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Regulation of the yeast DNA replication genes through the *Mlu* I cell cycle box is dependent on *SWI6*

RATI VERMA*, JEAN SMILEY*, BRENDA ANDREWS†, AND JUDITH L. CAMPBELL*

*Braun Laboratories, California Institute of Technology, Pasadena, CA 91125; and †Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada

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ABSTRACT **In *Saccharomyces cerevisiae*, at least 17 DNA replication genes are coordinately expressed at the G_1/S boundary during the cell cycle. All of these genes have the DNA sequence element ACGCGT in their 5′ upstream regulatory regions. This sequence has been shown to be essential for periodic expression of the *POL1*, *CDC9*, and *TMP1* genes. The cyclin (*CLN1* and *CLN2*) and *HO* genes are another subset of genes that are expressed with the same timing as the DNA replication genes. Their periodic expression requires the participation of two well-characterized transcriptional activators: the *SWI4* and *SWI6* gene products. In this study, we present evidence that *SWI6* contributes to the regulation of DNA replication genes as well. Surprisingly, a preferential requirement for *SWI6* over *SWI4* is observed in our studies of ACGCGT-dependent reporter gene expression *in vivo*. This selectivity has not been observed for the other G_1/S genes. Correlating with the *in vivo* results, protein–DNA complexes formed *in vitro* on multimeric ACGCGT elements are either abolished or reduced in *swi6*Δ deletion mutants.**

In *Saccharomyces cerevisiae*, at least 17 DNA replication genes are induced coordinately at the G_1/S boundary of the cell cycle. All of these genes have one copy or more of the consensus sequence ACGCGT in their upstream regulatory sequences (refs. 1 and 2; reviewed in ref. 3). That this conserved sequence is indeed required for periodic gene induction has been directly demonstrated for the *TMP1* (4), *POL1* (5), and *CDC9* (6) genes. Multimeric copies of the conserved hexamer can function as an upstream activation sequence as well as confer periodic activation on the heterologous *lacZ* gene (6). Since this sequence conforms to the recognition site for the *Mlu* I restriction endonuclease, it has been named the *Mlu* I cell cycle box (MCB).

In addition to the DNA replication genes, a number of other genes are known whose transcription is dependent on passage through Start. The cell cycle-regulated transcription of this second group of genes is known to be controlled by two transcription factors, encoded by *SWI4* and *SWI6*. The genes controlled by *SWI4* and *SWI6* include *CLN1* and *CLN2*, which encode two known G_1 cyclins, *HCS26*, which encodes a putative G_1 cyclin, and *HO*, whose product initiates cell type switching (7–9). *SWI4* and *SWI6* are known to act through a repeated sequence element, the *SWI4,6*-dependent cell cycle box (SCB), found 2–10 times in the upstream regulatory sequences of target genes. The consensus sequence for the SCB (CACGGAAAA), as originally defined through studies of the *HO* upstream regulatory region (10), is clearly different from the MCB sequence (ACGCGT). However, a consideration of the SCB elements of other genes as well as mutational analysis of the SCB has allowed deduction of a revised consensus sequence that reveals a core similarity between the two elements: aCGCGt (MCB) compared with (C)(G)CGa (matches between the two sequences are capitalized whereas parentheses indicate that the residue is one possible choice for that position) (11).

Given the core resemblance between the SCB and MCB sequences and the similar timing of expression of genes controlled by the two elements, we have investigated the potential role of *SWI4* and *SWI6* in the regulation of the DNA replication genes. We have tested the ability of the MCB sequence to function as an upstream activation sequence (UAS) in *swi4*Δ and *swi6*Δ deletion mutants. We find that MCB-dependent UAS activity is totally abolished in *swi6*Δ strains but is affected much less (2- to 8-fold) by deletion of *swi4*. The behavior of the MCB sequence in *swi4*Δ and *swi6*Δ mutants differs from that of the SCB element, which exhibits an absolute requirement for both *SWI4* and *SWI6* in order to function to activate transcription. Using gel mobility-shift assays, we have observed several protein complexes from crude yeast extracts that are specific for the MCB sequence. Formation of one complex is abolished and formation of a second complex is reduced in an *swi6*Δ deletion mutant. Taken together, the *in vitro* and *in vivo* data support a role for the Swi6 protein in regulating periodic transcription of the DNA replication genes.

MATERIALS AND METHODS

Plasmid Constructs and β-Galactosidase Assays. Plasmids containing the MCB consensus sequence were constructed by insertion of a synthetic MCB cassette. Two complementary oligomers with *Xho* I sticky ends, 5′-TCGAGCTTGACGCGTTAAG-3′ and 5′-TCGACTTAACGCGTCAAGC-3′, were annealed and cloned into the *Xho* I site upstream of the *CYC1* TATA box in the vector pΔSS (7). pΔSS contains on a yeast vector the *CYC1* promoter fused to *lacZ* but lacking UAS elements. Plasmid pBA486 carries three tandem copies of this cassette upstream of the *CYC1* promoter; plasmid pBA487 carries four copies of the cassette. A second, smaller cassette carrying the MCB sequence was also inserted into the UAS assay system. This cassette was formed of the self-complementary oligonucleotide 5′-TCGAGACGCGTC-3′ and is present in two copies upstream of the *CYC1* promoter in pBA487.

Yeast transformations were performed by a modification of the lithium acetate protocol of Ito *et al.* (12). For β-galactosidase assays, individual yeast transformants were grown to saturation in synthetic medium lacking uracil (SD-ura) to select for the tester plasmids. Cultures were diluted 1:50 in fresh SD-ura and grown to midlogarithmic phase (OD_{600} 0.6–0.8). The cultures were harvested (1.5 ml) by centrifugation for 1 min in an Eppendorf centrifuge. After suspension of the cell pellets in 150 μl of Z buffer (13), 50 μl of chloroform and 20 μl of 0.1% SDS were added. The mixture was mixed vigorously for 30 sec to permeabilize the yeast cell walls. To

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Abbreviations: MCB, *Mlu* I cell cycle box; SCB, *SWI4,6*-dependent cell cycle box; UAS, upstream activation sequence.

initiate the β-galactosidase reaction, 700 μl of a 1.2-mg/ml solution of *o*-nitrophenyl β-D-galactopyranoside (Sigma) was added to each cell suspension. Reaction mixtures were incubated at 30°C until a faint yellow color was visible, at which time the reaction was quenched by addition of 500 μl of 1 M sodium carbonate. The cell debris was pelleted and the OD_{420} of the supernatant was recorded. The β-galactosidase activity in each reaction was calculated as Miller units (13), $(OD_{420} \times 1000)/[OD_{600} \times$ volume of cells (ml) $\times$ time (min)].

Detergent Method of Extract Preparation. Yeast cells were grown at 30°C to an OD_{600} of 0.2–0.5 in YPD medium unless otherwise indicated. Cells were harvested by centrifugation in a GSA rotor (Sorvall) at 5000 rpm for 5 min at 4°C and the pellet was washed with ice-cold water. All subsequent steps were performed on ice. The pellet was suspended in 2 volumes of lysis buffer containing 50 mM Tris (pH 7.5) 0.2% Nonidet P-40, 0.3 M KCl, 10% (vol/vol) glycerol, 5 mM EDTA, leupeptin, chymostatin, and antipain at 10 μg/ml, pepstatin at 5 μg/ml, 5 mM dithiothreitol, 0.5 mM phenylmethanesulfonyl fluoride, and 0.5 mM benzamidine. Ice-cold, acid-washed and baked glass beads were added, and the cells were lysed by mixing each tube for 4 × 30 sec with intermittent cooling. The lysate was then spun at 20,000 rpm for 20 min in a 50 Ti rotor (Beckman). The clear supernatant was frozen in a dry ice/ethanol mixture and stored at either −70°C or in liquid nitrogen.

High-Salt Method of Yeast Extract Preparation. Exponentially growing yeast cells were harvested as above and then resuspended in lysis buffer containing 20 mM Hepes (pH 8.0), 1 mM EDTA, 10% glycerol, 5 mM dithiothreitol, 1.0 M KCl, and the protease inhibitors described above. Cells were lysed using glass beads and the cell debris was pelleted by spinning in a microcentrifuge for 5 min. The cleared supernatant was then dialyzed against the lysis buffer, except that the salt concentration was reduced to 50 mM KCl.

Mobility-Shift Assay. A 45-base-pair (bp) oligonucleotide containing three *Mlu* I sites in tandem was 5′ end-labeled using T4 kinase and used as a binding probe throughout. The sequence of the top strand was 5′-TCGACTCAAACGCGTTCGACAACGCGTTCGACAACGCGTTCGAGA-3′. The binding reaction mixture typically contained 20 μg of protein, 50 mM Tris (pH 7.5), 15% glycerol, 25 mM $MgCl_2$, and poly(dI·dC) at 0.25 μg/ml and was incubated on ice for 10 min. Reaction products were resolved in a 4% polyacrylamide gel in 44.5 mM Tris/44.5 mM boric acid/1.0 mM EDTA. The gels were dried and autoradiographed at −70°C with intensifying screens.

Northern Blot Analysis. RNA was prepared from logarithmic-phase cultures and blotted essentially as described by Gordon and Fantes (14). Each sample contained 30 μg of RNA. The probes were pPOL1-4 (15) and the *Eco*RI–*Hin*dIII fragment of the actin gene (5). They were labeled with a random primed DNA labeling kit (Boehringer Mannheim).

RESULTS

MCB-Driven UAS Activity Is Dependent on *SWI6*. To test whether *SWI4* or *SWI6* might act at the MCB sequences to activate transcription, we made use of the ability of synthetic MCB elements to activate transcription of a test gene (4–6). We have constructed plasmids similar to those of McIntosh *et al.* (4) and Lowndes *et al.* (6) in which two (pBA489), three (pBA486), or four (pBA487) copies of the MCB sequence are tandemly arranged upstream of a *CYC*–lacZ fusion gene lacking its own UAS. These constructs were transformed into wild-type cells and *swi4*Δ or *swi6*Δ mutant cells. MCB-mediated transcription was assayed by measuring β-galactosidase activity in the yeast transformants (Table 1). The MCB sequence was able to function efficiently as an UAS in wild-type and *swi4*Δ strains but was completely unable to function in the *swi6*Δ mutant strain. Although reductions in the UAS activity of the MCB sequence were found in the *swi4*Δ strain (2- to 8-fold), the requirement for *SWI6* in activating transcription through the MCB was absolute. These results contrast with studies of the SCB sequence (pBA249; Table 1), which is completely dependent on both *SWI4* and *SWI6* in order to function as an activating sequence in an identical assay (7, 16). We interpret the large difference in the requirement for *SWI4* and *SWI6* for the activation of transcription from the MCB sequences as indicating a specific requirement for *SWI6* in this process.

Table 1. The MCB sequence acts as an SWI6-dependent UAS

Plasmid	β-Galactosidase units JO14 (wild-type)	JO22 (*swi4*Δ)	JO23 (*swi6*Δ)
pBA489 (two MCBs)	16.1	1.95	0.45
pBA486 (three MCBs)	43.5	7.75	0.42
pBA487 (four MCBs)	118	60.2	0.25
pBA249 (two *HO* SCBs)	22.5	0.842	0.52
pBA67 (ribosomal protein 39 UAS)	838	838	820
pΔSS vector	0.26	0.13	0.14

Wild-type, *swi4*Δ, and *swi6*Δ cells were transformed with MCB::*lacZ* plasmids (pBA489, -486, and -487) carrying two, three, or four copies of a synthetic MCB sequence inserted upstream of a *CYC*::*lacZ* fusion gene. β-Galactosidase activity of the plasmids is dependent on the inserted MCB sequences. Results are the average of three to four assays using independent yeast transformants. The β-galactosidase activity of a nonspecific UAS (pBA67) is reduced somewhat in *swi4*Δ and *swi6*Δ mutant strains due to their compromised growth. All numbers were normalized to the activity of pBA67 in the wild-type strain. Both plasmid pBA67 and plasmid pBA249 have been described (7, 16). pBA249 contains two copies of the SCB sequence and serves as a *SWI4*- and *SWI6*-dependent control. Strains JO14, JO22, and JO23 have been described (8).

DNA–Protein Complexes Formed on the MCB *in Vitro* with Detergent or High-Salt Lysate. To investigate the molecular basis of the *in vivo* results presented above, we developed a biochemical assay for the formation of specific DNA–protein complexes on the MCB. Exponentially growing cells were harvested and disrupted in the presence of detergent (Fig. 1*A*) or high salt (Fig. 1*B*). Mobility-shift assays performed with a labeled probe containing three tandem MCB elements revealed several DNA–protein complexes. The slowest moving complex, complex 1, was observed only in extracts prepared with detergent (Fig. 1*A*). Complex 1 was judged to be MCB-specific, since its formation was blocked by a 15-fold excess of self sequence or the *CDC6* promoter-derived oligonucleotide C6. (Oligonucleotide C6 contains one *Mlu* I site and a near match in close proximity.) Unrelated oligonucleotides containing the binding site for the yeast heat shock transcription factor, as well as mutant self-sequence, failed to compete for complex formation even when present in the reaction mixture at a 30-fold molar excess relative to the labeled probe. Complex 2 was observed in extracts prepared by either method. Complex 2 was also judged to be MCB-specific on the basis of competition assays. There was complete competition by a 30-fold excess of self or C6 oligonucleotides in the high-salt extracts (Fig. 1*B*) and partial competition by the same oligonucleotides in detergent extracts. A third major complex (complex 3 in Fig. 1) was judged to be nonspecific, since nonradioactive MCB oligonucleotide failed to abolish its formation.

Complexes of higher mobility than complexes 1–3 were C6-specific but the trimeric tandem MCB sequence was a poor competitor. It is possible that these complexes recognize MCB in the monomeric form, because their formation was also blocked by an oligonucleotide derived from the

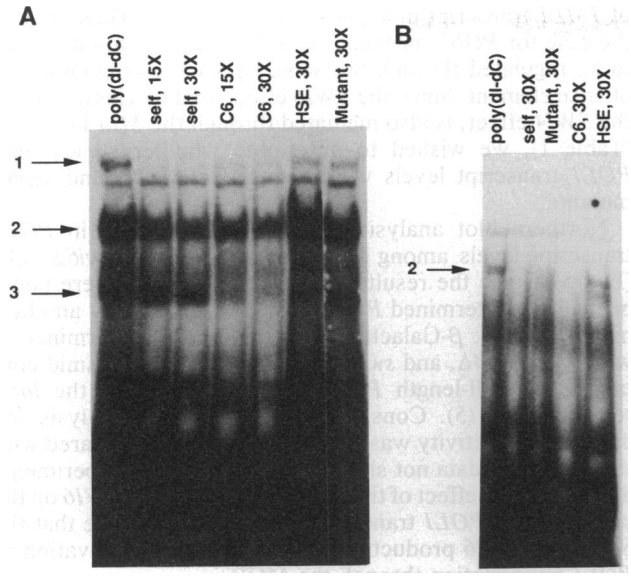

FIG. 1. Binding specificity of complexes formed on the trimeric MCB (ACGCGT)-containing DNA probe. (*A*) Mobility-shift assays were performed with 20 μg of yeast extract, prepared by the detergent method, in the presence of poly(dI·dC) or various competitors present at either 15- or 30-fold in excess over labeled probe. C6 oligonucleotide is derived from the *CDC6* promoter and contains one *Mlu* I site and one near match [. . . GACGCGAGGCCT-CACGCGT . . . (18, 19)]. The mutant oligonucleotide has each of the three *Mlu* I sites mutated to ACtaGT. The HSE oligonucleotide contains the heat shock recognition element for the heat shock transcription factor (20). (*B*) Binding conditions and competitors were as described for *A* except that the yeast extract was prepared by the high-salt method.

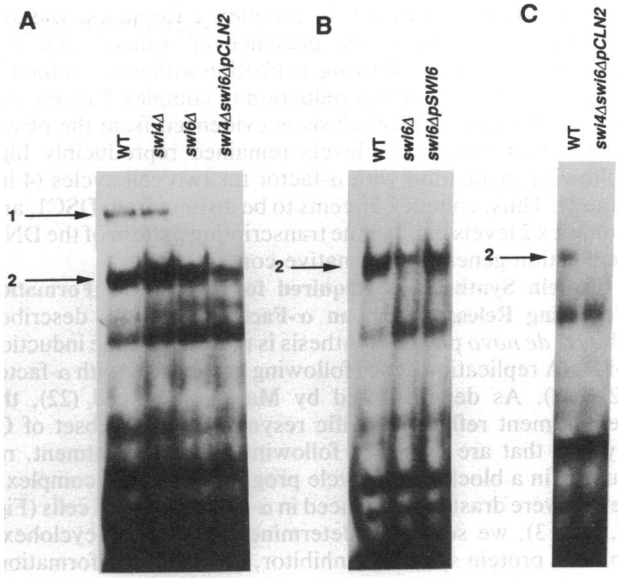

FIG. 2. Requirement for the *SWI6* gene product for *Mlu* I-specific binding activity. Exponential populations of wild-type cells (WT) and *swi4Δ*, *swi6Δ*, *swi6Δ*/pSWI6, and *swi4Δswi6Δ*/pCLN2 mutant strains growing at 30°C were harvested, and yeast extract was prepared by the detergent (*A*) or the high-salt (*B* and *C*) method. Mobility-shift assays were carried out using 20 μg of extract protein.

POL1 oligonucleotide, which contained two *Mlu* I sites spaced 19 bp apart (data not shown).

Lack of Complex 1 Formation in *swi6Δ*, but Not *swi4Δ*, Mutant Cells. Both *SWI4* and *SWI6* are known to function as components of a protein complex that binds to the SCB sequences upstream of the *HO* and cyclin genes. To examine the possibility that the requirement for *SWI6*, and to a lesser degree, *SWI4*, for the UAS activity of the MCB sequence (as demonstrated in Table 1) was due to the participation of Swi6 and Swi4 in protein complex formation at the MCB sequence, we prepared extracts from isogenic wild-type, *swi4Δ*, and *swi6Δ* strains. The results shown in Fig. 2*A* were obtained using extracts prepared by the detergent method, conditions optimal for visualization of complex 1. Complex 1 was absent from *swi6Δ* extract but was present in *swi4Δ* extract. Thus, complex 1 formation shows the same genetic requirements as UAS activity of the MCB sequence *in vivo*.

Complex 2 formation was also sensitive to *SWI6*, since levels were reduced in *swi6Δ* mutants relative to the wild type when extracts were prepared by the high-salt method (Fig. 2*B*). The decrease in complex 2 was specific to the absence of the *SWI6* gene product: transformation of a *swi6Δ* deletion mutant by the wild-type *SWI6* gene restored binding activity to wild-type levels (Fig. 2*B*). Although complex 2 did not appear to be affected in a *swi4Δ* mutant, in that levels were comparable to wild type (data not shown), an effect of deleting *swi4* was unmasked in a *swi4Δswi6Δ* double mutant (Fig. 2*C*). The effect of the *swi4* deletion on complex 2 formation in extracts from the double mutant was greater in extracts prepared by the high-salt method than in detergent extracts (compare Fig. 2 *A* and *C*). It may be that preparation of extracts in high salt renders Swi6 protein (and whatever else is involved) much less stable.

Complex 1 Formation Is Induced and Complex 2 Formation Is Abolished Following α-Factor Arrest. A binding activity, termed DSC1, that specifically recognizes the MCB sequence was previously identified (6). DSC1 activity was found to increase during incubation of cells with α-factor, a pheromone that arrests cells in G_1. An MCB-driven reporter gene was found to exhibit a similar increase in activity when cells were arrested with α-factor. This behavior stands in contrast to the transcription of genes driven by the MCB in the context of the native promoter: transcription of genes such as *POL1*, *RNR1*, and *CDC9* decreases during cell cycle arrest with α-factor and requires new protein synthesis to resume (21, 22). To investigate whether complexes 1 and 2 corresponded to DSC1 or exhibited new properties, we prepared extracts from cells growing exponentially (Fig. 3, lane 2) or arrested with α-factor for 2 hr (lane 1) or 4 hr (lane 3). Complex 1 formation was increased by incubation in the presence of α-factor. The competition binding data and the effect of α-factor thus suggest that complex 1 might contain DSC1. DSC1 binding has been reported to be periodic following an aberrant first cycle after α-factor release.

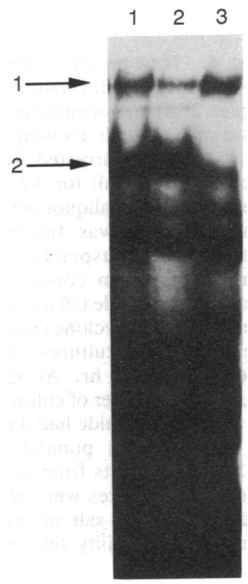

FIG. 3. Analysis of *Mlu* I-specific binding activity in cells arrested in G_1 with α-factor. An aliquot of *bar1* cells (for strain description see ref. 5; the *bar1-1* mutation reduces degradation of α-factor) growing exponentially at 30°C was harvested (lane 2) while another aliquot was arrested with α-factor (300 ng/ml) for 2 hr (lane 1) or 4 hr (lane 3) before harvest. Yeast extracts were prepared from each culture by the detergent method and mobility-shift assays were performed. Equal amounts of extract were loaded in each lane.

In contrast to complex 1, complex 2 formation was decreased after 2 hr in the presence of α-factor and was drastically reduced following incubation with α-factor for 4 hr (Fig. 3, lane 3). That this reduction in complex 2 levels was not due to general proteolysis is evidenced from the observation that complex 1 levels remained reproducibly high following incubation with α-factor for two cell cycles (4 hr, lane 3). Thus, complex 2 seems to be distinct from DSC1, and complex 2 levels parallel the transcription pattern of the DNA replication genes in their native context.

Protein Synthesis Is Required for Complex 2 Formation Following Release From an α-Factor Block. As described above, *de novo* protein synthesis is required for the induction of DNA replication genes following arrest in G_1 with α-factor (21, 22). As demonstrated by Marini and Reed (22), the requirement reflects specific resynthesis of a subset of G_1 cyclins that are degraded following α-factor treatment, resulting in a block in cell cycle progression. Since complex 2 levels were drastically reduced in α-factor-arrested cells (Fig. 3, lane 3), we sought to determine the effect of cycloheximide, a protein synthesis inhibitor, on complex 2 formation. Complex 2 binding activity in high-salt extracts was compared for α-factor-arrested cells and cells that were released from G_1 arrest by washing out the α-factor in the presence or absence of cycloheximide. Complex 2 was completely absent from α-factor-arrested cells (Fig. 4, lane 2). Complex 2 reappeared within 2 hr after release from G_1 arrest in the absence of cycloheximide (lane 5) but was completely blocked in the presence of cycloheximide (lane 4). Thus, complex 2 exhibits two properties consistent with the behavior of MCB-driven genes in their native configuration: (*i*) sensitivity to arrest by pheromone and (*ii*) a requirement for new protein synthesis for the reestablishment of complex formation following pheromone arrest.

Steady-State *POL1* Transcript Levels in *swi4*Δ and *swi6*Δ Mutant Cells. Using *POL1*::*lacZ* constructs, Gordon and Campbell (5) showed that when both *Mlu* I sites were eliminated from a synthetic *POL1* promoter, *lacZ* expression was greatly reduced in asynchronous cultures. Periodicity was also completely lost (5). Similar results were obtained with the *TMP1* gene. *TMP1*, which has two *Mlu* I (ACGCGT) sites spaced 30 bp apart, preferentially utilizes the 5′ distal site to drive periodic transcription (4). Site-directed mutagenesis resulting in a single G → T transversion in the 5′ site (ACtCGT) leads to a gross reduction in the steady-state levels of *TMP1* transcript in asynchronous cultures. Thus, as was the case for *POL1*, periodicity, as well as activation, seems to be regulated through the same *Mlu* I-site-containing sequence element. Since the *SWI6* effect, and to a lesser extent the *SWI4* effect, is also mediated through the *Mlu* I element (Table 1), we wished to determine whether steady-state *POL1* transcript levels were reduced in *swi4*Δ and *swi6*Δ mutants.

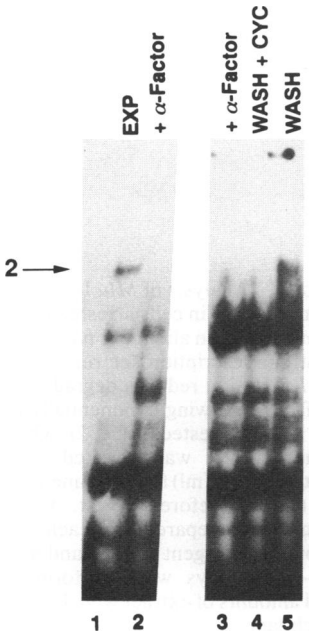

FIG. 4. New protein synthesis is required for formation of complex 2. An exponential population of *bar1* cells growing at 30°C (lane 1) was arrested with α-factor (300 ng/ml) for 4.5 hr (lanes 2 and 3). An aliquot of the arrested culture was filtered, washed, and resuspended in fresh YPD medium containing either cycloheximide (50 μg/ml) (lane 4) or no cycloheximide (lane 5). Released cultures were harvested after 2 hr. At this time, the cell number of cultures with no cycloheximide had doubled and budding population was >50%. Extracts from each of the above cultures were prepared by the high-salt method and used for mobility-shift assays.

Northern blot analysis showed no difference in *POL1* transcript levels among wild-type, *swi4*Δ, and *swi6*Δ cells (Fig. 5). Since the results of the RNA analysis were unexpected, we determined *POL1* promoter activity by an alternative method. β-Galactosidase activity was determined in wild-type, *swi4*Δ, and *swi6*Δ strains carrying a plasmid containing the full-length *POL1* promoter fused to the *lacZ* reporter gene (5). Consistent with the RNA analysis, no difference in activity was seen in the mutants compared with the wild type (data not shown). Although these experiments did not test the effect of the absence of *SWI4* and *SWI6* on the periodicity of *POL1* transcription, we can conclude that the *SWI4* and *SWI6* products are not required for activation of *POL1* transcription through the MCB.

DISCUSSION

The results define another role for the well-studied transcriptional activator encoded by the yeast *SWI6* gene. Although the MCB motif (ACGCGT) conferring periodicity on the DNA replication genes is different from the originally defined consensus site of Swi4/Swi6 protein interaction (7, 10), we have found that MCB-dependent UAS activity is completely dependent on *SWI6*. Consistent with the *in vivo* data, we observed two MCB-specific complexes *in vitro* that were either abolished (complex 1) or significantly reduced (complex 2, Fig. 2) in a *swi6*Δ mutant.

Complex 1 formation represents a biochemical correlate of the *in vivo* data in that complex 1 is formed when extracts are prepared from *swi4*Δ mutants but not from *swi6*Δ mutants. These observations indicate that *SWI6* can act independently of *SWI4* to promote binding to DNA and transcriptional activation. In its previously recognized role in activating transcription through the SCB sequences upstream of *HO* and certain cyclin genes, *SWI6* functions together with *SWI4* (7–9).

Previous studies of cell cycle-specific expression of the DNA replication genes have documented the absence of expression of these genes in α-factor-arrested cells. Reentry into the cell cycle following α-factor removal is accompanied

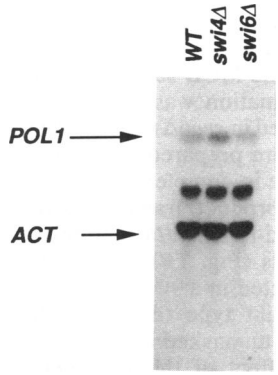

FIG. 5. Northern analysis of *POL1* message in wild-type, *swi4*Δ, and *swi6*Δ strains. Total RNA was prepared and blotted. The blot was probed for both *POL1* and actin (*ACT*) transcripts. Message sizes were determined by comparison with 18S and 25S rRNA. A shorter exposure (not shown) was used to normalize for *POL1* levels against actin transcript levels, and no significant difference was found among the three strains.

by an increase in expression that requires a fresh round of protein synthesis (21, 22). Mirroring this observation, we have found that binding activity contributing to complex 2 is absent from α-factor-arrested cells and is not restored following removal of the cell cycle block in the presence of cycloheximide (Fig. 4). By contrast, complex 1 is increased in α-factor-arrested cells. Thus, complex 2 may more closely reflect DNA–protein interactions important *in vivo* following G_1 arrest. Further evidence for the physiological significance of complex 2 is our observation that complex 2, while absent from cells arrested at the start of the cell cycle by α-factor (Fig. 4), is present in *cdc28*^{ts} arrested cells (data not shown). Marini and Reed (22) have shown that protein synthesis is not required for induction of transcription of *CDC9* after release from a *cdc28*^{ts} block in G_1. These data suggest that the DNA-binding proteins must already be present during a *cdc28* arrest and that activation following release may require posttranslational modification by Cdc28 protein kinase.

Our results indicate an important difference between the group of genes known to be directly regulated by *SWI6* (SCB-driven genes such as *HO*, *CLN2*, and *HCS26*) and the DNA replication genes. In the former case, the steady-state levels of *HO*, *CLN1*, and *CLN2* transcripts are reduced in a *swi6*Δ mutant, ranging from a modest effect on *CLN2* to a larger effect on *HO* (9, 17). In the case of the DNA replication genes, our data indicate an absolute dependence on *SWI6* for transcriptional activation through isolated MCB sequences and a requirement for *SWI6* in the optimal formation of protein complexes at this sequence. However, we see no effect of a *swi6*Δ deletion mutant on the steady-state levels of *POL1* transcripts. That is, *SWI6* is not required for activation of transcription through the MCB sequences upstream of *POL1*. This result was somewhat surprising, given our *in vivo* and *in vitro* evidence establishing a role for *SWI6* in activating transcription through the MCB. As stated earlier, previous work has demonstrated a requirement for the MCB, both for efficient UAS activity and for periodicity for the *POL1* promoter (5). In addition, McIntosh *et al.* (4) observed that steady-state levels of *TMP1* transcripts were drastically reduced by mutations in the MCB, suggesting a central role for this sequence in driving transcription of *TMP1*. If Swi6 were the key factor binding to the MCB sequence and activating transcription, we would have expected to see a reduction in *POL1* transcription in the *swi6*Δ mutant. Since this is clearly not the case, and yet a role for the MCB sequences in the *POL1* UAS in periodic expression has been firmly established (5), we suggest that another protein (or proteins) is binding to the MCB and activating transcription. *SWI6* may serve to regulate the formation and activity of the above complex, perhaps thus providing a link to cell cycle position. A candidate for a direct MCB-binding factor, MCBF, has been purified from yeast extracts (18). It remains to be seen whether MCBF and Swi6 interact. In addition, we expect that periodic transcription of *POL1* is deregulated in *swi6*Δ mutants, since our data demonstrate a role for *SWI6* in MCB activity (Table 1).

The Swi6 protein bears functional and structural similarity to the cdc10 protein from the fission yeast *Schizosaccharomyces pombe* (24). Of particular relevance to this discussion is the recent finding that cdc10 is a component of a transcription factor that forms on the MCB sequences of the *cdc22*$^{+}$ gene of *Sch. pombe* (23). Like *SWI4* and *SWI6*, the *cdc10*$^{+}$ gene is known to be involved in key events at the beginning of the mitotic cell cycle since mutants in *cdc10* arrest at start. Given the recent findings demonstrating a role for cdc10 in recognizing the MCB sequences, it seems likely that one of the essential functions of *cdc10*$^{+}$ for passing the cell cycle start is to induce transcription of DNA synthesis genes. The similarity between the protein sequences of cdc10 and Swi6, as well as results presented here demonstrating a role for *SWI6* in the function of MCB sequences in budding yeast, suggests that *SWI6* and *cdc10*$^{+}$ may represent functional homologues from distantly related yeasts. It seems likely that homologues of *SWI6* remain to be discovered in other eukaryotes.

Note Added in Proof. Since this manuscript was submitted, two papers (25, 26) have been published demonstrating the presence of Swi6 in complexes formed on the MCB. Aberrant regulation of the DNA replication gene family was observed in a *swi6*Δ mutant.

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