of the unlabeled wild-type (lanes d to g, respectively) or mutant (T308; lanes h to k, respectively) *SWI5* competitor binding site (reference 18 and Fig. 5A) was included in band shift reactions. Positions of the T, M, and B complexes and the free probe (F) are indicated.

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). 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 *SWI5* 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 *SWI5* 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$_{CLB2}$ against the *CLB2* probe is reminiscent of that of the SFF factor, which also cannot bind the *SWI5* site in the absence of Mcm1. A *SWI5* 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 *SWI5* 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 *SWI5*, *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$_{CLB2}$) 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 *CLB2-ubiYlacZ* reporter in vivo in a sequence-specific manner. Finally, point mutations which abolished or reduced binding of Mcm1 to the *CLB2* UAS in vitro had similar effects on transcription in vivo. A role for TCF$_{CLB2}$ is implied by the observation that a single-base substitution which blocked ternary complex formation in vitro also severely reduced UAS activity in vivo. We were unable to detect changes in the formation of these complexes throughout the cell cycle, and so if this complex is involved in regulating UAS activity in vivo, its involvement is probably not due to the binding activity of the components in the ternary complex alone.

Although the −362 to −131 UAS was shown to be necessary and sufficient for cell cycle control, deletion of this Mcm1 site 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$_{CLB2}$ 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$_{CLB2}$ complexes regulate multiple genes in G$_2$. 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 *MATα* cells, Mcm1 forms a ternary complex with the coactivator α1 to activate transcription of α-specific genes involved in determining cell identity, such as *STE3* (4), and with the corepressor α2 to silence **a**-specific genes, such as *STE6* (15). In *MAT***a** cells, Mcm1 binds to the promoters of **a**-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***a** cells (in which α1 and α2 are absent) and *MATα* 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 *SWI5* regulation, SFF (18). Several lines of evidence suggest that *CLB2* transcription and *SWI5* 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 *SWI5* (such as *CDC5*, *CLB1*, and *ACE2*) are also under Mcm1-TCF$_{CLB2}$–SFF control. We have identified putative Mcm1 binding sites however, in the *CLB1* promoter, but their functional role has not been established (our unpublished observation).

We have presented evidence which suggests that the *SWI5* and *CLB2* genes are coregulated by a transcription factor complex consisting of Mcm1 and a ternary complex factor, SFF-TCF$_{CLB2}$. Although molecular and biochemical characterization of SFF-TCF$_{CLB2}$ has not been performed, we believe it most likely that SFF-TCF$_{CLB2}$ 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*–TCF 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 stability does not appear to be regulated with the cell cycle (6a), and so transcriptional control is most likely the only major determinant of *CLB2* transcript levels. If a positive feedback loop exists, it is likely to act on *CLB2* transcription via the Mcm1-TCF$_{CLB2}$ transcription complex. A possible mechanism for this could involve either a direct or indirect effect of Clb-Cdc28 kinase activity on Mcm1-TCF$_{CLB2}$; the decrease in *CLB2* transcription seen at the end of mitosis could then be explained as a consequence of Clb destruction. An alternate hypothesis is that periodic transcription is governed by a repressor protein and that the ternary complex is required only for activated transcription. There is no evidence to support this model, and in *SWI5* transcription, a role for a cell cycle-regulated repressor has been ruled out (18). Moreover, a direct role for B-type cyclins in control of G$_1$ cyclin transcription (SCB-dependent pathway) has previously been shown (2), so it is possible that mitotic cyclins have two roles in the control of transcription in the cell cycle: repression of SCB-regulated transcripts and activation of Mcm1-TCF$_{CLB2}$-regulated transcripts in the late S and G$_2$ phases. We are currently investigating this possibility.

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.
2. **Amon, A., M. Tyers, B. Futcher, and K. Nasmyth.** 1993. Mechanisms that make the yeast cell cycle clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. Cell **74:**993–1007.
3. **Bachmair, A., D. Finley, and A. Varshavsky.** 1986. In vivo half-life of a protein is a function of its amino-terminal residue. Science **234:**179–186.
4. **Bender, A., and G. F. Sprague.** 1987. *MAT*a1 protein, a yeast transcriptional activator, binds synergistically with a second protein to a set of cell-type specific genes. Cell **50:**681–691.
5. **Booher, R. N., R. J. Deshaies, and M. W. Kirchner.** 1993. Properties of *Saccharomyces cerevisiae* wee1 and its differential regulation of p34^{CDC28} in response to G1 and G2 cyclins. EMBO J. **12:**3417–3426.
6. **Dalton, S.** 1992. Cell cycle regulation of the human cdc2 gene. EMBO J. **11:**1797–1804.
6a. **Dalton, S.** Unpublished data.
7. **Dalton, S., and R. Treisman.** 1992. Characterization of SAP-1, a protein recruited by serum response factor to the c-*fos* serum response element. Cell **68:**597–612.
8. **Dohrmann, P. R., G. Butler, K. Tamai, S. Dorland, J. R. Greene, D. J. Thiele, and D. J. Stillman.** 1992. Parallel pathways of gene regulation: homologous regulators SWI5 and ACE2 differentially control HO and chitinase transcription. Genes Dev. **6:**93–104.
9. **Dubois, E., J. Bercy, and F. Messenguy.** 1987. Characterization of two genes, ARGR1 and ARGR111, required for specific regulation of arginine metabolism in yeast. Mol. Gen. Genet. **207:**142–148.
10. **Fitch, I., C. Dahmann, U. Surana, A. Amon, K. Nasmyth, L. Goetsch, B. Byers, and B. Futcher.** 1992. Characterization of four B-type cyclin genes of the budding yeast *Saccharomyces cerevisiae*. Mol. Biol. Cell **3:**805–818.
11. **Ghiara, J. B., H. E. Richardson, K. Sugimoto, M. Henze, D. J. Lew, C. Wittenberg, and S. I. Reed.** 1991. A cyclin B homolog in *S. cerevisiae*: chronic activation of the Cdc28 protein kinase by cyclin prevents exit from mitosis. Cell **65:**163–174.
12. **Grandin, N., and S. I. Reed.** 1993. Differential function and expression of *Saccharomyces cerevisiae* B-type cyclins in mitosis and meiosis. Mol. Cell. Biol. **13:**2113–2125.
13. **Guarente, L., B. Lalonde, P. Gifford, and E. Alani.** 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *cyc1* gene of *S. cerevisiae*. Cell **36:**503–511.
13a. **Hopwood, B., and S. Dalton.** Unpublished data.
14. **Jarvis, E. E., K. L. Clark, and G. F. Sprague, Jr.** 1989. The yeast transcription activator PRTF, a homolog of the mammalian serum response factor, is encoded by the *MCM1* gene. Genes Dev. **3:**936–945.
15. **Keleher, C. A., C. Goutte, and A. D. Johnson.** 1988. The yeast cell-type-specific repressor α2 acts cooperatively with a non-cell type-specific protein. Cell **53:**927–936.
16. **Kitada, K., A. T. Johnson, L. H. Johnston, and A. Sugino.** 1993. A multicopy suppressor gene of the *Saccharomyces cerevisiae* G$_1$ cell cycle mutant gene *dbf4* encodes a protein kinase and is identified as *CDC5*. Mol. Cell. Biol. **13:**4445–4457.
17. **Lew, D. J., and S. I. Reed.** 1993. Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins. J. Cell Biol. **120:**1305–1320.
18. **Lydall, D., G. Ammerer, and K. Nasmyth.** 1991. A new role for MCM1 in yeast: cell cycle regulation of SWI5 transcription. Genes Dev. **5:**2405–2419.
19. **Marais, R. M., J. J. Hsuan, C. McGuigan, J. Wynne, and R. Treisman.** 1992. Casein kinase II phosphorylation increases the rate of serum response factor-binding site exchange. EMBO J. **11:**97–105.
20. **Meeks Wagner, D., and L. Hartwell.** 1986. Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell **44:**43–52.
21. **Nasmyth, K., A. Seddon, and G. Ammerer.** 1987. Cell cycle regulation of *SWI5* is required for mother-cell-specific *HO* transcription in yeast. Cell **49:**549–558.
22. **Norman, C., M. Runswick, R. Pollock, and R. Treisman.** 1988. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds the c-*fos* serum response element. Cell **55:**989–1003.
23. **Richardson, H. E., D. J. Lew, M. Henze, K. Sugimoto, and S. I. Reed.** 1992. Cyclin-B homologs in *Saccharomyces cerevisiae* function in S phase and in G2. Genes Dev. **6:**2021–2034.
24. **Schwarz-Sommer, Z., P. Huijser, W. Nacken, H. Saedler, and H. Sommer.** 1990. Genetic control of flower development by homeotic genes in *Antirrhinum majus*. Science **250:**931–936.
25. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
26. **Sommer, H., J.-P. Beltran, P. Huijser, H. Pape, W.-E. Lonnig, H. Saeddler, and Z. Schwarz-Sommer.** 1990. *deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homology to transcription factors. EMBO J. **9:**605–613.
27. **Surana, U., A. Amon, C. Dowzer, J. McGrew, B. Byers, and K. Nasmyth.** 1993. Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. EMBO J. **12:**1969–1978.
28. **Surana, U., H. Robitsch, C. Price, T. Schuster, I. Fitch, B. Futcher, and K. Nasmyth.** 1991. The role of CDC28 and cyclins during mitosis in the budding yeast *S. cerevisiae*. Cell **65:**145–161.
29. **Treisman, R.** 1994. Ternary complex factors: growth factor regulated transcription complexes. Curr. Opin. Genet. Dev. **4:**96–101.
30. **Wynne, J., and R. Treisman.** 1992. SRF and MCM1 have related but distinct DNA binding specificities. Nucleic Acids Res. **20:**3297–3303.
31. **Yanofsky, M. F., H. Ma, J. L. Bowman, G. N. Drews, K. A. Feldmann, and E. M. Meyerowitz.** 1990. The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature (London) **346:**35–39.