The Transcription Factor Swi5 Regulates Expression of the Cyclin Kinase Inhibitor p40\textsuperscript{SIC1}

DUNJA KNAPP,\textsuperscript{1} LEENA BHOITE,\textsuperscript{2} DAVID J. STILLMAN,\textsuperscript{2} AND KIM NASMYTH\textsuperscript{1}\textsuperscript{*}

Research Institute of Molecular Pathology, A-1030 Vienna, Austria,\textsuperscript{1} and Division of Molecular Biology and Genetics, Department of Oncological Sciences, University of Utah Health Sciences Center, Salt Lake City, Utah 84132\textsuperscript{2}

Received 21 March 1996/Returned for modification 22 April 1996/Accepted 25 July 1996

DNA replication in budding yeast cells depends on the activation of the Cdc28 kinase (Cdkl1 of \textit{Saccharomyces cerevisiae}) associated with B-type cyclins Clb1 to Clb6. Activation of the kinase depends on proteolysis of the Cdk inhibitor p40\textsuperscript{SIC1} in late G\textsubscript{1}, which is mediated by the ubiquitin-conjugating enzyme Cdc34 and two other proteins, Cdc4 and Cde53. Inactivation of any one of these three proteins prevents p40\textsuperscript{SIC1} degradation and causes cell arrest in G\textsubscript{1} with active Cln kinases but no Clb-associated Cdc28 kinase activity. Deletion of \textit{SIC1} allows these mutants to replicate DNA in the absence of Cdc4 function. Mutations in three loci (\textit{SIC1}, \textit{SWI5}, and \textit{RIC3}) were identified. We have shown that high \textit{SIC1} transcript levels at late M phase depend on Swi5. Swi5 accumulates in the cytoplasm during S, G\textsubscript{2}, and M phases of the cell cycle but enters the nuclei at late anaphase. Our data suggest that cell cycle-regulated nuclear accumulation of Swi5 is responsible for the burst of \textit{SIC1} transcription at the end of anaphase. This transcriptional control may be important for inactivation of the Clb/Cdk1 kinase in G\textsubscript{2}/M transition and during the subsequent G\textsubscript{1} period.

DNA replication in budding yeast cells depends on the activation of Cdk1/Cdc28 kinase associated with six different B-type cyclins, Clb1 to Clb6. Clb-associated Cdk1 kinase is regulated by transcription of cyclin proteins, and by accumulation of inhibitory proteins. Due largely to the onset of \textit{CLB5} and \textit{CLB6} transcription in late G\textsubscript{1} (\textit{CLB1} to \textit{CLB4} RNAs do not appear until later), DNA replication is normally triggered by the appearance of active Cln5 or Cln6/Cdc28 kinases (10, 26). In the absence of Clb5 and Clb6, Cln1 to Cln4 trigger replication. Normally, DNA replication is simultaneous with bud formation, but in the clb\textsubscript{5}, clb\textsubscript{6} mutant, DNA replication is delayed relative to bud emergence (26). Cln/Cdc28 kinase activity is also regulated by changes in cyclin stability (1) and by binding of the inhibitory protein p40\textsuperscript{SIC1}. p40\textsuperscript{SIC1} does not inhibit Cdc28 kinase associated with Cln cyclins which also appear in late G\textsubscript{1} and are necessary for activating \textit{CLB} gene transcription, turning off Clb proteolysis, and triggering p40\textsuperscript{SIC1} proteolysis (19, 22, 25). p40\textsuperscript{SIC1} protein accumulates to high levels in G\textsubscript{1}, disappears at the G\textsubscript{1}/S transition, and does not reaccumulate until cells complete nuclear division and reenter G\textsubscript{1}. To understand how p40\textsuperscript{SIC1} regulates the activity of cyclin B/Cdc28 kinases, we need to know how p40\textsuperscript{SIC1} accumulation is regulated. It has been demonstrated that the appearance of Cln1 and Cln2/Cdc28 kinases in late G\textsubscript{1} leads to the rapid proteolysis of p40\textsuperscript{SIC1} (25). This instability of p40\textsuperscript{SIC1} persists until cells undergo anaphase (24). It is not understood why it becomes stable in the subsequent G\textsubscript{1} period. p40\textsuperscript{SIC1} proteolysis depends on three genes: \textit{CDC4}, \textit{CDC34}, and \textit{CDC53} (25). \textit{CDC34} encodes an E2-type ubiquitin-conjugating enzyme (11). The functions of the Cdc4 and Cde53 proteins are currently not understood. Mutants with temperature-sensitive alleles of \textit{CDC4}, \textit{CDC34}, and \textit{CDC53} fail to degrade p40\textsuperscript{SIC1} at the restrictive temperature and arrest in G\textsubscript{1} with little or none of the Cln/Cdc28 kinase activity needed for entry into S phase (25). The Cln/Cdc28 kinases, in contrast, are active during this arrest (32), and cells duplicate their spindle pole bodies and form buds—events that are not dependent on Clb/Cdc28 kinases. Deletion of the \textit{SIC1} gene allows these mutants to activate the Clb kinases and to enter S phase (25).

To investigate the role of Cdc4 in cell cycle progression, we set out to isolate mutations that would allow the cdc4-1 mutants to activate the Clb kinases and thereby enter S phase. We isolated mutations in three genes, \textit{RIC1} to \textit{RIC3}, that allow cells to partially overcome the replication defect of a cdc4-1 mutant. \textit{RIC2} corresponds to \textit{SIC1}, and \textit{RIC1} corresponds to \textit{SWI5}, which encodes a G\textsubscript{1}-specific transcription factor first identified because of its role in activating the \textit{HO} endonuclease gene (29). Our data suggest that in addition to controlling the stability of p40\textsuperscript{SIC1}, transcriptional control of the \textit{SIC1} gene contributes to reaccumulation of p40\textsuperscript{SIC1} protein during G\textsubscript{1}. \textit{SIC1} transcripts are present at a low level throughout the cycle, but they accumulate transiently to high levels as cells exit mitosis. We have shown that Swi5 and the related factor Ace2 both bind, in vitro, to specific sites within the \textit{SIC1} gene promoter. However, while Swi5 has a major role in transiently activating \textit{SIC1} during anaphase, Ace2 plays a minor role.

\textbf{MATERIALS AND METHODS}

\textbf{Strains and media.} All yeast strains were derivatives or were backcrossed at least three times to W303 (MAT\textalpha{} HML\textalpha{} HM\textalpha{}Ra ho ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 ssd1). The strains used in this study are listed in Table 1. Cells were grown in yeast extract-peptone medium (YPE) supplemented with 2% glucose (YPEP) or raffinose (YPE8) unless otherwise stated.

\textbf{Linkage analysis.} To demonstrate linkage between mutations isolated in the screen and the \textit{SIC1} and \textit{SWI5} genes, mutants were crossed with K4163 and D69, respectively, and sporulated, and the phenotype of spores from at least 10 tetrads was analyzed by fluorescence-activated cell sorting (FACS) with a FACScan.

\textbf{BrdU incorporation assay.} Stationary-phase cells of strains D6 and D8 were...
TABLE 1. List of the strains and their genotypes used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6</td>
<td>MATα cdc4-1 TRPI GPD-HPV-1</td>
</tr>
<tr>
<td>D8</td>
<td>MATα cdc4-1 HIS3 GPD-HPV-1</td>
</tr>
<tr>
<td>D50</td>
<td>MATα cdc4-1 TRPI GPD-HPV-1</td>
</tr>
<tr>
<td>D51</td>
<td>MATα cdc4-1 HIS3 GPD-HPV-1</td>
</tr>
<tr>
<td>D52</td>
<td>MATα cdc4-1 TRPI GPD-HPV-1</td>
</tr>
<tr>
<td>D58</td>
<td>MATα cdc4-1 HIS3 swi5::URA3</td>
</tr>
<tr>
<td>D69</td>
<td>MATα cdc4-1 HIS3 swi5::URA3</td>
</tr>
</tbody>
</table>

 mutagenized by ethyl methanesulfonate to 50% survival and plated on YEPA at 25°C. Colonies per 13-mm-diameter plate. The background signal coming from cells which had passed the Cdc4 step before the temperature shift but had not replicated yet, colonies were grown for 4 to 5 days (i.e., until more than 90% of the cells in colony were in the G1 stationary phase). mutants were then replica plated onto nitrocellulose filters (Schleicher & Schuell) on fresh YEPA plates containing 200 μg of bromodeoxyuridine (BrDU) per ml and incubated at 37°C until more than 50% of the cells were released from stationary phase (checked by budding). The wild-type, budding and replication happen almost simultaneously. We reasoned that the mutants with an abolished phase (checked by budding) The incubation time on YEPA-BrDU plates was kept short to minimize the background of mitochondrial replication. BrDU incorporation into DNA was detected by a filter assay as described earlier (5), except that the first incubation step with sorbitol, EDTA, and diethiothreitol was done at 37°C.

Mutants positive in the BrDU filter assay were rechecked for the replication phenotype in the following way: patches were grown until cells were mostly in stationary phase (overnight) on YEPA plates at 25°C. They were then in inoculated into fresh YEPA at 37°C and analyzed by FACScan after 4 h.

Isolation and analysis of RNA from synchronous cultures. YEPR-grown wild-type (K4719) and swi5 (K4798) cells were synchronized by centrifugal elutriation as described previously (26). In the cdc15 release experiment, SW5 ACE2 (K1998), swi5Δ (K3774), and ace2Δ (K3659) cultures were grown in YEPA at 25°C until they reached an optical density of 0.60 nm of 0.2. They were then transferred to 25°C for 2.5 h (i.e., until they were arrested [dumbbell shaped]) and then released from the block by addition of an appropriate amount of YEPA at 4°C so that the resulting temperature was 25°C. Cultures were grown further at 25°C; total RNA was isolated, and 10 μg was subjected to Northern (RNA) transfer as described previously (4).

For detection of the SIC1 transcript, membranes were probed with the 20-bp Asp 718-Nde1 fragment of SIC1. CMD1 and PCL1 transcripts were used as internal controls for loading and for the cell cycle progression, respectively. Quantitations were done with a Molecular Dynamics PhosphorImager.

In vitro DNA-binding assays. Purification of Swi5 (6×His-tagged Swi5 fusion protein) and Ace2 (glutathione S-transferase–Ace2 fusion protein), gel retardation assays, and DNase I footprinting were performed as described previously (7). DNA probes were end labeled with T4 polynucleotide kinase and [γ-32P] ATP (3000 Ci/mmol). The SIC1 probe for gel retardation was a 221-bp Asp 718-TagI fragment from the SIC1 promoter. The HO probe was a 206-bp BglII-PvuII fragment from plasmid M1403. Plasmid M1403 contains a 46-bp region from the HO promoter, including Swi5 binding site B, cloned into the pUC19 vector; thus, the HO probe also contains plasmid sequences. The CTS1 probe was a 206-bp EcoRI-HindII fragment from plasmid M1818 (7) that contains two Ace2 binding sites. The SIC1 probe for DNase I footprinting was a 46-bp- EcoRI-Asp 718 fragment from the SIC1 promoter, labeled at the Asp 718 end.

RESULTS

Screen for suppressors of cdc4 mutation. To identify genes required for the inhibition of the Cib/Cdc28 kinase in cdc4 mutants, we set out to identify mutations that allow a cdc4-1 strain to grow at the restrictive temperature (35°C). After mutagenesis of 5 × 10^6 cells of the cdc4-1 strains (K3995 and K3996), only 108 colonies grew at 35°C, and only 8 of them contained recessive suppressor mutations. Contrary to our expectations that the recessive suppressors would be due to a loss of an inhibitory function, genetic analysis showed that all eight recessive mutations were cdc4 alleles. Eight dominant mutations were tested in a similar manner and were also found to be tightly linked to the CDC4 locus. These results suggest that there may be more than one essential target for Cdc4.

Screen for mutants which replicate DNA in the absence of Cdc4 function. Having established that it is difficult or impossible to suppress the proliferative defect of cdc4 mutants by loss-of-function mutations in other genes, we instead set out to identify mutations that merely allow cdc4-1 mutant cells to replicate DNA at the restrictive temperature. We used strains containing seven copies of the herpes simplex virus thymidine kinase expressed from the constitutive GPD promoter, which allowed us to measure the incorporation of BrDU into DNA. cdc4-1 strains were mutagenized by ethyl methanesulfonate plated onto YEPA plates at 25°C, replica plated onto a nitrocellulose filter on fresh YEPA plates containing BrDU, and incubated at the restrictive temperature for cdc4-1. After screening of approximately 170,000 colonies, 274 mutants capable of incorporating BrDU were examined by FACScan to determine their ability to replicate DNA (see Materials and Methods). Ten mutants showed a strong ability to replicate DNA at a nonpermissive temperature for cdc4-1, and genetic analysis showed that these mutants form three allelic groups, RIC1 to RIC3 (for replicates in cdc4). We identified six alleles of RIC1, two alleles of RIC2, and two alleles of RIC3. The FACScan profiles of representative mutants from each group are shown in Fig. 1A. The mutations allow about one-half of the cdc4-1 mutant cells to fully replicate their genomes.

Since by this stage it had been shown that deletion of SIC1 enables a cdc4-1 strain to replicate DNA (see reference 25 and Fig. 1A), SIC1 was a likely candidate for one of the RIC genes. The various ric mutants were crossed to a sic1α cdc4 double mutant strain (K4163), and the resulting spores were tested by FACScan for the ability to replicate DNA at the nonpermissive temperature. We found that RIC2 (two alleles) corresponds either to SIC1 itself or to a closely linked gene. Interestingly, the sic1 mutation is partially dominant in terms of the DNA replication phenotype. As shown in Fig. 1B, a diploid that is heterozygous SIC1/sic1Δ but homozygous cdc4/cdc4, is able to replicate DNA at the nonpermissive temperature. This suggests that the SIC1 gene product is haplo-insufficient, which is consistent with the observation that pB90C′ acts stoichiometrically in binding to and inhibiting the Cib/Cdk1 kinase (16, 25).

It was found accidentally that overexpression of CLB5 from the GAL promoter is toxic in swi5 mutant cells (reference 9 and data not shown), as it is in a sic1 mutant. This suggested that the Swi5 transcriptional activator plays a role, either directly or indirectly, in regulating Clb5 kinase expression or activity. Therefore, we tested whether a swi5 null mutation suppresses the DNA replication defect associated with cdc4. The FACScan analysis in Fig. 1A shows that a swi5 deletion confers a ric− phenotype. To determine whether the ric1 and ric3 mutant strains contain mutations in SWI5, genetic crosses were performed. Linkage analysis demonstrated that all 48 spores from 12 tetrads from the cross of ric1-1 cdc4-1 with swi5 Δ cdc4-1 (D69) had the parental (replicating) phenotype.

We concluded that RIC1 corresponds to SWI5.

There are quantitative differences in the ability of sic1 and swi5 to suppress the DNA replication defect caused by cdc4. A sic1 deletion allows more than 90% of the cdc4-1 mutant cells to complete S phase, while a deletion of SWI5 enables only 50% of the cells to complete S phase at 37°C (Fig. 1A). The
same is true for the effect on cell morphology. After 4 h at the nonpermissive temperature, all of the cdc4-1 single mutants have multiple elongated buds, presumably because Cln/Cdc28 kinases which stimulate bud emergence are active during this arrest and cause this phenotype (1, 32). Multiple buds were not seen in sic1Δ cdc4-1 mutants when cells were arrested at 37°C; most cells arrest with a dumbbell shape. When swi5Δ cdc4-1 mutants were shifted to 37°C, we found a mixture of dumbbell-shaped cells with single buds and cells with multiple elongated buds.

Mutations at the RIC3 locus proved to have no obvious phenotype in a CDC41 background, and, therefore, we have not attempted to clone the gene.

**SIC1 transcription is regulated by Swi5.** *SWI5* encodes a transcription factor needed for the expression of the *HO*endonuclease involved in mating-typeswitching (29, 30). Recent work suggests that it is also needed for the transcription of *EGT2*, whose transcripts appear as cells exit mitosis (15). *SWI5* is transcribed during G₂ and M phases, but Swi5 protein stays in the cytoplasm and does not accumulate in nuclei until the end of mitosis, when Cln2-associated kinases are destroyed by cyclin proteolysis. Swi5 protein is rapidly degraded upon its entry into nuclei, with the result that Swi5 protein accumulates to high levels in nuclei only transiently as cells exit from mitosis (17). *SIC1* as well as *EGT2* is transcribed at the time Swi5 enters the nucleus (8, 25). To test whether Swi5 is involved in the transcription of *SIC1*, we compared *SIC1* transcript levels in *SWI5* and *swi5Δ* cycling cultures. In *swi5Δ* mutant, the level of *SIC1* RNA is decreased to 50% of that of the wild-type RNA (Fig. 2). We also tested whether *SIC1* transcription depends on *ACE2*, which encodes a related transcription factor that also enters nuclei only at the end of mitosis. *SIC1* transcripts are reduced to about 80% of the wild-type levels in *ace2Δ* cells. However, deletion of both *SWI5* and *ACE2* genes reduced *SIC1* RNA levels to 20% of that of the wild type, suggesting that Swi5 and Ace2 share the task of activating *SIC1*, as they do for *EGT2* (15).

To test whether Swi5 is required specifically for the accumulation of *SIC1* RNAs at the end of mitosis, we compared *SIC1* transcript levels of *SWI5* and *swi5Δ* strains synchronized by centrifugal elutriation (Fig. 3). The basal levels of *SIC1* transcripts were similar, but the transient accumulation at the M-to-G₁-phase transition was reduced by two- to threefold in *swi5Δ* cells (zero-minutetime point after elutriation and 140 to 170 min later in the subsequent M/G₁ phases). In the *swi5Δ* mutant, a small increase in *SIC1* expression is still seen at the M/G₁ period of the cell cycle, and it is likely that Ace2 is responsible for this activation. We could not, however, test the effect of an *ace2* mutation by using centrifugal elutriation to synchronize cells, because *ace2Δ* strains are too clumpy and do not separate into single cells. We, therefore, compared the *SIC1* transcript levels after release from a cell cycle arrest in late mitosis due to a temperature-sensitive *cdc15-2* allele. In this case, deletion of *SWI5* abolished cell cycle-regulated *SIC1*
RNA accumulation (Fig. 4). In SWI5 ACE2 cells, SIC1 RNAs accumulated during the cdc15 arrest, increased further after the release from the block, soon afterwards fell as cells proceeded through the cell cycle, and then reaccumulated again. In contrast, in the swi5 mutant, SIC1 RNAs remained at a low constant level throughout this time course. Deletion of ACE2 had a modest effect on SIC1 RNA regulation with this protocol. The synchrony of the releases was checked by measuring the levels of the G1 cyclin PCL1 (HCS26) mRNA. We conclude that Swi5 has a major role in activating SIC1 transcription at the end of mitosis and that Ace2 has a modest one.

Swi5 and Ace2 bind to the SIC1 promoter in vitro. To test whether Swi5 activates SIC1 directly by binding to its promoter, we searched for potential Swi5 binding sites in the sequences between SIC1’s AUG codon and the upstream gene BOS1. We identified two putative Swi5 binding sites, on the basis of the similarity to the DNA sequence recognized by Swi5 at the HO and the CTS1 promoters (Fig. 5A). Although Swi5 and Ace2 differentially activate transcription of HO and CTS1 in vivo (6), in vitro Swi5 and Ace2 each bind to both promoters with similar affinities (7).

Swi5 and Ace2 proteins purified from an Escherichia coli expression system were used to examine DNA binding to SIC1 promoter sequences in vitro. When a 221-bp fragment from the SIC1 promoter was used in a gel retardation assay with Swi5, a single protein-DNA complex was observed (data not shown). This probe contains two possible Swi5 binding sites. Thus, it was not clear whether a single molecule of Swi5 bound to this probe or whether two Swi5 molecules bound in a highly cooperative fashion. To distinguish between these possibilities, we prepared probes of similar sizes from SIC1, HO (site B), and CTS1. Only one molecule of Swi5 binds to this HO probe, while two molecules of Swi5 bind noncooperatively to CTS1 (7). These three probes were incubated with increasing concentrations of purified Swi5 protein, and the protein-DNA complexes were analyzed by gel retardation (Fig. 5B). The mobility of the Swi5-SIC1 protein-DNA complex was similar to that of the Swi5-HO complex, but quite different from that of the Swi5-CTS1 complex. This suggests that a single molecule of Swi5 binds to the SIC1 promoter fragment. Interestingly, the affinity of Swi5 for the SIC1 probe is threefold higher than that for the HO probe.

Gel retardation experiments were also carried out with the Ace2 protein and the three promoter fragment probes. Unlike...
the results with Swi5, incubation of the highest concentration of Ace2 with the \textit{SIC1} promoter showed two slowly migrating complexes (Fig. 5C, lane 5). The mobilities of these two bands are comparable to that seen with the \textit{CTS1} probe (Fig. 5C, lane 15), suggesting that two molecules of Ace2 can bind \textit{SIC1}. Additionally, Ace2 binds to all three promoter fragments with similar affinities. Although roughly threefold more Swi5 protein is required than for Ace2 to achieve a specific degree of binding in vitro, we do not know what fraction of each protein preparation is active for DNA binding. Thus, we cannot make any conclusions about the relative affinities of Swi5 and Ace2 for these sites.

To further define the regions bound by Swi5 and Ace2 at the \textit{SIC1} promoter, DNase I footprinting was performed (Fig. 6). With Swi5, there was one region of protection that covered the downstream binding site and extended to include two residues of the upstream site. At the highest protein concentrations, several hypersensitive sites also appeared in the region 5' of the upstream site (indicated by arrows in Fig. 6). Thus, both the gel retardation and DNase I footprinting assays identified a single binding site for Swi5 on the \textit{SIC1} promoter spanning nucleotides −163 to −133. In contrast to the data for Swi5, the region in the \textit{SIC1} promoter protected from DNase I digestion by Ace2 was larger, extending from nucleotide −172 to nucleotide −133. Thus, Ace2, at the highest protein concentration...
tested, protects both of the putative binding sites from DNase I digestion; at this protein concentration, the gel retardation assay showed that two molecules of Ace2 can bind simultaneously. We conclude that Swi5 and Ace2 can each bind to the SIC1 promoter, but there are significant differences in how they bind.

**DISCUSSION**

It has been known for many years that the CDC4, CDC34, and CDC53 genes are needed for DNA replication but not for other simultaneous cell cycle events like spindle pole body duplication or bud formation. The discovery that CDC34 encodes a ubiquitin-conjugating enzyme involved in protein degradation suggested that yeast cells must destroy proteins in order to initiate DNA replication. The study described here was initiated by the finding that Cbl/Cdk1 kinases are inactive in cdc4, cdc34, and cdc53 mutants. Our working hypothesis was that these mutants were all defective in destroying an inhibitor of Cbl/Cdk1 kinases. We set out to identify this inhibitor or factors necessary for its activity by isolating mutations that allow cdc4 mutants to overcome their G1 arrest and enter S phase. We identified three genes, RIC1 to RIC3, which are necessary to prevent DNA replication in cdc4 mutants. RIC2 proved to be identical to SIC1, which encodes the Cbl/Cdk1 inhibitor recently shown to be responsible for the G1 arrest of cdc4, cdc34, and cdc53 mutants (25). RIC1 proved to be identical to SWI5, which encodes a transcription factor responsible for activating the EGT2 (15), ASH1 (2), CDC6 (21), and RME1 (31) genes at the end of mitosis and the HO endonuclease gene in late G1. We have shown that Swi5 and its related factor, Ace2, are both capable of binding to specific SIC1 promoter sequences in vitro and that they share the task of activating SIC1 transcription transiently at the end of mitosis. Swi5 is a much more efficient activator of SIC1 transcription than Ace2, which is opposite to the situation at the CTS1 promoter, where Ace2 is much more potent, even though both proteins, Swi5 and Ace2, can bind to the CTS1 promoter in vitro. This specificity in transcriptional activation may be conferred by additional regulatory proteins (7).

**Genes regulated by Swi5.** EGT2, ASH1, CDC6, RME1, and SIC1 are transcribed transiently as cells undergo anaphase. This pattern is presumably due to the transient accumulation of Swi5 and Ace2 within nuclei at this stage of the cell cycle. Both accumulate in the cytoplasm during G2 and only enter the nuclei upon inactivation of Cbl/Cdk1 protein kinases during anaphase (6, 17, 18). Swi5 is then rapidly degraded during G1. HO, however, is not transcribed until late G1 because its transcription requires, in addition to Swi5, the late G1-specific transcription factor SBF (3). Thus, HO differs from other genes activated by Swi5 in that it does not require a high concentration of Swi5 within the nucleus when it is transcriptionally activated by SBF. HO is only transcribed in mother cells; however, this is not due to differences in the accumulation of Swi5 in mother and daughter nuclei but instead is due to the preferential accumulation of Ash1 protein in daughter nuclei (2, 28). Therefore, there is no reason to believe that, like HO, SIC1 is preferentially expressed in mother nuclei. Indeed, p40\(^{53}SCC\) function seems especially important for daughter cells (19).

**Switching cell cycle states.** Transcriptional regulation by Swi5 is just one aspect of the system controlling p40\(^{53}SCC\) accumulation during the yeast cell cycle. p40\(^{53}SCC\) is rapidly degraded during G1 and M phases, becomes more stable as cells enter G2, but is again rapidly degraded in late G1 upon activation of Cdk1 kinase by the Cln1 and Cln2 cyclins (24). It is thought that phosphorylation of p40\(^{53}SCC\) regulates its stability both as cells start the cell cycle and during G1 (22, 25). It is conceivable that Cln/Cdk1 kinases target p40\(^{53}SCC\) for proteolysis during late G1 and S phases, whereas Cbl/Cdk1 kinases target its proteolysis during G2 and M phases. If this is correct, Cbl/Cdk1 kinases and p40\(^{53}SCC\) live lives of mutual hostility; Cbl/Cdk1 inhibits accumulation of p40\(^{53}SCC\), whereas p40\(^{53}SCC\) inhibits Cbl/Cdk1 kinases. The outcome of this battle between Cbl/Cdk1 and its inhibitor switches as cells move through the cell cycle, or rather key cell cycle transitions are triggered by reversing the roles of victor and vanquished. p40\(^{53}SCC\) becomes the victor during anaphase but later loses its supremacy in late G1. Our discovery that Swi5 regulates transcription of SIC1 fits nicely within this scheme. Swi5 is prevented from entering nuclei during S, G2, and metaphase by phosphorylation of its nuclear localization signal by Cbl/Cdk1 kinases and only accumulates within the nuclei upon the inactivation of these kinases during anaphase. This is therefore another mechanism by which Cbl/Cdk1 kinases inhibit p40\(^{53}SCC\) accumulation during S, G2, and metaphase. It is another means by which changes in the outcome of the battle between p40\(^{53}SCC\) and Cbl/Cdk1 kinases are reinforced; reductions in the activity of Cbl/Cdk1 kinases during anaphase increase SIC1 transcription and thereby lead to lower Cbl/Cdk1 kinase levels.

How cells trigger the transition from a state in which Cbl/Cdk1 kinases hold sway to one in which p40\(^{53}SCC\) rules is not understood. Activation of cyclin B proteolysis via its ubiquitination by the anaphase-promoting complex, or APC (12), presumably plays an important part. Several genes encoding kinases such as CDC15 (23), CDC5 (14), and DBF2 (13) and a Ras-like GTPase, Tem1 (27), are needed for this process and for the efficient accumulation of Swi5 within nuclei, but their mode of action is not understood. More is known about how cells reverse this state of affairs in late G1. They synthesize specialized cyclins, Cln1 and Cln2, which form complexes with Cdk1 that are only weakly or not at all inhibited by p40\(^{53}SCC\) (25). It would not be surprising if yet other genes, for example, RIC3, were involved in deciding the outcome of the battle between p40\(^{53}SCC\) and Cdk1 kinases.

**ACKNOWLEDGMENTS**

We thank Christian Dahmann for initial help with the screen and Ralf Jansen, Christian Koch, and Stefan Irriger for comments on the manuscript. Ralf Jansen is also thanked for data presented in Fig. 2. We are also grateful to Etienne Schwob for useful discussions. D.J.S. was supported by National Institutes of Health grant GM48624, and K.N. was supported by the Austrian Industrial Research Promotion Fund.

**REFERENCES**

cyclin dependent kinases prevent re-replication by inhibiting the transition of origins to a pre-replicative state. Curr. Biol. 5:1257–1269.
9. Dowzer, C. Personal communication.
24. Schwob, E. Personal communication.