Two promoter elements have been defined that activate G1/S-specific transcription in *Saccharomyces cerevisiae*. SCB elements (CAGCGTNA) are activated by the Swi4-Swi6 complex, and MCB elements (ACGCGTNA) are activated by the Mbp1-Swi6 complex. *CLN1* encodes a cyclin which is expressed during this interval, and requires Swi4 and Swi6 for peak transcription, but it has no consensus SCB elements in its promoter. Two SCB-like sequences had been previously noted and suggested to be the functional promoter elements. Our studies indicate that these sequences are unable to activate transcription of a *lacZ* reporter construct, or to bind Swi4-Swi6 complexes in vitro. However, a cluster of three sequences resembling MCB sequences are active promoter elements, sufficient to confer G1/S-specific transcription to a reporter. These sites are the predominant activation elements in the *CLN1* promoter, and despite their resemblance to MCB elements, they bind Swi4-Swi6 complexes in vitro and require Swi4 and Swi6 for their activity in vitro. This indicates that the sequences that promote Swi4/Swi6 binding have not been fully defined, or that there are multiple Swi4- and Swi6-containing complexes with distinct DNA binding specificities. In addition to these novel Swi4/Swi6-binding sites, these studies also show that there must be at least one novel promoter element that can confer G1/S-specific transcription to *CLN1*, because when all the potential SCB- and MCB-like sequences are eliminated the transcript is still cell cycle regulated.

Passage of cells through the cell cycle of *Saccharomyces cerevisiae* requires the activation of the Cdc28 kinase. Cdc28 is activated by association with different regulatory subunits, called cyclins. As the name implies, cyclins are unstable proteins whose levels vary within the cell cycle, primarily by the cell cycle regulation of transcription (1, 2). During late G1, there are two predominant cyclins (Cln1 and Cln2) which can form active complexes with Cdc28. The promoters of both of these genes show strong cell cycle regulation which is reflected both at the level of the protein and associated Cdc28 kinase activity (3, 4).

The timing of expression of *CLN1* and *CLN2* is similar to that of the *HO* endonuclease and many of the DNA synthesis genes of *S. cerevisiae*. Transcription of the *HO* endonuclease is absolutely dependent on the activity of two transcription factors, Swi4 and Swi6, which form a complex and bind to repeated sequence elements within the *HO* promoter known as Swi4/Swi6-regulated cell cycle boxes (SCBs) (5–7). Swi4 is the DNA-binding component of the complex (6, 8) and brings Swi6 to the DNA via association between their COOH termini (8–10). Many of the DNA synthesis genes are also transiently expressed in late G1, and their cell cycle regulated transcription is also dependent on Swi6 (11, 12). However, transcription of these genes seems to be mediated by an alternative DNA sequence element, the Mtu1 cell cycle box (MCB) (13, 14), which is bound by a complex of Swi6 (11, 12, 15) with a different DNA binding partner. Mbp1. MBP1 was identified through its homology to Swi4, and like Swi4, Mbp1 binds DNA through the amino-terminal region of the protein, and associates with Swi6 through its COOH terminus (16).

Deletion of *SWI4* and *SWI6* is lethal (17) and deletion of *SWI4* alone is lethal in some strains (18). To identify the essential transcriptional targets of the Swi4-Swi6 complex, high copy suppressors of this lethality were sought. *CLN1* and *CLN2* were identified in these screens (18, 19) and their transcription was shown to be largely dependent upon Swi4 and Swi6. Furthermore, complexes formed in vitro on the SCB sequences within the *HO* and *CLN* promoters include the Swi4 and Swi6 proteins (6, 18, 19).

Although *CLN1* has been considered to be coordinately regulated with *CLN2*, no direct analyses of the *CLN1* promoter have been performed. Within the *CLN1* promoter, there are two SCB-like sequences, CTCGAAA, which differ from the consensus SCB sequence at the second position, with T replacing A. Because of the similarity of the SCB-like sequence to the consensus, and because of the transcriptional induction of *CLN1* by the Swi4 and Swi6 factors, it has been widely thought that the *CLN1* SCB-like sequences are functional in binding Swi4/Swi6 and mediating cell cycle-regulated expression. However, mutational analysis has indicated that all the bases within the SCB element are critical for its activity (20). To clarify this issue, we have characterized the *CLN1* promoter and found that the SCB-like sequences play little or no role in transcriptional induction of *CLN1*. Instead there are three MCB-like sequences that are critical for activation and cell cycle regulation of this promoter. Furthermore, we find that Swi4 plays a more important role in binding and activating these MCB-like sites than does the “MCB-binding protein” Mbp1. In addition to the novel Swi4-Swi6 complexes that form on the *CLN1* promoter, our studies show that there is at least

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The abbreviations used are: SCB, Swi4/Swi6-regulated cell cycle box; MCB, Mtu1 cell cycle box; PCR, polymerase chain reaction; UAS, upstream activation sequence.
Cell Cycle Regulated Transcription of CLN1

one other cell cycle regulatory element which also confers G$_i$/S-specific transcription to the CLN1 promoter.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Conditions**—Strains BY 602 (MATa, ade2-1, trp1-1, can1-100, leu2-3, 112, his3-11,15, ura3, met, HO::lacZ46) and the related swi4 and swi6 deletion strains (BY606 and BY600) have been described previously (21), as have the mbp1 deletion strain K3294 and its isogenic wild type strain K1107 (16). All these strains were derived from W303-1a, but they are not isogenic with it. The BY1231 wild type is isogenic with W303-1a (MATa, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3). Yeast cultures were grown in YEP medium or YC minimal media supplemented with essential nutrients as required and containing 2% glucose (22). For cell cycle synchronization, cells were grown overnight in media lacking either uracil or tryptophan, transferred to rich media and grown to an A$_{600}$ of 0.2, before addition of 4–5 μg/ml α-factor. α-Factor arrests were monitored by phase microscopy. For measurement of transcript levels in logarithmically growing cells containing plasmids, the cells were grown under conditions that selected for the plasmid to an A$_{600}$ of about 0.15. Then the cells were transferred to rich media and allowed to double once before RNA was isolated.

**Clones and Constructs**—Region of the CLN1 promoter encompassing nucleotides -618 to -479 was amplified by PCR from YEp13 (23) with oligonucleotide primers BD1497 (GCCAGCCTGAGGCCTGGAGCAGAGAGCGGCGCTTTAAGGCTTCAAGGCTTCAATCTCC) and BD1507 (GCCAGCCTGAGGCCTGGAGGCGGCCACATTCCATCTTC). The -480 to -367 region of the CLN1 promoter was amplified from the same plasmid with BD1495 (GCCAGCCTGAGGCCTGGAGGCGGCCACATTCCATCTTC) and BD1496 (GCCAGCCTGAGGCCTGGAGGCGGCCACATTCCATCTTC). Both sets of primers incorporate XhoI and NotI restriction sites so that they could be subcloned into pBD1442, which is a derivative of pSH414 (24) with a NotI restriction site inserted downstream of the XhoI restriction site by introduction of the annealed linker oligonucleotides BD1392 (TCCGAAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
transcription, in that it induces a 70-fold increase in \( \text{lacZ} \) expression. The promoter containing the similar to the full-length promoter, we synchronized cells carrying the \( \text{CLN1} \) gene by the Swi4/Swi6 protein, known to activate transcription in this reporter context (5), it is likely that the T in the second position prevents recognition of the activator sequences in this region. Since SCB elements are known to activate transcription above background, this suggests that there are no upstream activation sequences in this region. Since SCB elements are known to activate transcription in this reporter context (5), it is likely that the T in the second position prevents recognition of these sites by the Swi4/Swi6 protein.

To see if either of these regions are upstream activation sequences (UAS) for \( \text{CLN1} \) transcription, we cloned these regions into a UAS-deficient promoter, which was fused to \( \text{lacZ} \) (pBD1442), and measured \( \text{lacZ} \) transcript levels. The level of \( \text{MATa1} \) transcript was also measured and served as an internal control for the amount of total RNA present in each sample. These measurements (see Table I) show that the SCB-like region of the promoter does not activate \( \text{lacZ} \) expression above background. This suggests that there are no upstream activation sequences in this region. Since SCB elements are known to activate transcription in this reporter context (5), it is likely that the T in the second position prevents recognition of these sites by the Swi4/Swi6 protein.

**Table I**

| Plasmid       | BY602 K1107 |
|---------------|-------------|
| pBD1442 UAS-less/lacZ | 0.19 0.30 |
| pBD1513 CLN1 SCB/lacZ   | 0.49 0.23 |
| pBD1512 CLN1 MCB/lacZ   | 14.38 21.5 |

...modest effect, together with the inability of the \( \text{CLN1} \) SCB-like region to significantly activate \( \text{lacZ} \) expression in the reporter construct, suggests that these SCB-like sequences are minor contributors to the transcriptional activation of \( \text{CLN1} \).

**Fig. 2. MCB core sequences are the predominant activation elements in the \( \text{CLN1} \) promoter.** Plasmids containing either the wild type \( \text{CLN1:HIS3} \) fusion (pBD1584), the mcb scb (pBD1632), or the mcb mutant derivative (pBD1586) of it were transformed into wild type BY1231 cells. Three independent transformants of each type were subjected to quantitative S1 protection to measure transcript levels. A single probe covering the \( \text{CLN1:HIS3} \) transcript protects the fusion transcript as well as the endogenous \( \text{CLN1} \) and \( \text{HIS3} \) transcripts, which resolved as indicated after electrophoresis. The radioactivity in each band was quantitated by PhosphorImaging. Background values were obtained from the gel section immediately above each band and subtracted. The \( \text{CLN1:HIS3} \) transcript levels were then normalized to endogenous \( \text{HIS3} \) mRNA and the three samples were averaged. This image, and all others in this article, were scanned from autoradiographs with a Sharp scanner JX-325 v2.0, into Adobe Photoshop where they were cropped and minimally adjusted for background uniformity. Pic files were then transferred to Canvas 3.0, labeled and printed on a Tektronix Phaser II SDX printer.

**Swi4 and Swi6 Are the Principle Activators of Transcription from the \( \text{MCB Region of the CLN1 Promoter} **— Our \textit{in vivo} results show that the MCB core sequences served as a major source of transcriptional activation of \( \text{CLN1} \). This result was unexpected because peak levels of \( \text{CLN1} \) transcription are dependent upon both Swi4 and Swi6 (18, 19), and these proteins have been characterized as activators of expression through SCB sequences (5). To see if Swi4 and Swi6 activate \( \text{CLN1} \) transcription through the MCB core sequences, we transformed \( \text{swi4} \) and \( \text{swi6} \) deletion strains with the \( \text{CLN1} \) MCB: \( \text{lacZ} \) reporter plasmid, and assayed \( \text{lacZ} \) transcription in asynchronous cell populations by \( S_2 \) nuclear protection as before (Table II). Transcription from the \( \text{CLN1} \) MCB: \( \text{lacZ} \) reporter dropped about 5-fold in the absence of either Swi4 or Swi6 activity, suggesting that these factors could be responsible for...
trans-activation via the MCB core sequences. In contrast, deletion of MBP1, the transcription factor thought to have MCB-binding specificity (16), caused less than a 2-fold reduction in lacZ transcription. These results indicate that Swi4 and Swi6 are the principle activators of transcription from the MCB region of CLN1 in vivo. Mbp1 participates to a lesser extent in this activation.

We then asked if Swi4 and Swi6 could bind to the CLN1 MCB elements directly by performing gel-retardation assays on whole cell extracts prepared from wild type and mutant yeast. To ensure that the extracts had the potential to form Swi4-Swi6 and Mbp1-Swi6 complexes, we first assayed complex formation on fragments from the HO and TMP1 promoters. HO was the first promoter in which SCB sequences were identified (30), and binding of Swi4/Swi6 to these sequences is well established (6, 7, 9). Consistent with previous studies, a low abundance high molecular weight complex (denoted by the arrow in Fig. 4A) was formed with wild type cell extracts on the HO promoter. This complex was absent from the swi4 or swi6 extracts, but was unaffected by mutation of MBP1. This confirms that our wild type extracts are competent to form SCB binding complexes. The lower complexes observed were comparable to those previously observed and were not specific for the SCB elements.

The TMP1 promoter contains MCB sequences which are critical for cell cycle-dependent transcription (12, 13), and activation of these MCB elements is Swi6- (11, 12) and Mbp1-dependent (16). Again, consistent with previous findings (16), a low abundance high apparent molecular weight complex was formed on the TMP1 MCB elements upon incubation with wild type extract that was not formed with the swi6 and mbp1 mutant cell extracts (Fig. 4B). This confirmed that the wild type extracts were competent to form standard MCB binding complexes. The MCB specificity of these binding complexes was verified by competition studies with wild type and mutant MCB sequences (data not shown). It is worth noting that complex formation is reduced in the swi4 strain. This could indicate that Swi4 also binds to the TMP1 MCB elements, however, Swi4 antibodies have no apparent effect on these binding complexes (see Fig. 5C).

Knowing that our extracts and conditions permitted formation of both SCB- and MCB binding complexes, we repeated the assay with the SCB-like region of CLN1. With the CLN1 SCB probe, no complexes were formed that were Swi4-, Swi6-, or Mbp1-dependent (Fig. 4C). In contrast, the CLN1 MCB probe formed high molecular weight complexes with proteins from wild type extracts (Fig. 4D) that resembled the HO and TMP1 specific complexes in mobility. These complexes did not form with swi6 and swi4 mutant extracts, but they were as abundant with the mbp1 mutant extract as they were with wild type extracts. This indicates that the CLN1 MCB binding complex is Swi4- and Swi6-dependent, but that Mbp1 is not required.

To address whether Swi4 and Swi6 are present in the CLN1 MCB binding complex, we used affinity purified antibodies raised against recombinant Swi4 and Swi6 proteins (9) in the gel retardation assays. Addition of the Swi4 antibody to the CLN1 MCB binding reaction (Fig. 5A) caused retardation of all of the upper complexes. In fact, the pattern of supershifting on CLN1 MCB was very similar to that produced on incubation of Swi4 antisera with the HO DNA binding reaction using the same wild type extracts (Fig. 5D) (9). This suggests a direct involvement of Swi4 protein in the CLN1 MCB binding complex. The Swi6 antibody also affected the CLN1 MCB complex in that it either shifted the complexes to a higher position in the gel or prevented binding altogether. These results also support a direct involvement of Swi6 in the CLN1 MCB binding complex. In fact, all of the specific CLN1 MCB binding complex is affected by both the Swi4 and Swi6 antibodies. This would not be expected if a significant fraction of the complexes included Mbp1 instead of Swi4 or if they lacked Swi6. Thus, both the in vitro and in vivo data indicate that Swi4-Swi6 complexes are the predominant trans-activators of the CLN1 MCB region.

The Swi4 and Mbp1 proteins are related (16), therefore it was possible that the Swi4 antisera could cross-react with Mbp1. To address this possibility, we incubated Swi4 antibody in a TMP1 binding reaction in which Mbp1-Swi6 is the predominant complex. In this case the Swi4 antibodies had no impact on the mobility or the abundance of the TMP1 binding complexes (see Fig. 5C).
complex (Fig. 5C). The Swi4 antibody does not cross-react with Mbp1 under the conditions of the gel retardation assay, so it must be detecting only Swi4 in the CLN1 MCB binding complex.

Finally, we wanted to confirm that the MCB core sequences were responsible for the Swi4-Swi6 complex formation observed with the CLN1 MCB probe. To do this, we performed competition experiments using either the CLN1 MCB(2) probe (see Fig. 1) which includes the first two CGCG core sequences, or the analogous but mutated competitor (CLN1 mcb(2)), in which both CGCG cores of the elements are mutated to CTCT. Titration of the wild type competitor into the binding reaction caused a loss of complex formation, whereas even high levels of the mutant competitor failed to compete for binding to CLN1 MCB (Fig. 6). Interestingly, a single SCB element from the HO promoter could also compete for complex formation, whereas the homologous sequence with a mutated scb element failed to compete even at high concentrations. These experiments show that the CGCG core sequences are important for complex formation with the CLN1 MCB probe, but that binding of Swi4-Swi6 to the CLN1 MCB probe can also be competed by an SCB consensus binding sequence (CACGAAA).

DISCUSSION

We have shown that the MCB core sequences are the predominant transcription activation elements in the CLN1 promoter during logarithmic growth. The SCB-like sequences in this promoter contribute much less to CLN1 activation. They are unable to activate reporter gene constructs, and they do not serve as Swi4-Swi6-binding sites in vitro. These SCB-like sequences differ from the known SCB sites at the second position, with CTCGAAA instead of the consensus CACGAAA. An A to C transversion at this position causes a 5-fold drop in UAS activity (20), so it is not surprising that a Ti s also deleterious at the second position.

One of the surprising results of these studies is that transcriptional activation of CLN1 is, in large part, derived from MCB-like sequences which are activated by Swi4-Swi6 complexes. Antibodies to Swi4 react with and supershift all the specific protein complexes formed on the CLN1 MCB core sequences, indicating that Swi4 is present in all of these complexes. This is not due to cross-reactivity of the Swi4 antibodies with Mbp1 (Fig. 6), nor is it due to the absence of Mbp1 from the whole cell extracts because the Mbp1-dependent band shift
on TMP1 readily forms under these conditions (Fig. 5B). Mbp1 simply is not a detectable component of the CLN1 MCB complexes that form in vitro. Why, then, is the UAS activity of the CLN1 MCB region reduced at all in an mbp1 mutant strain? Two explanations seem plausible. Mbp1 may participate directly in a subset of the DNA binding complexes instead of Swi4, but our in vitro conditions may not favor its detection. Alternatively, the 2-fold drop in CLN1 MCB transcription that we observe in mbp1 strains may be an indirect effect. Swi4 has three MCB-like elements in its promoter that are critical for full SWI4 transcription (24). Thus, the loss of Mbp1 activity could reduce SWI4 transcription or affect its timing and that could indirectly cause the drop in CLN1 MCB transcription. Whether Mbp1 participates directly or indirectly, it is clear that it plays a minor role compared with Swi4 in these complexes. Swi4-Swi6 complexes form on the MCB core sequences and are the predominant trans-activators of the CLN1 gene.

This is the first clear demonstration that Swi4-Swi6 complexes can activate transcription through MCB-like elements in vivo, but there is other evidence that supports this idea. Cross-competition between SCB and MCB binding complexes has been demonstrated in vitro, but this requires 50-fold molar excess of competitor (12). In addition, in vitro translated fragments of Swi4 can bind to MCB sequences, and fragments of Mbp1 can bind to SCB sequences (16). Interpretation of these experiments is complicated by the fact that only fragments of the Swi4 and Mbp1 proteins were used, and they were present at far higher concentrations than the wild-type proteins. Thus, their binding could be an artifact of protein concentration or conformational differences. These fragments are not competent to bind Swi6, so they certainly do not reflect the binding properties of the native complex. Swi4 overproduction in vivo promotes Swi6-independent transcription at SCB (17) and MCB sites (31), but this activity depends upon overproduction of Swi4, and the vast majority of the Swi4 present in such cells is also fragmented (9). With wild-type levels of Swi4, both binding and activation of SCB elements absolutely depends upon Swi6 activity (5, 32). So, while these studies are useful for defining what the DNA binding fragment of Swi4 is capable of interacting with, the physiological significance of this interaction could not be assessed until such an activity was demonstrated in vivo with wild-type cells. This study shows that the Swi4-Swi6-binding sites in the CLN1 promoter are different and more MCB-like than the ones that have been identified in the CLN2 and HO promoters. This raises the possibility that the complexes themselves may be fundamentally different, and that the Cln1 and Cln2 cyclins may be differentially regulated.

The only other known promoter at which functional overlap between Swi4 and Mbp1 may exist is the CLB5 promoter. CLB5 is also a cyclin, whose transcription peaks at the same time as SCB- and MCB-regulated genes. Interestingly, the CLB5 promoter contains five MCB core sequences (CGCG), but it has no perfect matches to either the MCB or SCB consensus (33). It has been suggested that these sites may be activated by both Swi4- and Mbp1-containing complexes because CLB5 transcription is cell cycle-regulated in both swi4 and mbp1 mutants (16, 33). However, the critical cell cycle regulatory elements in the CLB5 promoter that have not been identified, so it is also possible that these CGCG sequences play no role in CLB5 transcription at all. If they do, they may be conventional Mbp1-Swi6-binding sites and the residual cell cycle regulation of CLB5 that is observed in mbp1 mutants may be due to a novel cell cycle regulatory element that has not yet been identified. The existence of novel cell cycle-specific regulatory elements has been demonstrated in all three of the G1-specific promoters that have been carefully analyzed (SWI4 (24), CLN2 (34, 35), and CLN1 (Fig. 3)). However, since activation at CGCG sequences occurs principally via Swi4-Swi6 complexes at the CLN1 promoter, it will be interesting to determine if CLB5 is regulated in a similar fashion, or if the CGCG sites in its promoter represent a fourth type of site at which binding by both Swi4-Swi6 and Mbp1-Swi6 complexes is equally likely.

The SCB binding complex, which includes Swi4 and Swi6, has been referred to as SBF, and the MCB binding complex, including Mbp1 and Swi6, is commonly called MBF (12, 36). Our data indicates the existence of a third type of complex, where Swi4 and Swi6 bind to CGCG sequences. This finding makes it clear that the binding site specificity of Swi4-Swi6 complexes is not restricted to SCB sequences as they are currently perceived, so it may be premature to apply one name to all of them. The lack of a clear consensus sequence for Swi4 and Mbp1-mediated transcription also makes it impossible to deduce by inspection the elements or factors required for the expression of any given promoter.

There are several possible hypotheses that could account for the altered binding specificity of Swi4 in the CLN1 MCB complexes. One possibility is that sequences adjacent to the CGCG sequences directly specify the binding of Swi4—rather than Mbp1-containing complexes. Alternatively, an accessory factor may bind to an adjacent site on the DNA that promotes this binding. A third possibility is that another protein binds directly to or modifies a subset of the Swi4-Swi6 complexes.
specificity of the Oct1 transcription factor is altered by the presence of the Herpes viral tegument protein, VP16, from ATGCAAT to TAATGARAAT (38). We cannot rule out any of these possibilities at the moment. Determining the binding site specificity of Swi4-containing complexes by site selection (39) or by mutational analysis (20) is difficult because a single SCB element does not form a stable band shift complex2 or activate sufficient transcription to be reliably measured (5). The involvement of other proteins also cannot be addressed easily because full-length Swi4 cannot be efficiently produced in the expression systems that have been tested or by in vitro translation (8, 10). However, resolving these issues will be important for understanding the large number of G1/S-specific transcription factors.

The other surprising result of this study is that the CGCG promoter which activates G1/S-specific transcription. Elimination of these sequences as well as the SCB-like sequences did not eliminate the cell cycle regulation of the CLN1 promoter. In fact, the timing of the residual cell cycle regulated transcription from the mutant promoter is similar to the timing of the expression from the wild type promoter. There are no other obvious cell cycle regulatory elements within this promoter, so there must be another unrecognized cell cycle regulatory element within the G1/S-specific CCGG element. Redundancy is a commonly employed strategy for regulating cell cycle-specific events. Cyclin-CDK complexes are typically regulated at the level of transcription, post-translational phosphorylation, and ubiquination, and by the binding of inhibitors which are in turn highly regulated (40). Here is another example, where even the transcriptional control exerted on a cyclin is redundant. It will be interesting to determine how their mechanisms of activation are equivalent, or whether they respond to different regulatory signals. The latter would afford more protection from deregulation, and more capacity to respond to different environmental cues.

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