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

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\textit{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 \textit{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 \textit{G2}-regulated transcripts.

In the budding yeast \textit{Saccharomyces cerevisiae}, the \textit{CDC28} gene encodes a 34-kDa protein (p34\textit{CDC28}) which serves as the catalytic subunit for a cell cycle-regulated protein kinase. This kinase regulates the \textit{G2-M} transition, and passage through a control point in \textit{G1}, known as Start, where cells prepare for DNA replication and become irreversibly committed to a further round of cell division. Changes in p34\textit{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 \textit{G1} phase, Cdc28 complexes with one of three functionally redundant \textit{G1} cyclins (Cln1, Cln2, or Cln3), which together are required for execution of Start and for the \textit{G1-to-S} transition. Regulation of the \textit{G2-M} transition by p34\textit{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}: 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 \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 \textit{S} phase, \textit{CLB1} and \textit{CLB2} transcripts begin to accumulate late in \textit{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 \textit{G2}-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 Mmc1 (18). This is similar to the recruitment of ternary complex factors by the mammalian counterpart of Mmcm1, the serum response factor SRF (7, 29). Through its interactions with cell type-specific coactivators and corepressors, Mmc1 also regulates genes not under cell cycle control, such as cell type-specific phenomone and receptor genes. It is therefore likely that Mmc1 has no intrinsinc 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). Other yeast manipulations were done as described previously (7). 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 pLG-178 (13) as a Sall-BamHI fragment (sites introduced by PCR); this generated a fusion between the CLB2 5′-flanking region and the lacZ gene, which reads 5′-ATGTTATAGTACC-GGA-TGG-CGA-ACT-TGG-lacZ-3′ at the junction (initiator ATG in boldface, C for 60 min. Quantitation of signals on filters after probing was performed with a Molecular Dynamics Phosphorimager.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<tbody>
<tr>
<td>S1</td>
<td>MATα his3-11,15 trp1-1 ade2-1 leu2-3,112 ura3-100 can1-100; W303 1b</td>
<td>R. Rothstein</td>
</tr>
<tr>
<td>S129</td>
<td>MATα his3-11,15 trp1-1 ade2-1 leu2-3,112 ura3-100 can1-100; W303 1a</td>
<td>R. Rothstein</td>
</tr>
<tr>
<td>S130</td>
<td>MATα trp1 leu2 GAL1 trp1-1 pep3-4 prb1-1-112 prc1-407</td>
<td>U. Surana</td>
</tr>
<tr>
<td>S131</td>
<td>MATα ade2-1 trp1 can1-100 leu2-3,112 his3-11,15 GAL psi+ ura3 bar1-1; his; from W303 1a</td>
<td>U. Surana</td>
</tr>
<tr>
<td>S226</td>
<td>MATα mcml-1; LEU2 ADH-mcm1-98:URA3; from W303 1a</td>
<td>G. Ammerer</td>
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<tr>
<td>S270</td>
<td>MATα ade2-1 trp1 can1-100 leu2-3,112 his3-11,15 GAL psi+ URA3 bar1-1; his; from S131</td>
<td>This study</td>
</tr>
<tr>
<td>S500</td>
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<td>This study</td>
</tr>
<tr>
<td>S501</td>
<td>MATα bar1 CLB2-lacZ::URA3; from S270</td>
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<td>S504</td>
<td>MATα bar1 CLB2-lacZ::URA3; from S270</td>
<td>This study</td>
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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 S19 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 PBS0 (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 PBS0 and PBS100. 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-
moter function by measuring β-gal activities from CLB2 promoter-lacZ fusion genes. This analysis defined a region from positions −236 to −2131 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 −236 to −2131 region. Smaller deletions within this region also resulted in substantial decreases in promoter activity but to a lesser extent than the −236 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 −236 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 of S131 (MATα bar1 ura3). (A) CLB2, lacZ, H2A, and Prt1 transcript levels in a synchronous culture. 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).

FIG. 1. Sequence and features of the CLB2 promoter. (A) The 5′-flanking region of the CLB2 gene from a 5.5-kb HindIII 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 close 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).
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 reporter 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 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 G1) or by the cell cycle inhibitor α-factor (G1) or nocodazole (metaphase), and one-half of the culture was induced with 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.

![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 (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/~305G; ▲, ~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.](http://mcb.asm.org/)

![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 S80 (CLB2-UAS-WT-ubiYlacZ::ura3), S91 (CLB2-UAS-318C-ubiYlacZ::ura3), S502 (CLB2-UAS-307G-ubiYlacZ::ura3), S503 (RP39-UAS-ubiYlacZ::ura3), or S504 (CLB2-UAS-306A-ubiYlacZ::ura3). Cells were originally grown in YEPD medium plus 2% raffinose (Raf) 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.](http://mcb.asm.org/)
where activity of the uninduced (cells grown in raffinose medium) culture was very low. A point mutation in the \( \text{CLB2} \) UAS shown to severely reduce Mcm1 binding (see Fig. 7) also showed severely reduced \( \text{ubiYlacZ} \) activity compared with the wild-type control, showing that \( \text{trans-activation} \) 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 \( \text{CLB2} \) UAS, the same experiment was performed with a strain carrying a \( \text{ubiYlacZ} \) gene driven by the non-cell cycle-regulated \( \text{RP39} \) UAS. In this strain, Mcm1-Vp16 failed to increase reporter gene activity, confirming that \( \text{trans activation} \) requires an intact Mcm1 binding site.

Mcm1 recruits a ternary complex factor to the \( \text{CLB2} \) UAS in vitro. To determine if we could detect binding of Mcm1 to the cell cycle-regulated \( \text{CLB2} \) UAS from crude whole-cell extracts, we performed band shift experiments by using the wild-type \( \text{CLB2} \) UAS as a probe. In this assay, we detected several specific DNA-protein complexes (Fig. 7A), designated here 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 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 d, g, j, and m, and 7B, lanes 4 and 5). Hence, a strong correlation exists between the ability of the \( \text{CLB2} \) UAS to bind factors from crude cell extracts in vitro and its ability to function as a cell cycle-regulated UAS in vivo.

![Image of ternary complexes formation on the CLB2 UAS](http://mcb.asm.org/ on July 11, 2017 by guest)
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(318C)) 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 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 band shift 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 band shift 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 able to form a ternary complex with rMcm1-DNA complexes. This activity, which we refer to as TCFCLB2, 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). Addition of the wild-type SWI5 probe 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_{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_{CLB2}) identified here exhibits no autonomous DNA binding activity but is recruited into a ternary complex by DNA-bound Mcm1. Several pieces of evidence suggest 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 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 Go.** 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 *MATa* 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 α-specific genes, such as *STE6* (15). In *MATa* 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 *MATa* cells (in which α1 and α2 are absent) and *MATa* 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 SW5 regulation, SFF (18). Several lines of evidence suggest that CLB2 transcription and SW5 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 SW5 (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 SW5 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 complex transcription 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}–TAF complex. A possible mechanism for this could involve either a direct or indirect effect of Cib–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 Cib 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 that the ternary complex is required only for activated transcription of the SRF–elk1 protein, a yeast transcriptional activator, binds synergistically with a second protein to a set of cell-type specific repressor genes. Nature (London) 2113–2125.

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