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Multiple SWI6-Dependent *cis*-Acting Elements Control *SWI4* Transcription through the Cell Cycle

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The *Saccharomyces cerevisiae SWI4* gene encodes an essential transcription factor which controls gene expression at the G_1/S transition of the cell cycle. *SWI4* transcription itself is cell cycle regulated, and this periodicity is crucial for the normal cell cycle regulation of *HO* and at least two of the G_1 cyclins. Since the regulation of *SWI4* is required for normal cell cycle progression, we have characterized *cis*- and *trans*-acting regulators of *SWI4* transcription. Deletion analysis of the *SWI4* promoter has defined a 140-bp region which is absolutely required for transcription and can function as a cell cycle-regulated upstream activating sequence (UAS). The *SWI4* UAS contains three potential *Mlu*I cell cycle boxes (MCBs), which are known cell cycle-regulated promoter elements. Deletion of all three MCBs in the *SWI4* UAS decreases the level of *SWI4* mRNA 10-fold in asynchronous cultures but does not abolish periodicity. These data suggest that MCBs are involved in *SWI4* UAS activity, but at least one other periodically regulated element must be present. Since SWI6 is known to bind to MCBs and regulate their activity, the role of SWI6 in *SWI4* expression was analyzed. Although the MCBs cannot account for the full cell cycle regulation of *SWI4*, mutations in *SWI6* eliminate the normal periodicity of *SWI4* transcription. This suggests that the novel cell cycle-regulated element within the *SWI4* promoter is also SWI6 dependent. The constitutive transcription of *SWI4* in *swi6* mutant cells occurs at an intermediate level, which indicates that SWI6 is required for the full activation and repression of *SWI4* transcription through the cell cycle. It also suggests that there is another pathway which can activate *SWI4* transcription in the absence of SWI6. The second activator may also target MCB elements, since SWI4 transcription drops dramatically when they are deleted.

The *Saccharomyces cerevisiae SWI4* gene encodes an essential transcription factor which is required for the expression of the *HO* endonuclease and a subset of the G_1 cyclins (6, 23, 24). The *SWI* genes were initially identified as regulators of *HO* transcription (6, 12, 29). SWI4 and SWI6 control the periodic expression of *HO* and are specifically required for the activity of the SWI4/SWI6-dependent cell cycle box (SCB), an 8-bp ($CACGA_4$) sequence which is repeated 10 times in the *HO* promoter (6, 22). The SCB is sufficient to confer cell cycle-regulated, START-dependent transcription on heterologous genes (6). SWI4 and SWI6 associate with each other in vitro (3a, 25) and in vivo (27) and bind to the SCB sequence (2, 3, 23, 24). SCB-related elements have also been found in the promoters of the *CLN1*, *CLN2*, and *HCS26* G_1 cyclin genes (23, 24). Maximal expression of these genes is *SWI4* dependent, and SWI4/SWI6 protein complexes have been detected on the SCB-related sequences in *CLN2* and *HCS26* promoter fragments in vitro (23, 24). Thus, *HO* and the periodically expressed G_1 cyclins are probably regulated by related mechanisms.

The SWI6 protein also functions in the regulation of a second class of genes which are maximally expressed at the G_1/S boundary. The periodic expression of the DNA synthesis genes is controlled by the *Mlu*I cell cycle box (MCB; ACGCGTNA), another promoter element which is both necessary and sufficient for cell cycle-regulated transcription (1, 10, 17). MCB elements have also been found in the *CLB5* promoter, which is a B-type cyclin that is also transcribed specifically at the G_1/S boundary (9a). Functional SWI6 is required for the periodic activity of the MCB, and SWI6 is a component of the DNA-binding complex that forms on the MCB (9, 15). Thus, SWI6 is involved in two distinct control mechanisms which direct gene expression at the G_1/S transition.

Cell cycle regulation of *HO* transcription is due in part to the fact that *SWI4* mRNA levels are also regulated through the cell cycle. *SWI4* mRNA is maximally expressed in late G_1, and peak accumulation of *SWI4* mRNA immediately precedes the peak of *HO* transcription (5). When *SWI4* is expressed constitutively through the cell cycle, the periodicity of *HO* transcription is largely lost, indicating that the cell cycle regulation of *HO* is a direct consequence of the cell cycle regulation of *SWI4* (5). SWI4 overproduction also deregulates the normal cell cycle regulation of *CLN1* and *CLN2* expression (4a). Thus, the regulated expression of *SWI4* is crucial for the regulated expression of at least three genes whose products act at the start of the cell cycle.

In this work, we have analyzed the regulation of *SWI4* and have shown that the periodic transcription of *SWI4* results from control of transcription initiation. We have identified a 140-bp upstream activating sequence (UAS) in the *SWI4* promoter which is essential for *SWI4* transcription and which contains all of the information necessary for the cell cycle regulation of *SWI4*. The *SWI4* UAS contains three MCB elements. Deletion of all three of these sequences dramatically reduces UAS function but does not eliminate the cell cycle-regulated UAS activity. These data suggest that an unknown target of cell cycle regulation, distinct from the MCBs (and SCBs), resides within the 140-bp UAS and contributes to the periodic expression of *SWI4*. The cell cycle regulation of *SWI4* is virtually eliminated by *swi6* mutations, and the constitutive *SWI4* transcription that results is at a level intermediate to that observed during the cell cycle in wild-type cells. This indicates that SWI6 is required to maintain both the repressed and activated state

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of the *SWI4* UAS. It also shows that there is a SWI6-independent pathway for activating *SWI4* transcription. This second activator may also bind to MCB elements, since their deletion lowers SWI4 transcription at least 10-fold.

MATERIALS AND METHODS

Strains and plasmids. Strain W303 (*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3,115 his3-11 ura3 ho*) was used as the wild-type strain in all experiments. BY1232 is isogenic to W303 and carries a disruption allele of *SWI6* in which the 1.4-kb *Xho*I-*Acc*I fragment of the *SWI6* open reading frame (7) has been replaced by the 1.6-kb *Xho*I-*Acc*I fragment from the *LEU2* gene (23a).

Plasmid p*SWI4*ΔL:*HIS3* was generated by site-directed mutagenesis of a pRS314 (28)-derived plasmid which carried 2.0 kb of sequences upstream of the *SWI4* translation initiation codon joined in frame to the complete *HIS3* open reading frame. To link the *HIS3* protein-coding sequence directly to the *SWI4* transcription initiation site, the 300-bp *SWI4* untranslated leader was removed by oligonucleotide-directed mutagenesis. Plasmids used in the construction of exonuclease III deletions carried a 2.3-kb *Nde*I-*Bam*HI fragment spanning the *SWI4* upstream regulatory region and 300 nucleotides of the open reading frame inserted into either pRS314 or pRS316 (28) to generate plasmids Bd909 and Bd1007, respectively. To remove the *Mlu*I site at nucleotide 561 in the *SWI4* promoter, Bd909 (wild type [WT]) was digested with *Mlu*I, treated with S1 nuclease and Klenow fragment, and recircularized. The resulting plasmid (Bd974; Δ*Mlu*I-1) was subjected to site-directed mutagenesis to remove the CGCG sequences at nucleotides 548 and 579 and generate plasmid Bd998 (Δ*Mlu*I-3). The plasmid p*GAL*:*SWI4* has been described elsewhere (5). The p*GAL*:*SWI4*(*HIS3*) plasmid is a derivative of p*GAL*:*SWI4* which carries the *HIS3* selectable marker in place of *URA3*.

Growth of yeast cells. The growth media and conditions used have been described before (5). In the synchrony experiments depicted in Fig. 5 and 6, cells carrying plasmids were grown to early log phase in selective minimal medium containing 2% raffinose. The cultures were transferred to YEP plus 2% raffinose, grown to an optical density at 660 nm of 0.2, and then shifted to YEP plus 2% galactose. After 30 min, α-factor (4 μg/ml) was added to the culture. When arrest was complete, the cells were collected by filtration and resuspended in fresh YEP plus 2% galactose medium to release them from the α-factor block.

S1 nuclease protection. The analysis of transcript levels by S1 nuclease protection has been described before (21). The *MAT***a***1*, *SIR3*, *lacZ*, *HIS3*, *H2B*(*HTB1*), and *SWI4* probes have all been described previously (5, 6, 18, 19). The *SWI4*:*HIS3* probe used in Fig. 2B was derived from an M13mp18 clone carrying the *SWI4*:*HIS3* fusion from p*SWI4*ΔL:*HIS3* on a *Sal*I-*Pst*I fragment. The *SWI4* probe used in Fig. 3 included the 4-kb *Nde*I-*Xba*I *SWI4* fragment inserted into M13mp19. The *CDC9* probe was provided by D. Barker and L. Johnston. It was derived from an M13mp11 clone carrying the 800-bp *Nde*I fragment from *CDC9* (4). The S1 protection data in Fig. 3 were quantitated by video densitometry with the Bioimage, Inc., Visage 2000 system. All other quantitation was carried out with a PhosphorImager 400A (Molecular Dynamics Corp., Sunnyvale, Calif.).

***SWI4* promoter sequence.** Both strands of a 1.7-kb *Hin*dIII fragment containing the *SWI4* promoter and transcription start site were sequenced with chemically synthesized oligonucleotides as primers by the dideoxynucleotide sequencing method (26). The GenBank accession number for this sequence is M97918.

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GGAATTTGCCTTTTTCATAGTACAGGAAACTGGCGGTAAAGGAAAAGTAG          50
TACTTTAAACACATTTAAAATTACTTATCACTATGAATATCTTTAAATTA          100
                    1L
TTGTTATACATAATAAACTACCTATTAAAAAAAAGATGCTACCCAATTCA          150
                 2L
CGTTGTCCATGGTTATTTTAGGGCCTAAAAATAAAAGGCAGTAGGTTTAG          200
                      3L    1R
AGGGACTTCAGTACAAGTAGATAACGAACAAAGCAATTACTCTGTGTACG          250
                      2R
GTAGCGGAAAAAATGAAATGTTACCTAAGAAGGAGGTAATATAAGTACAT          300
TACATTTCAACCGCATTGTGATTGCGTACTTTAAACGATAGGTGACTGCG          350
                                                 3R
GATCTTGGGTCGTTTAGCAGCATACGAAAATTAGTTTTACTAAACTAGAC          400
                   4L
ATCTAGTAAAAGGCTATCGAAATCTCTCAATTTAAAAAGAATCTGCTACT          450
                                        4R
GACTATAAGTTTTGTCACTACAAATTATTGGTCTAATCTAGCCTTCATTA          500
                      5L                6L
TGGATACAATTACCTTCGGCGGCTAAAAACAACTTGGTTTTGTTACCCGC          550
GTTTGAAGTGACGCGTCACTAACCATGACGCGAAAGTTATTATATTCAGC          600
                                        5R
TCCCCGCCTGCTTATTTCCCGTTTAGGAAATAAATGGTTAACCTAACAAA          650
  6R  7L
AAAAAAAGAGGAACAAGGACTAAGAAGCACGTCAACGTGAAAAACGCACA          700
                                          8L
AAAGATATTATAGCTATTGCCATCCTTTAGCATTGGTTTAGCATATCAAC          750
                                             7R, 8R
TAATTGACTAATATTTGCTGCTAAATTCAACTGGAAGCTTTTTTTTTTTT          800
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FIG. 1. Sequence of the *SWI4* promoter. An 800-bp region of the *SWI4* promoter is shown. The arrow at position 753 marks the major transcription initiation site. The start of translation is at nucleotide 1068. The endpoints of the internal *SWI4* promoter deletions, numbered as in Fig. 3, are indicated (L = left endpoint; R = right endpoint). The *Mlu*I site at position 561 is underlined, as are the CGCG core sequences at nucleotides 548 and 579. The solid arrowheads designate the 140-bp *SWI4* promoter fragment analyzed in Fig. 4.

Construction of exonuclease III deletions. Deletions from either the 5′ or 3′ end of the *SWI4* promoter were generated in Bd909 and Bd1007, respectively. The *Xho*I site present in Bd1007 was removed by digestion with *Sal*I and *Xho*I and subsequent religation. The plasmids were digested to completion with either *Kpn*I and *Sal*I (Bd909) or *Bst*XI and *Bam*HI (Bd1007). Exonuclease III digestion of the linearized plasmids was carried out as described before (13). Phosphorylated *Xho*I linkers (New England Biolabs) were ligated to the exonuclease III-treated DNA prior to recircularization. The endpoint of each deletion was determined by DNA sequencing with Sequenase (United States Biochemical Corporation). Internal deletions of the *SWI4* promoter were generated by combining the appropriate *Xho*I-*Nae*I fragments and confirmed by DNA sequencing.

PCR amplification. Nucleotides 513 to 653 from the *SWI4* promoter region present in Bd909 (WT), Bd974 (Δ*Mlu*I-1), or Bd998 (Δ*Mlu*I-3) were amplified by the polymerase chain reaction (PCR) (20) with the primers 5′-GCCAGCTCGAGC CTTCGGCGGCTAA-3′ and 5′-GCCAGCTCGAGTTTTTT GTTAGGTT-3′. The resulting PCR products were cloned into the *Xho*I site of pSH144 to generate plasmids pWT:*lacZ*, pΔ*Mlu*I-1:*lacZ*, and pΔ*Mlu*I-3:*lacZ*, respectively. Plasmid pSH144 is a derivative of pLGΔ178 (12) in which a 30-bp *Xho*I-*Hae*III fragment has been deleted (12a).

RESULTS

***SWI4* expression is controlled at the level of transcription initiation.** To begin an analysis of *SWI4* regulation, we sequenced the *SWI4* promoter and mapped the transcription initiation site (Fig. 1). The *SWI4* translation initiation codon

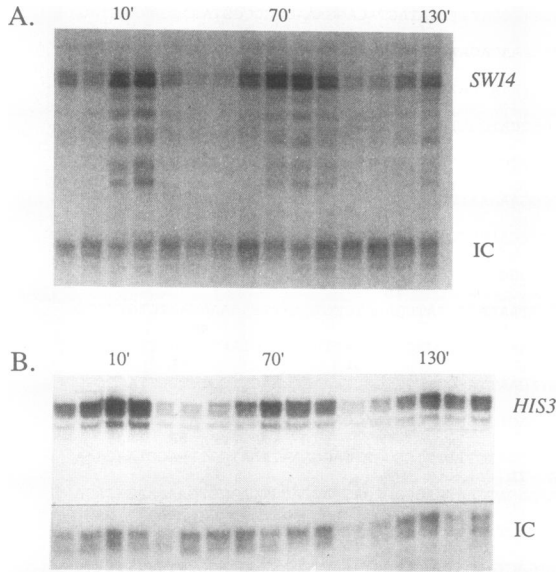

FIG. 2. *SWI4* expression is controlled at the level of transcription initiation. The transcription of endogenous *SWI4* mRNA (A) in wild-type cells (5) and of the plasmid-derived *HIS3* mRNA (B) in p*SWI4*ΔL:*HIS3*-transformed wild-type cells was analyzed by S1 nuclease protection. Transcript levels were measured over at least two synchronous cell cycles following release from α-factor arrest. In both panels and in all subsequent synchrony experiments, the first lane is the sample from α-factor-arrested cells. The subsequent lanes represent samples isolated at 5 and 10 min and then at 10-min intervals after release. The times of peak accumulation of *SWI4* and *HIS3* mRNAs in each cell cycle following release are indicated. In panel A, *SIR3* mRNA was monitored as an internal control (IC) to measure the amount of RNA analyzed in each sample. The internal control in panel B is the protected 310-bp *MAT***a***1* mRNA (18).

is at nucleotide 1068. The arrow at position 753 indicates the major transcription initiation start site as determined by S1 nuclease mapping of the *SWI4* mRNA 5′ end (data not shown). This initiation site produces a 315-bp untranslated leader sequence which contains no ATG codons.

To determine whether the cell cycle regulation of *SWI4* is conferred by the promoter, 1.7 kb of upstream sequence was fused at the transcription initiation site to the *HIS3* open reading frame. A centromere vector carrying the *SWI4*:*HIS3* fusion (p*SWI4*ΔL:*HIS3*) was transformed into a wild-type strain. Exponentially growing cells were synchronized in the G_1 phase of the cell cycle with the mating pheromone α-factor. Samples were taken at specific time points after release from the arrest, and the level of *HIS3* mRNA present in each sample was analyzed by S1 nuclease protection. The level of *MAT***a***1* mRNA was also measured and served as an internal control for the amount of RNA present in each sample. The results of that experiment are shown in Fig. 2B. The periodic fluctuation of the plasmid-derived *HIS3* mRNA was comparable to the fluctuation of the endogenous *SWI4* gene (Fig. 2A) (5) through three synchronous cell cycles. In both cases, peak mRNA levels were observed at 10, 70, and 130 min after release from α-factor, and there was an approximate 10-fold fluctuation between peak (70 min) and trough (40 min) levels. From this, it is clear that the *SWI4* promoter is sufficient to confer cell cycle regulation on the *HIS3* mRNA.

Identification of the *SWI4* UAS. To identify *cis*-acting sequences required for the periodic transcription of *SWI4*, we generated a series of deletions in the *SWI4* promoter (see Materials and Methods). The level of *SWI4* mRNA produced from each deletion during exponential growth was quantitated and compared directly with the level produced from the wild-type promoter (Fig. 3). The first set of deletions analyzed produced essentially wild-type levels of plasmid-derived *SWI4* mRNA until sequences 3′ to nucleotide 488 were deleted from the *SWI4* promoter. This finding was confirmed by analysis of *SWI4* mRNA produced from small internal deletions constructed across the *SWI4* promoter. A normal level of plasmid-derived *SWI4* transcript was maintained until either nucleotides 523 to 640 or nucleotides 540 to 653 were deleted from the promoter. Although the deletion of nucleotides 657 to 792 and 741 to 792 eliminated production of the normal *SWI4* transcript, both promoters were active and directed significant levels of transcription from alternative start sites. This was not observed with the plasmids carrying deletions of nucleotides 523 to 640 and 540 to 653. The results obtained with these two deletions show that nucleotides 523 to 653 are required for the full transcription of *SWI4*.

To determine whether this region of the *SWI4* promoter contains a UAS which is sufficient for activating transcription, we cloned nucleotides 513 to 653 into a vector carrying a UAS-deficient *CYC1* promoter linked to *lacZ*. Both β-galactosidase activity assays (data not shown) and quantitation of *lacZ* mRNA levels by S1 nuclease protection (Table 1) indicate that this region contains a UAS which is approximately 25% as active as the *CYC1* UAS.

Role of the MCBs. The MCB (ACGCGTNA) is located in the promoters of many yeast genes involved in DNA synthesis which, like *SWI4*, are both cell cycle regulated and maximally expressed at the G_1/S boundary of the cell cycle (1). In the promoters of these genes, the MCB elements are often clustered and positioned within 200 nucleotides of the translation start site. The MCB was defined as being both necessary and sufficient for the periodic expression of the DNA synthesis genes (10, 17), and point mutagenesis studies determined that the CGCG core is critical for full UAS activity of the MCB element (17). The essential UAS that we have defined within the *SWI4* promoter contains one perfect MCB (ACGCGTCA) at position 561 and two CGCG core sequences. These are positioned between 174 and 205 bp upstream from the transcription start site. To determine whether these sequence elements are important in *SWI4* transcription, we precisely deleted either the central MCB or all three elements from either the *SWI4* promoter or the *SWI4* UAS:*lacZ* construct (pWT:*lacZ*). The transcriptional activity of each deletion mutant was compared directly with that of the wild type, and the results of that analysis are shown in Table 1.

Deletion of the central MCB (Δ*Mlu*I-1) had no significant effect on UAS function, whereas deletion of all three elements (Δ*Mlu*I-3) resulted in an approximately 10-fold drop in UAS activity. This clearly indicates that at least one of the flanking CGCG elements in the *SWI4* UAS is an activator of transcription. The equivalent MCB deletions were also generated in the full-length *SWI4* promoter, where they had a quantitatively similar effect on *SWI4* transcription (Table 1). Again, deletion of the central MCB had no effect, whereas loss of all three MCBs reduced promoter activity 10-fold. These data show that the MCB elements provide most of the UAS activity resident in the full-length *SWI4* promoter.

We then asked whether the 140-bp essential UAS from the *SWI4* promoter could confer cell cycle regulation on the *lacZ* transcript in wild-type cells. Figure 4A shows that the *SWI4*

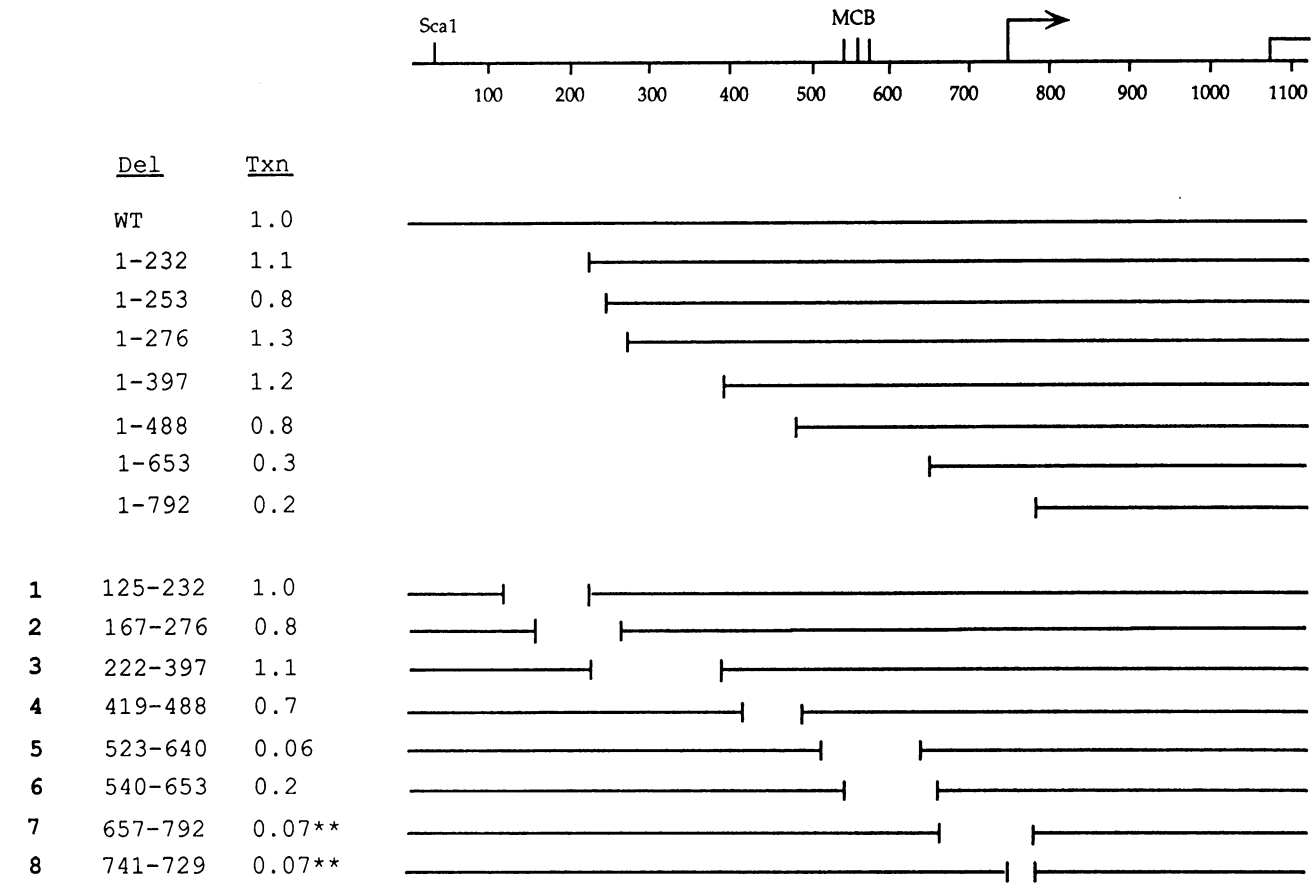

FIG. 3. Deletion analysis of the *SWI4* promoter. The positions of the MCBs in the *SWI4* promoter relative to the transcription initiation site (arrow) are shown. The *ScaI* site is at nucleotide 59 (see Fig. 1). The specific nucleotides deleted in each mutant promoter are indicated. Each deletion was carried on a centromere plasmid and transformed into wild-type cells. The level of *SWI4* mRNA produced from each deleted promoter in asynchronously growing cells was measured by S1 analysis and quantitated as described in Materials and Methods. The level is expressed relative to the amount of *SWI4* transcript produced from a plasmid-borne wild-type (WT) *SWI4* promoter in the Txn column. Deletions designated by ** directed transcription from alternative initiation sites.

UAS is clearly cell cycle regulated. The level of *lacZ* transcription driven by the 140-bp *SWI4* UAS fluctuated 10- to 20-fold over two complete cell cycles. Thus, nucleotides 513 to 653 of the *SWI4* promoter are not only an essential UAS but also contain the information required for the cell cycle regulation of *SWI4*.

TABLE 1. Transcriptional activity of wild-type and MCB-deleted variants of the *SWI4* promoter[a]

Plasmid	pUAS:*lacZ*	YCp*SWI4*
No UAS	0.03	0.09
WT *SWI4*	1.0	1.0
Δ*Mlu*I-1	0.89	1.2
Δ*Mlu*I-3	0.06	0.14

[a] Transcript levels were measured by S1 nuclease protection, quantitated, and normalized to the *HIS3* (central column) or *SIR3* (right column) mRNA level. The pUAS:*lacZ* column shows the relative *lacZ* mRNA levels in asynchronously growing wild-type cells transformed with either a UAS-deficient plasmid (pSH144) or the *SWI4:lacZ* fusion plasmid with the wild-type (WT) or MCB-deleted variants (Δ*Mlu*I-1 and Δ*Mlu*I-3) of the *SWI4* UAS inserted at the *Xho*I site of pSH144. The YCp*SWI4* column shows the relative levels of a truncated *SWI4* message produced by asynchronous wild-type cells transformed with a CEN plasmid carrying nucleotides −1973 to +334 from the ATG of the *SWI4* gene (WT *SWI4*) or equivalent plasmids lacking the entire *SWI4* UAS (deletion 5; see Fig. 3), the central MCB (Δ*Mlu*I-1), or all three MCBs (Δ*Mlu*I-3). The truncated *SWI4* mRNA produced from these plasmids can be distinguished from the genomic *SWI4* transcript by size.

The MCBs contained within this 140-bp region were the most likely source of the periodic activity. To determine whether the MCBs were responsible for the cell cycle-regulated UAS activity, we monitored the residual transcription from pΔ*Mlu*I-3:*lacZ* throughout the cell cycle (Fig. 4B). The level of *lacZ* mRNA produced from the MCB-deficient UAS was decreased overall, as expected, and peaked slightly earlier, but still fluctuated approximately eightfold through two complete cell cycles. These data are compared in Fig. 4C. Both the WT and Δ*Mlu*I-3 versions of the *SWI4* UAS direct periodic transcription of *lacZ*. These data suggest that another target of cell cycle regulation, distinct from the MCBs, is contained within the *SWI4* 140-bp UAS.

SWI6 is required for the cell cycle regulation of *SWI4* transcription. SWI6 is a required component of the DNA-binding complex on MCB elements (9, 14). In addition, mutations in *SWI6* can lead to reduced UAS activity of MCB-containing sequences and interfere with their cell cycle regulation (9, 14). To determine whether mutations in *SWI6* affect the periodic transcription of *SWI4*, we monitored the activity of both the full-length *SWI4* promoter and the 140-bp *SWI4* UAS (pWT:*lacZ*) in isogenic *SWI6* and *swi6*

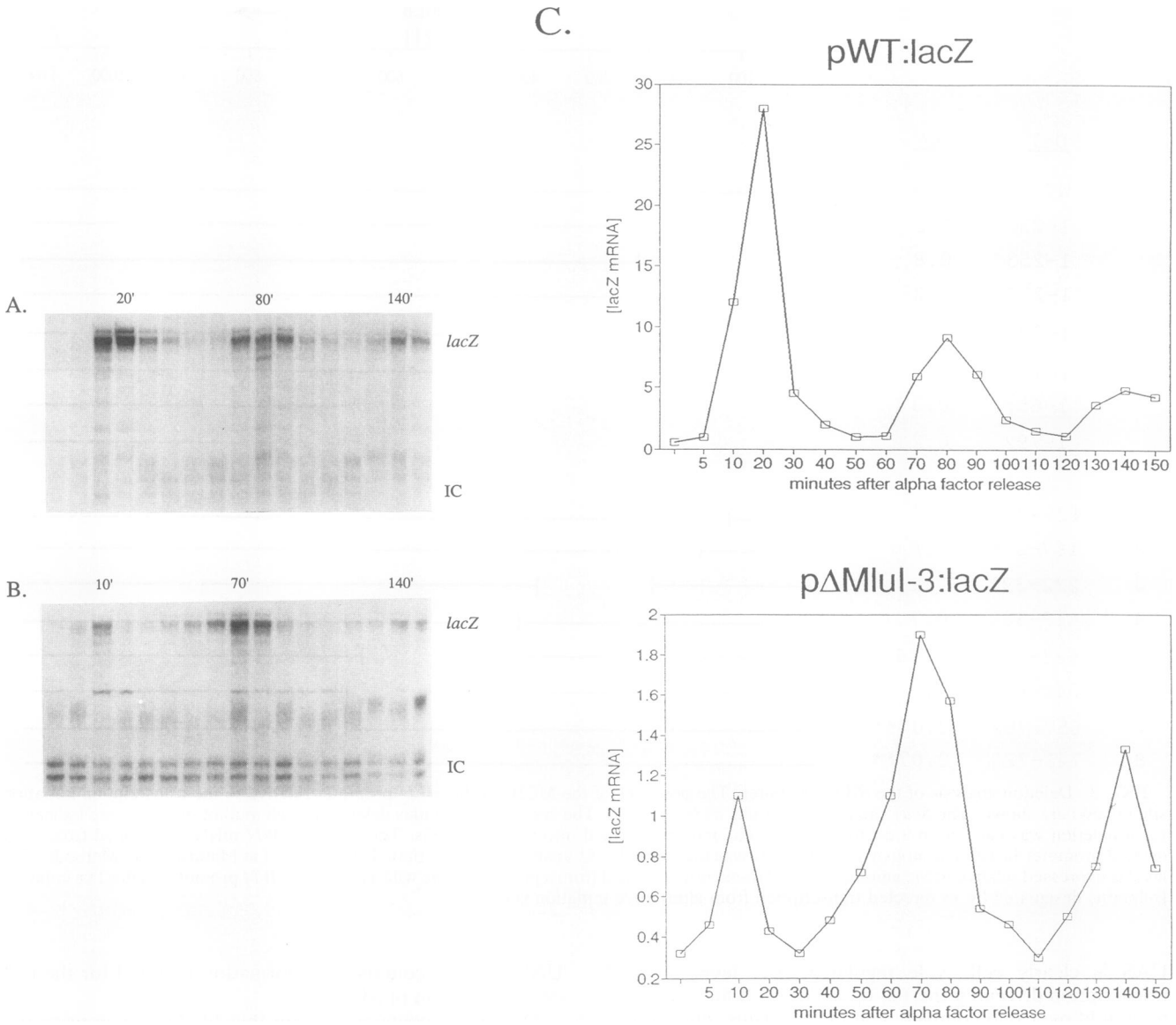

FIG. 4. The MCBs account for only part of the cell cycle-regulated activity of the *SWI4* UAS. The level of *lacZ* mRNA in wild-type cells carrying the pWT:*lacZ* (A) or p*Δ*MluI-3:*lacZ* (B) plasmid was measured over two synchronous cell cycles by S1 nuclease protection. The internal control in both experiments is *HIS3*. (C) Quantitation of *lacZ* levels normalized to that in the *HIS3* control. These experiments were carried out in parallel with the same labeled probes so that RNA levels could be compared directly.

strains. The activities of the full-length promoter and the 140-bp UAS were essentially equivalent in asynchronously growing cultures of wild-type and *swi6* mutant strains (0.84 versus 1.0 for the full promoter). The fact that the average level of *SWI4* transcription is not reduced in *swi6* mutant cells indicates that SWI6 is not the only positive regulator of *SWI4* transcription.

To determine whether SWI6 has a role in the cell cycle regulation of *SWI4*, we had to overcome the technical difficulties of synchronizing *swi6* mutant cells. Loss of *SWI6* function severely impairs recovery from the G_1 arrest induced by α-factor (14). In a screen for high-copy-number suppressors of this defect, we found that *SWI4* overexpression could expedite recovery of a *swi6* mutant (23b). To exploit this effect, we transformed BY1232 (*swi6*::*LEU2*) with p*GAL*:*SWI4*, a plasmid which carries the *SWI4* open reading frame under the control of the inducible *GAL1-10* promoter (5). We analyzed the ability of the transformed strain to arrest and recover synchronously from α-factor in the presence of high-level expression of *SWI4*. SWI4 overproduction does not impair the α-factor arrest and recovery pathway in wild-type cells, as judged from the fact that the periodic transcription of the histone *H2B* gene occurs normally (5).

We used the same criterion to evaluate the synchrony of recovery and progression through the cell cycle of the *swi6*(p*GAL*:*SWI4*) mutant cells. The results of that analysis are shown in Fig. 5A. Two clear peaks of histone transcription were observed, which indicates reasonable synchrony for at least two cell cycles. These peaks were slightly delayed compared with that seen in *SWI*(p*GAL*:*SWI4*) cells (5), but that is probably a result of the slower growth rate of

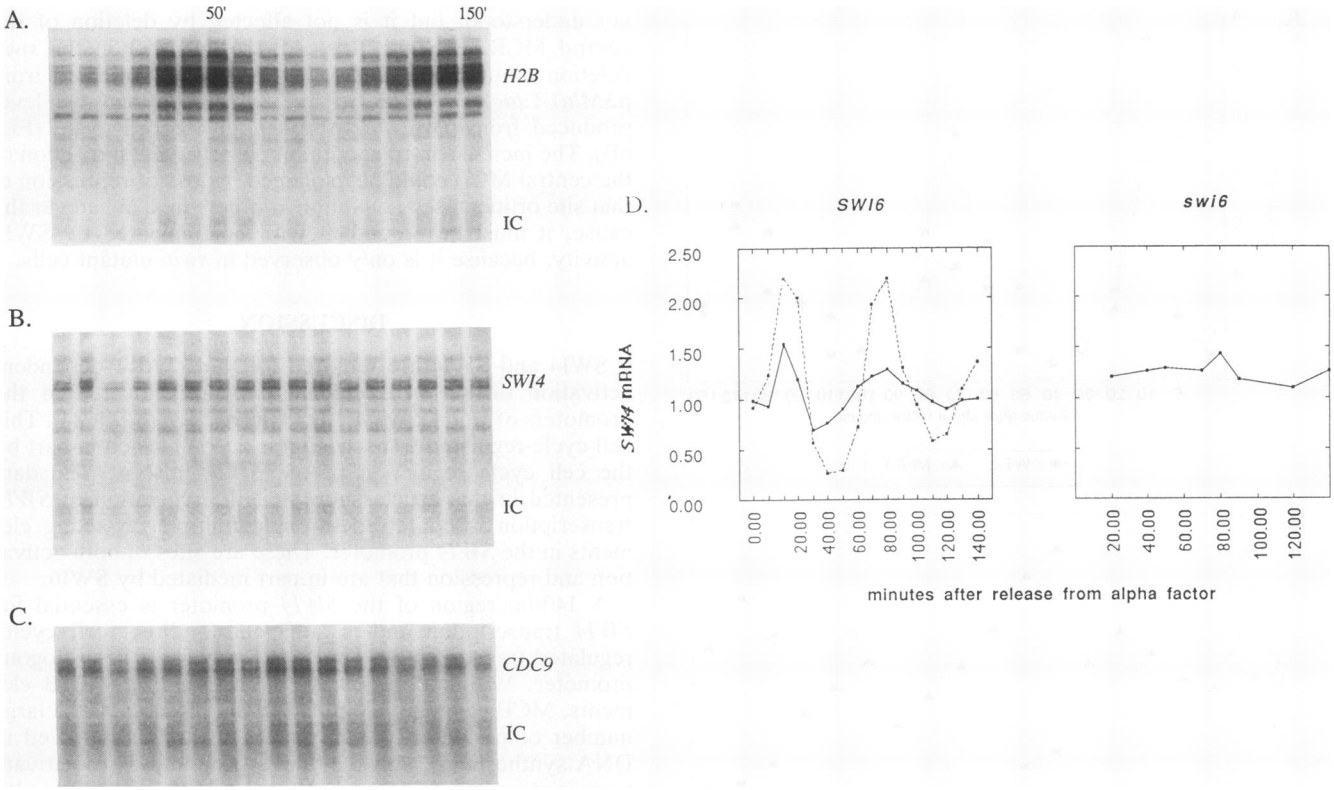

FIG. 5. Mutation of *SWI6* eliminates the normal periodicity of *SWI4* transcription. BY1232 (*swi6::LEU2*) was transformed with p*GAL:SWI4*. Synchronization with α-factor and release from arrest were carried out in galactose medium. Histone *H2B1* mRNA levels were monitored (A) to confirm that the cells were undergoing synchronous cell cycles. The levels of *SWI4* (B) and *CDC9* (C) transcripts in the same samples were analyzed similarly. The internal control in all four panels is *SIR3*. (D) Quantitation of the *SWI4* mRNA levels through the cell cycle in a galactose-grown *SWI*(p*GAL:HIS3*) strain (dashed line, left panel) or *SWI*(p*GAL:SWI4*) strain (solid line, left panel). The right panel shows the *SWI4* mRNA level in the *swi6*(p*GAL:SWI4*) strain. These experiments were carried out in parallel, with the same labeled probes, so that the level of *SWI4* mRNA can be compared directly.

swi6 cells (6). We then monitored transcription through the cell cycle of *SWI4* (Fig. 5B) and of *CDC9* (Fig. 5C), another MCB-driven gene, in the *swi6*(p*GAL:SWI4*) strain. In contrast to histone *H2B* mRNA, both the *SWI4* and *CDC9* messages were produced at the same level throughout two cell cycles.

To determine whether the level of *SWI4* transcription in the *swi6*(p*GAL:SWI4*) strain represented high or low constitutive expression, we quantitated the amount of *SWI4* mRNA in the *swi6*(p*GAL:SWI4*) synchrony experiment and compared it with the *SWI4* transcript levels detected through the cell cycle in *SWI*(p*GAL:SWI4*) and *SWI*(p*GAL:HIS3*) cells also grown in galactose (Fig. 5D). The constant level of *SWI4* mRNA in the *swi6* cells was intermediate between the peak and trough levels of *SWI4* mRNA in the control strains. A similar analysis showed that *CDC9* was also expressed at an intermediate level in the *swi6* mutant (14; data not shown). These results indicate that SWI6 activity is required for peak transcription of *SWI4* (and *CDC9*) in late G_1 and also for full repression of both genes at other times in the cell cycle. It also indicates that SWI6 is not the only positive activator of *SWI4* or *CDC9* transcription.

It should be noted that the periodic fluctuation of *SWI4* mRNA in the *SWI*(p*GAL:SWI4*) strain is diminished compared with the fluctuation seen in the *SWI*(p*GAL:HIS3*) strain (Fig. 5D). This probably reflects the slight loss of synchrony in the *SWI4*-overproducing strain that is also evident when histone *H2B* transcription is monitored (5). This difference is not due to an overall increase in *SWI4* promoter activity, because peak synthesis of endogenous *SWI4* mRNA in the *SWI*(p*GAL:SWI4*) strain is actually lower than that in the *SWI*(p*GAL:HIS3*) strain (Fig. 5D). Furthermore, we have quantitated the level of *SWI4* mRNA in asynchronous cultures of *SWI*(p*GAL:SWI4*) and *SWI* (p*GAL:HIS3*) cells, and they are roughly equivalent (0.84 and 1.0, respectively).

The results presented in Fig. 5 show that loss of SWI6 activity results in an intermediate constitutive level of *SWI4* transcription. This suggests that SWI6 acts both positively and negatively to regulate *SWI4* transcription throughout the cell cycle. Since MCB elements are known targets of SWI6 binding, we wondered whether the three MCB sequences found in the *SWI4* UAS respond to SWI6 in an equivalent manner. Our initial analysis showed that deletion of the central MCB (Δ*Mlu*I-1) did not affect the overall level of *SWI4* UAS activity in a wild-type strain (Table 1). We then compared the UAS activity of the Δ*Mlu*I-1 and wild-type *SWI4* promoters throughout the cell cycle.

The *SWI4* transcript produced from each was monitored through two synchronous cell cycles by S1 nuclease protection, and the level of *SWI4* mRNA was quantitated (Fig. 6A). The profiles of WT and Δ*Mlu*I-1 promoter activity were superimposable throughout the cell cycle. This indicates that deletion of the central MCB does not affect the cell cycle

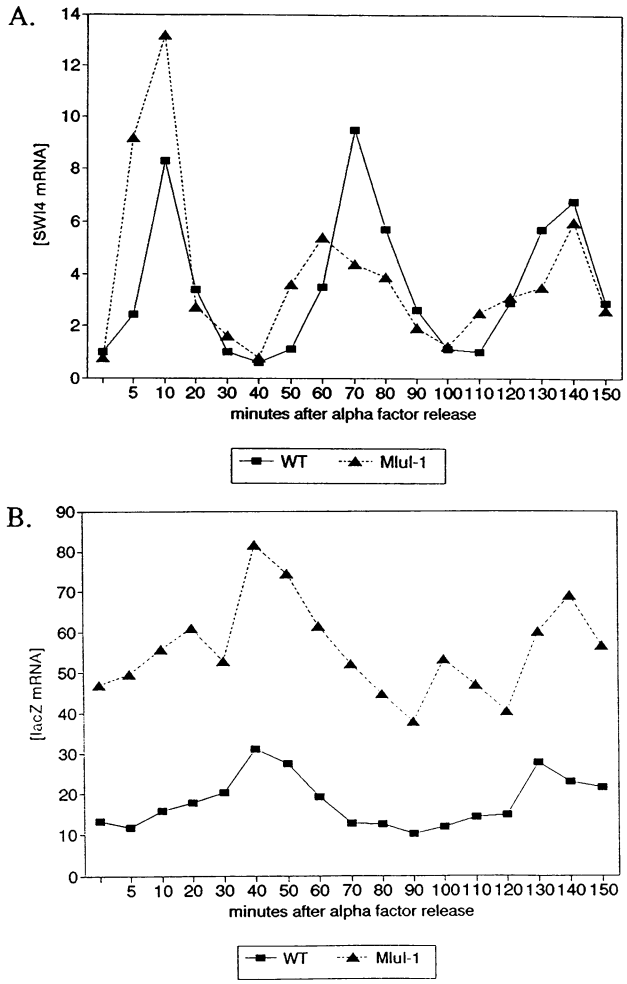

FIG. 6. Deletion of the central MCB is phenotypically silent in *SWI* cells and increases transcription in *swi6* mutant cells. Transcription was monitored for at least two cell cycles after release from α-factor arrest. Message levels were quantitated, normalized to an invariant mRNA, and then plotted as a function of time after release from arrest. Message levels in each panel can be compared directly because the analysis was carried out in parallel with the same labeled probes. (A) *SWI4* mRNA levels produced from the wild-type *SWI4* promoter on Bd909 (■) or from the Δ*Mlu*I-1 plasmid Bd974 (▲). (B) Same comparison of the wild-type *SWI4* UAS activity on pWT:*lacZ* (■) with that of the central MCB deletion on p*Mlu*I-1:*lacZ* in BY1232 *swi6*::*LEU2*(p*GAL*:*SWI4*) cells (▲).

regulation of the *SWI4* promoter in wild-type cells. Thus, the ACGCGTNA sequence was not required for the normal repression or activation of the *SWI4* promoter. However, when we carried out analogous experiments with the isogenic *swi6* mutant, we obtained very different results. BY1232 (*swi6*::*LEU2*) was transformed with p*GAL*:*SWI4* (*HIS3*) and either pWT:*lacZ* or pΔ*Mlu*I-1:*lacZ*, and the level of *lacZ* transcript produced from each was quantitated (Fig. 6B). As expected, neither the WT nor Δ*Mlu*I-1 UAS displayed normal cell cycle-regulated activity in the *swi6* deletion mutant. In both cases, the *lacZ* mRNA level still fluctuated about twofold, but the small peak that we observed was delayed by approximately 30 min compared with the peak of wild-type *SWI4* and coincided with the peak of *HTB1* transcription (Fig. 5A). The cause of this fluctuation is not understood, but it is not affected by deletion of the central MCB sequence. Instead, we find that in the *swi6* deletion mutant, the level of *lacZ* mRNA produced from pΔ*Mlu*I-1:*lacZ* is three- to fivefold higher than the level produced from pWT:*lacZ* throughout the cell cycle (Fig. 6B). The increase in transcription observed upon deletion of the central MCB could be explained by loss of repression at that site or increased activation at other sites. Whatever the cause, it must be redundant with or eliminated by SWI6 activity, because it is only observed in *swi6* mutant cells.

DISCUSSION

SWI4 and SWI6 are required for the START-dependent activation of the SCB element which is located in the promoters of *HO*, *CLN1*, *CLN2*, and *HCS26* (6, 23, 24). This cell cycle-regulated transcription is accomplished in part by the cell cycle regulation of the *SWI4* mRNA. The data presented in this article show that the regulation of *SWI4* transcription is conferred through multiple *cis*-acting elements in the *SWI4* promoter. These are sites of both activation and repression that are in part mediated by SWI6.

A 140-bp region of the *SWI4* promoter is essential for *SWI4* transcription and is sufficient to direct cell cycle-regulated transcription from a UAS-deficient, heterologous promoter. Within this domain, there are three MCB elements. MCB elements are found in the promoters of a large number of periodically expressed yeast genes involved in DNA synthesis (1), and they have been shown to activate transcription in late G_1-early S phase of the cell cycle (10, 17). The MCB elements in the *SWI4* promoter probably also contribute to the cell cycle regulation of *SWI4*. The *SWI4* MCB elements are responsible for 90% of the *SWI4* promoter UAS activity, and that UAS activity is regulated within the cell cycle. However, the MCB elements in the *SWI4* promoter are not necessary for the periodic transcription of *SWI4*. The residual transcription from an MCB-deficient *SWI4* UAS still fluctuates nearly 10-fold during the cell cycle and peaks at the same time as transcription from the wild-type *SWI4* UAS. Thus, there must be another form of periodic regulation conferred by a novel element within this 140-bp UAS. This element could be the target of negative or positive regulation of transcription and is not related to any of the other known elements involved in cell cycle regulation of yeast transcription. The presumption that the MCBs contribute to the cell cycle regulation of *SWI4* cannot be addressed until this novel element is identified and then deleted from the *SWI4* UAS. It should then be possible to determine whether the MCBs are sufficient to confer cell cycle regulation or whether these elements provide a constant UAS activity which is regulated within the cell cycle by repression.

Since SWI6 is known to bind to and regulate transcription from MCB elements (9, 15), we examined the cell cycle regulation of *SWI4* in a *swi6* mutant. Although the MCBs present in the *SWI4* promoter cannot account for all of the cell cycle regulation of *SWI4* transcription, mutations in *swi6* virtually eliminate periodicity. This suggests that the novel cell cycle-regulated element in the *SWI4* promoter is SWI6 dependent. We still observe an approximately twofold fluctuation in *SWI4* UAS activity in the *swi6* mutant, but the timing of peak transcription is delayed by approximately 30 min relative to the timing in *SWI6* cells. The source of this fluctuation is unknown.

The level of *SWI4* mRNA in the *swi6* strain is intermediate between the normal peak and trough transcript levels ob-

served in wild-type cells. This indicates that SWI6 function is required for full activation of transcription at the G_1/S border and full repression of *SWI4* transcription throughout the rest of the cell cycle. Whether both of these activities are carried out by SWI6 or by a SWI6-associated protein is unknown. Since SWI4 is efficiently expressed in *swi6* mutant cells, there must be another SWI6-independent mechanism of *SWI4* transcription. This second activator may also act at MCB elements, because loss of the three MCB elements reduces *SWI4* transcription 10-fold. SWI6-independent transcription of other MCB-regulated genes has been observed (9, 14), and the presence of a second activation pathway for these genes may explain why deletion of SWI6 is not lethal.

The three MCB elements in the *SWI4* promoter differ in that only the central one is a perfect consensus MCB sequence (ACGCGTNA). The two flanking MCBs have the CGCG core sequence and either the 5′ A or the 3′ T. We have not generated all possible combinations of MCB deletion variants to determine whether these three MCB sequences are equivalent. However, we have shown that deletion of the perfect consensus MCB does not affect UAS activity or cell cycle regulation of *SWI4* in wild-type cells. When we delete all three MCBs, UAS activity drops 10-fold. This shows that at least one of the two flanking CGCG sequences can function as a UAS and that the necessary repression and/or activation responsible for cell cycle control can occur at these nonconsensus sites. These data also show that UAS function is not additive, as there is no loss of activity when the central MCB is deleted. Indeed, it is possible to argue from these data that the central MCB has no role in *SWI4* regulation. However, the loss of this site results in a three- to fivefold increase in UAS activity in the *swi6* mutant strain. One possible explanation for this observation is that deletion of the central MCB (Δ*Mlu*I-1) generates a new, more active UAS. However, this is not likely, since the increase in UAS activity is only detected in the *swi6* strain. Alternatively, the Δ*Mlu*I-1 deletion may place the two flanking MCBs in a better position for synergistic function. This is plausible, since we know that there is another mechanism of *SWI4* activation which may act at MCB sites and which we detect only in *swi6* cells. This other activity may be more efficient when the flanking MCBs are repositioned in the Δ*Mlu*I-1 promoter.

Another possibility is that there is a repressor that acts specifically on the central MCB element. The flanking CGCG sites, which are present in the Δ*Mlu*I-1 UAS, may not be targets for this repressor, but they are targets for repression by SWI6. In wild-type cells, the repressing activity of SWI6 at the CGCG sequences could mask the loss of the ACGCGT-specific repression, so cell cycle regulation and UAS activity would remain unchanged. If both forms of repression were eliminated, as in the *swi6* Δ*Mlu*I-1 strain, transcription would be increased. This could explain why loss of the central MCB element causes an increase in transcription in *swi6* mutant cells. Point mutations that eliminate either the 5′ A or 3′ T of an MCB element in the *TMP1* promoter lead to an apparent increase in transcription (17). This led McIntosh et al. (17) to suggest that the A and T residues are specifically required for binding by a repressor. Our data are consistent with this idea, except that we see this effect only in *swi6* mutant cells.

Figure 7 is a working model which diagrams the regulation of *SWI4* gene expression and its relation to the start of the cell cycle. The central feature of this model is the positive feedback loop which was first proposed by Cross and Tinkelenberg (8), who noted that *CLN* expression is dependent upon at least one functional CLN protein and the CDC28 kinase. Included in this model is the observation that SWI4 and SWI6 bind to and activate SCB elements which are found in the promoters of *HO* (22) and the periodically expressed *CLN* genes (23, 24). SWI6 now appears to have multiple roles at the start of the cell cycle. In addition to its role at the SCB elements, SWI6 activates the MCB elements which regulate the transcription of many genes involved in DNA synthesis (1, 9, 14, 17) and which also appear in the *CLB5* promoter (9a). SWI6 also regulates the transcription of *SWI4* through both the MCBs and a novel regulatory element in the *SWI4* promoter.

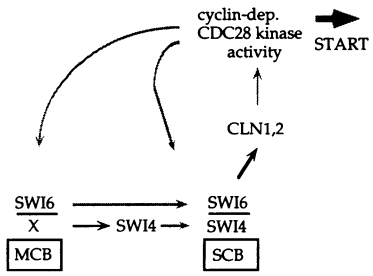

FIG. 7. SWI6 has multiple roles at the start of the cell cycle. SWI6 regulates the periodic transcription of *SWI4* through the MCB elements and another unidentified element in the *SWI4* promoter. SWI6 also associates with SWI4 (3a, 25, 27), binds to SCB elements, and activates transcription of *CLN1* and *CLN2* (2, 23, 24). These G_1 cyclins associate with the CDC28 protein to produce an active cyclin-dependent (cyclin-dep.) kinase (32). This kinase activity is required to further activate transcription of MCB- and SCB-regulated promoters through a positive-feedback loop and to start the cell cycle (8, 16, 23). Solid arrows represent induction at the level of transcription, and stippled arrows represent activation by association and/or modification. Two arrows have been drawn to represent the positive activation of MCB- and SCB-regulated promoters by the CDC28 kinase, but the target(s) of this regulation is unknown.

The unique role of SWI6 in this regulatory cascade suggests a rather simple competition model to explain the periodic waves of transcription that we observe. At the start of the cell cycle, in response to an unknown signal, SWI6/X-mediated transcription at MCB elements induces high-level expression of *SWI4*. This nascent SWI4 protein would compete with X in order to form the SWI4/6 complex. If SWI6 is limiting, this would result in decreased *SWI4* transcription and increased SCB-directed transcription. Assuming that SWI4 is unstable, this decrease in *SWI4* transcription would lead to the decay of the SWI4/6 complex and loss of SCB-activating potential. The timing of *SWI4* transcription has been compared directly with that of *HO* transcription, and it is consistent with this model. The peak of *SWI4* transcription precedes the peak of *HO* transcription by approximately 5 min (5). SWI4 is also an unstable protein (25a). However, there are several facts that cannot be explained by this model.

First, there is no evidence that SWI6 is limiting in the cell. Vast overproduction of SWI6 from the *GAL* promoter has no impact on the cell cycle regulation of *SWI4* or *HO* (5). Similarly, SWI4 overproduction does not reduce or deregulate MCB transcription. It does increase SCB transcription, but apparently not at the expense of MCB transcription (4a, 5). Second, SWI6 is required for both the activation and repression of *SWI4* transcription throughout the cell cycle. Although there are alternative explanations, the most likely one is that SWI6 remains bound to the *SWI4* promoter

throughout the cell cycle. Third, SWI6 can form complexes on SCB elements and MCB elements throughout the cell cycle in vitro (9, 30). Thus, all the components of these binding complexes must be present and functional in extracts from cells collected throughout the cell cycle. However, it is worth noting that Lowndes et al. (15) have detected an MCB-binding activity that is cell cycle regulated and is at its peak during α-factor arrest and very early in the cell cycle. This is inversely related to another SCB-binding complex on the *HO* promoter, which is absent in α-factor-arrested cells and whose level rises only at the time of SCB transcription (30). Thus, there could be an element of competition between these two binding activities as cells recover from pheromone-mediated G_1 arrest.

Another possibility is that the requirement for SWI6 in MCB and SCB binding provides a common target for coordinate regulation. Renaturation of a temperature-sensitive CDC28 kinase is sufficient to induce a pulse of SCB and MCB transcription in the presence of cycloheximide (16). This indicates that the proteins that are absolutely required for SCB- and MCB-directed transcription persist at some level in this arrest and that at least one component of each pathway is activated directly or indirectly by the CDC28 kinase. Since SWI6 is the common component of both MCB and SCB transcription, it is a likely target.

SWI6 deletion mutants are abnormally large and grow very slowly (6). They are also very defective in recovery from stationary phase and from pheromone-induced G_1 arrest (14, 23b). These phenotypes suggest an impaired ability to progress through the cell cycle and to restart the cell cycle after prolonged arrest. However, *SWI6* is not an essential gene (7). SWI6 modulates the activity of SCB- and MCB-containing promoters but is not absolutely required for their transcription. In the absence of SWI6 function, all of the known SWI6-regulated promoters except the nonessential *HO* gene are expressed at levels which are sufficient for viability. In the case of MCB-regulated genes, there appears to be at least one other activation pathway which ensures that these genes will be expressed in the absence of SWI6 activity. *SWI4*, in particular, is transcribed at an intermediate constitutive level in *swi6* mutant cells. The SCB-containing promoters are less active (23, 24), and the cell cycle regulation of *CLN1* and *CLN2* transcription is severely dampened but not eliminated in *swi6* mutant cells (9, 14). This is consistent with our previous conclusion that the cell cycle regulation of *SWI4* transcription is important for the periodicity of SCB-regulated genes but is not the only source of control (5). Clearly, the CDC28 kinase plays a role in activation of both MCB and SCB pathways (16), but its targets are unknown. The fact that the cell cycle regulation of *CLN1* and *CLN2* transcription persists in *swi6* mutant cells (in which *SWI4* transcription is deregulated and SWI6 is absent) suggests that the SWI4 protein may be one of the targets of CDC28-dependent activation.

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REFERENCES

1. **Andrews, B. J.** 1992. Dialogue with the cell cycle. Nature (London) **355**:393–394.
2. **Andrews, B. J., and I. Herskowitz.** 1989. Identification of a DNA binding factor involved in cell-cycle control of the yeast *HO* gene. Cell **57**:21–29.
3. **Andrews, B. J., and I. Herskowitz.** 1989. The yeast SWI4 protein contains a motif present in developmental regulators and is part of a complex involved in cell-cycle-dependent transcription. Nature (London) **342**:830–833.

3a. **Andrews, B. J., and L. A. Moore.** 1992. Interaction of the yeast SWI4 and SWI6 cell cycle regulatory proteins *in vitro*. Proc. Natl. Acad. Sci. USA **89**:11852–11856.

4. **Barker, D. J., J. H. M. White, and L. H. Johnston.** 1985. The nucleotide sequences of the DNA ligase gene (*CDC9*) from *Saccharomyces cerevisiae*: a gene which is cell-cycle regulated and induced in response to DNA damage. Nucleic Acids Res. **13**:8323–8337.

4a. **Breeden, L.** Unpublished data.

5. **Breeden, L., and G. Mikesell.** 1991. Cell cycle-specific expression of the SWI4 transcription factor is required for the cell cycle regulation of HO transcription. Genes Dev. **5**:1183–1190.
6. **Breeden, L., and K. Nasmyth.** 1987. Cell cycle control of the yeast *HO* gene: *cis*- and *trans*-acting regulators. Cell **48**:389–397.
7. **Breeden, L., and K. Nasmyth.** 1987. Similarity between cell-cycle genes of budding yeast and fission yeast and the *Notch* gene of *Drosophila*. Nature (London) **329**:651–654.
8. **Cross, F. R., and A. H. Tinkelenberg.** 1991. A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. Cell **65**:875–883.
9. **Dirick, L., T. Moll, H. Auer, and K. Nasmyth.** 1992. A central role for *SWI6* in modulating cell cycle start-specific transcription in yeast. Nature (London) **357**:508–513.

9a. **Epstein, C. B., and F. R. Cross.** 1992. CLB5: a novel B cyclin from budding yeast with a role in S phase. Genes Dev. **6**:1695–1706.

10. **Gordon, C. B., and J. L. Campbell.** 1991. A cell cycle-responsive transcriptional control element and a negative control element in the gene encoding DNA polymerase α in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **88**:6058–6062.
11. **Guarente, L., and T. Mason.** 1983. Heme regulates transcription of the *CYC1* gene of *S. cerevisiae* via an upstream activation site. Cell **32**:1279–1286.
12. **Haber, J. E., and B. Garvik.** 1977. A new gene affecting the efficiency of mating type interconversions in homothallic strains of *S. cerevisiae*. Genetics **87**:33–50.

12a. **Hahn, S.** Unpublished data.

13. **Henikoff, S.** 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155**:156–165.
14. **Lowndes, N. F., A. L. Johnson, L. Breeden, and L. H. Johnston.** 1992. SWI6 protein is required for transcription of the periodically expressed DNA synthesis genes in budding yeast. Nature (London) **357**:505–508.
15. **Lowndes, N. F., A. L. Johnson, and L. H. Johnston.** 1991. Coordination of expression of DNA synthesis genes in budding yeast by a cell-cycle regulated *trans* factor. Nature (London) **350**:247–250.
16. **Marini, N. J., and S. I. Reed.** 1992. Direct induction of G_1-specific transcripts following reactivation of the CDC28 kinase in the absence of *de novo* protein synthesis. Genes Dev. **6**:557–567.
17. **McIntosh, E. M., T. Atkinson, R. K. Storms, and M. Smith.** 1991. Characterization of a short, *cis*-acting DNA sequence which conveys cell cycle stage-dependent transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:329–337.
18. **Miller, A. M.** 1984. The yeast *MAT*a*1* gene contains two introns. EMBO J. **3**:1061–1065.
19. **Miller, A. M., V. L. MacKay, and K. A. Nasmyth.** 1985. Identification and comparison of two sequence elements that confer cell-type specific transcription in yeast. Nature (London) **314**:598–603.
20. **Mullis, K. B., F. A. Faloona, S. J. Scharf, R. K. Saiki, G. T.**

Horn, and H. A. Erlick. 1986. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol. **51:**263–273.

21. **Nasmyth, K.** 1983. Molecular analysis of cell lineage. Nature (London) **302:**670–676.
22. **Nasmyth, K.** 1985. A repetitive DNA sequence that confers cell cycle START (*CDC28*)-dependent transcription of the *HO* gene in yeast. Cell **42:**225–235.
23. **Nasmyth, K., and L. Dirick.** 1991. The role of SWI4 and SWI6 in the activity of G1 cyclins in yeast. Cell **66:**995–1013.

23a. **Neary, K.** Unpublished data.

23b. **Neary, K., and L. Breeden.** Unpublished data.

24. **Ogas, J., B. J. Andrews, and I. Herskowitz.** 1991. Transcriptional activation of *CLN1*, *CLN2*, and a putative new G1 cyclin (*HCS26*) by SWI4, a positive regulator of G1-specific transcription. Cell **66:**1015–1026.
25. **Primig, M., S. Sockanathan, H. Auer, and K. Nasmyth.** 1992. Anatomy of a transcription factor important for the start of the cell cycle in *Saccharomyces cerevisiae*. Nature (London) **358:** 593–597.

25a. **Quinton, T., J. Sidorova, and L. Breeden.** Unpublished data.

26. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
27. **Sidorova, J., and L. Breeden.** 1993. Analysis of the SWI4/SWI6 protein complex which directs G_1/S-specific transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**1069–1077.
28. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
29. **Stern, M., R. Jensen, and I. Herskowitz.** 1984. Five *SWI* genes are required for expression of the *HO* gene in yeast. J. Mol. Biol. **178:**853–868.
30. **Taba, M. R. M., I. Muroff, D. Lydall, G. Tebb, and K. Nasmyth.** 1991. Changes in a SWI4,6-DNA-binding complex occur at the time of *HO* gene activation in yeast. Genes Dev. **5:**2000–2013.
31. **Wittenberg, C., and S. I. Reed.** 1988. Control of the yeast cell cycle is associated with assembly/disassembly of the CDC28 protein kinase complex. Cell **54:**1061–1072.
32. **Wittenberg, C., K. Sugimoto, and S. I. Reed.** 1990. G1-specific cyclins of *S. cerevisiae*: cell cycle periodicity, regulation by mating pheromone, and association with the $p34^{CDC28}$ protein kinase. Cell **62:**225–237.