The Transcription Factor Swi5 Regulates Expression of the Cyclin Kinase Inhibitor p40<sup>SIC1</sup>

DUNJA KAAPP, LEENA BHOITE, DAVID J. STILLMAN, AND KIM NASMYTH

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

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DNA replication in budding yeast cells depends on the activation of the Cdc28 kinase (Cdk1 of Saccharomyces cerevisiae) associated with B-type cyclins Clb1 to Clb6. Activation of the kinase depends on proteolysis of the Cdk inhibitor p40<sup>SIC1</sup> in late G<sub>1</sub>, which is mediated by the ubiquitin-conjugating enzyme Cdc34 and two other proteins, Cdc4 and Cdc53. Inactivation of any of these three proteins prevents p40<sup>SIC1</sup> degradation and causes cells to arrest in G<sub>1</sub> with active Cln kinases but no Clb-associated Cdc28 kinase activity. Deletion of SIC1 allows these mutants to replicate. p40<sup>SIC1</sup> disappears at the G<sub>1</sub>/S transition and reappears only after nuclear division. Cell cycle-regulated proteolysis seems largely responsible for this pattern, but transcriptional control could also contribute; SIC1 RNA accumulates to high levels as cells exit M phase. To identify additional factors necessary for the inhibition of the Cdk1/Cdc28 kinase in G<sub>1</sub>, we isolated mutants that can replicate DNA in the absence of Cdc4 function. Mutations in three loci (SIC1, SWI5, and RIC3) were identified. We have shown that high SIC1 transcript levels at late M phase depend on Swi5. Swi5 accumulates in the cytoplasm during S, G<sub>2</sub>, 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 SIC1 transcription at the end of anaphase. This transcriptional control may be important for inactivation of the Cdc2/Cdk1 kinase in G<sub>2</sub>M transition and during the subsequent G<sub>1</sub> period.

DNA replication in budding yeast cells depends on the activation of Cdk1/Cdc28 kinase associated with six different B-type cyclins, Cln1 to Cln4. Cln-associated Cdk1 kinase is regulated by transcription of cyclin genes, and by accumulation of inhibitory proteins. Due largely to the onset of CLB5 and CLB6 transcription in late G<sub>1</sub> (CLB1 to 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 Cln5 and Cln6, Cln1 to Cln4 trigger replication. Normally, DNA replication is simultaneous with bud formation, but in the cdc4-1 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<sup>SIC1</sup>. p40<sup>SIC1</sup> does not inhibit Cdc28 kinase associated with Cln cyclins which also appear in late G<sub>1</sub> and are necessary for activating CLB gene transcription, turning off Cln proteolysis, and triggering p40<sup>SIC1</sup> proteolysis (19, 22, 25). p40<sup>SIC1</sup> protein accumulates to high levels in G<sub>1</sub>, disappears at the G<sub>1</sub>/S transition, and does not reaccumulate until cells complete nuclear division and reenter G<sub>1</sub>. To understand how p40<sup>SIC1</sup> regulates the activity of cyclin B/Cdc28 kinases, we need to know how p40<sup>SIC1</sup> accumulation is regulated. It has been demonstrated that the appearance of Cln1 and Cln2/Cdc28 kinases in late G<sub>1</sub> leads to the rapid proteolysis of p40<sup>SIC1</sup> (25). This instability of p40<sup>SIC1</sup> persists until cells undergo anaphase (24). It is not understood why it becomes stable in the subsequent G<sub>1</sub> period. p40<sup>SIC1</sup> proteolysis depends on three genes: CDC4, CDC34, and CDC53 (25). CDC34 encodes an E2-type ubiquitin-conjugating enzyme (11). The functions of the Cdc4 and Cdc53 proteins are currently not understood. Mutants with temperature-sensitive alleles of CDC4, CDC34, and CDC53 fail to degrade p40<sup>SIC1</sup> at the restrictive temperature and arrest in G<sub>1</sub> 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 Cln/Cdc28 kinases. Deletion of the SIC1 gene allows these mutants to activate the Cln 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 Cln kinases and thereby enter S phase. We isolated mutants in three genes, RIC1 to RIC3, that allow cells to partially overcome the replication defect of a cdc4-1 mutant. RIC2 corresponds to SIC1, and RIC1 corresponds to SWI5, which encodes a G<sub>1</sub>-specific transcription factor first identified because of its role in activating the HO endonuclease gene (29). Our data suggest that in addition to controlling the stability of p40<sup>SIC1</sup>, transcriptional control of the SIC1 gene contributes to reaccumulation of p40<sup>SIC1</sup> protein during G<sub>1</sub>. 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 SIC1 gene promoter. However, while Swi5 has a major role in transiently activating SIC1 during anaphase, Ace2 plays a minor role.

MATERIALS AND METHODS

Strains and media. All yeast strains were derivatives or were backcrossed at least three times to W303 (MATa HMLa HMRa ade2-1 trpl-1 can1-100 leu2-3,112 his3-11,15 ura3-1). The strains used in this study are listed in Table 1. Cells were grown in yeast extract-peptone medium (YPE) supplemented with 2% glucose (YPED) or raffinose (YPEB) unless otherwise stated.

Linkage analysis. To demonstrate linkage between mutations isolated in the screen and the SIC1 and 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.

BrdU incorporation assay. Stationary-phase cells of strains D6 and D8 were...
mutagenized by ethyl methanesulfonate to 50% survival and plated on YEPR at 25°C. 250,000 cells per 13-cm-diameter plate were plated. To minimize 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 a colony were in the G1 stationary phase). The colonies were then replica plated onto nitrocellulose filters (Schleicher & Schuell) on fresh YEPR 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). In the wild-type cells, budding and replication happen almost simultaneously. We reasoned that the mutants with an abolished requirement for Cdc4 function should replicate by the time 50% of the cells have budded. The incubation time on YEPR-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 diithiothreitol 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 YEPR plates at 25°C. They were then inoculated into fresh YEPR 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 (K4788) cells were synchronized by centrifugal elutriation as described previously (26). In the cdc15 release experiment, SWI5-1 HisG (K3774) and ace2::HisG (K3659) cultures were grown in YEPR at 25°C until they reached an optical density at 600 nm of 0.2. They were then transferred to 37°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 YEPR 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 0.7-kb Asp718-S61 fragment of SIC1. CMD1 and PCL1 transcripts were used as internal controls for loading and for the cell cycle progression, respectively. Quantifications were done with a Molecular Dynamics PhosphorImager.

In vitro DNA-binding assays. Purification of Swi5 (6xHis-tagged Swi5 fusion protein) and Ace2 (glutathione S-transferase–Ace2 fusion protein), gel retardation assays, and DNaF I footprinting were performed as described previously (7). DNA probes were end labeled with T4 polynucleotide kinase and [γ-32P] ATP (3,000 Ci/mmol). The SIC1 probe for gel retardation was a 221-bp Asp718-TagI fragment from the S15 promoter. The HO probe was a 206-bp BglII-EcoRII 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 EcoRHI-HindIII fragment from plasmid M1818 (7) that contains two Ace2 binding sites. The SIC1 probe for DNase I footprinting was a 461-bp EcoRII-Asp718 fragment from the S15 promoter, labeled at the Asp718 end.

RESULTS

Screen for suppressors of cdc4 mutation. To identify genes required for the inhibition of the Cbl/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 × 106 cells of the cdc4-1 strains (K3995 and K3996), only 108 colonies grew at 35°C, and 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 YEPR plates at 25°C, replica plated onto a nitrocellulose filter on fresh YEPR 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 allelism groups, RIC1 to RIC3 (for replicates in cdc4). We identified six alleles of RIC1, two alleles of RIC2, and two alleles of RIC3.

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 ric1A cdc4-1 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/sic1A 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 p40(Ca) 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 Cbl5 kinase expression or activity. Therefore, we tested whether a swi5 null mutation suppresses the DNA replication defect associated with cdc4. The FACS 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 swi5Δ cdc4-1 (D609) 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 CDC4Δ background, and, therefore, we have not attempted to clone the gene.

**SI C1 transcription is regulated by Swi5.** SWI5 encodes a transcription factor needed for the expression of the HO endonuclease involved in mating-type switching (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 Cln- and/or Cdc28-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 the 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 level 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-minute time point after elutriation and 140 to 170 min later in the subsequent M/G1 phases). In the swi5 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 ace2 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

**FIG. 1.** Mutations in RIC1, RIC2, and RIC3 genes as well as deletions of SIC1 and SWI5 partially suppress a cdc4-1 mutation. Stationary-phase cells from 1-day-old patches grown at 25°C were inoculated into fresh YEPD at 37°C. Samples for FACSscan were taken 0, 2, and 4 h after temperature shift. (A) DNA content of wild-type (WT [K699]), cdc4-1 (K3996), cdc4-1 sic1Δ (K4163), cdc4-1 swi5Δ (D69), cdc4-1 ric1-1 (D90), cdc4-1 ric2-1 (D58), and cdc4-1 ric3-1 (D52) strains. (B) DNA content of diploid of wild-type (WT), cdc4-1/cdc4-1, cdc4-1/cdc4-1 sic1/sic1, and cdc4/cdc4 sic1/sic1 strains.

**FIG. 2.** SIC1 transcript is regulated by Swi5. Results are from Northern blot analysis of the SIC1 mRNA levels of exponentially growing wild-type (WT [K699]), swi5Δ (K1998), ace2Δ (K3772), and swi5Δ ace2Δ (K3773) cultures. CMD1 RNA serves as an internal loading control.
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 SIC1 promoter showed two slowly migrating complexes (Fig. 5C, lane 5). The mobilities of these two bands are comparable to that seen with the CTS1 probe (Fig. 5C, lane 15), suggesting that two molecules of Ace2 can bind 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 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 SIC1 promoter spanning nucleotides -163 to -133. In contrast to the data for Swi5, the region in the 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 Clb/Cdk1 kinases are inactive in *cde4*, *cdc34*, and *cde53* mutants. Our working hypothesis was that these mutants were all defective in destroying an inhibitor of Clb/Cdk1 kinases. We set out to identify this inhibitor or factors necessary for its activity by isolating mutations that allow factors necessary for its activity by isolating mutations that code a ubiquitin-conjugating enzyme involved in protein degradation. We have shown that Swi5 and its related factor, *SWI5* genes at the end of mitosis and the *HO* was initiated by the finding that Clb/Cdk1 kinases are inactive in order to initiate DNA replication. The study described here was initiated by the finding that Clb/Cdk1 kinases are inactive in *cde4*, *cde34*, and *cde53* mutants. Our working hypothesis was that these mutants were all defective in destroying an inhibitor of Clb/Cdk1 kinases. We set out to identify this inhibitor or factors necessary for its activity by isolating mutations that allow factors necessary for its activity by isolating mutations that code a ubiquitin-conjugating enzyme involved in protein degradation.

**Switching cell cycle states.** Transcriptional regulation by Swi5 is just one aspect of the system controlling p40\textsubscript{SIC1} accumulation during the yeast cell cycle. p40\textsubscript{SIC1} is rapidly degraded during G\textsubscript{1}, and M phases, becomes more stable as cells enter G\textsubscript{1}, but is again rapidly degraded in late G\textsubscript{1} upon activation of Cdk1 kinase by the Cln1 and Cln2 cyclins (24). It is thought that phosphorylation of p40\textsubscript{SIC1} regulates its stability both as cells start the cell cycle and during G\textsubscript{1} (22, 25). It is conceivable that Cln/Cdk1 kinases target p40\textsubscript{SIC1} for proteolysis during late G\textsubscript{1} and S phases, whereas Clb/Cdk1 kinases target its proteolysis during G\textsubscript{2} and M phases. If this is correct, Clb/Cdk1 kinases and p40\textsubscript{SIC1} live lives of mutual hostility; Clb/Cdk1 inhibits accumulation of p40\textsubscript{SIC1}, whereas p40\textsubscript{SIC1} inhibits Clb/Cdk1 kinases. The outcome of this battle between Clb/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\textsubscript{SIC1} becomes the victor during anaphase but later loses its supremacy in late G\textsubscript{1}. Our discovery that Swi5 regulates transcription of *SIC1* fits nicely within this scheme. Swi5 is prevented from entering nuclei during S, G\textsubscript{2}, and metaphase by phosphorylation of its nuclear localization signal by Clb/Cdk1 kinases and only accumulates within the nuclei upon the inactivation of these kinases during anaphase. This is therefore another mechanism by which Clb/Cdk1 kinases inhibit p40\textsubscript{SIC1} accumulation during S, G\textsubscript{2}, and metaphase. It is another means by which changes in the outcome of the battle between p40\textsubscript{SIC1} and Clb/Cdk1 kinases are reinforced; reductions in the activity of Clb/Cdk1 kinases during anaphase increase SIC1 transcription and thereby lead to lower Clb/Cdk1 kinase levels.

How cells trigger the transition from a state in which Clb/Cdk1 kinases hold sway to one in which p40\textsubscript{SIC1} 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 G\textsubscript{1}. They synthesize specialized cyclins, Cln1 and Cln2, which form complexes with Cdk1 that are only weakly or not at all inhibited by p40\textsubscript{SIC1} (25). It would not be surprising if yet other genes, for example, *RIC3*, were involved in deciding the outcome of the battle between p40\textsubscript{SIC1} and Cdk1 kinases.

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3. Regulation of SIC1 by Swi5 or Ace2 may be not essential for proper timing of cell cycle progression; however, an inappropriate S1C1 level might have more subtle effects, like the ability of cells to establish prereplicative complex on the origins of replication at M/G\textsubscript{1} transition, a process inhibited by the Clb/Cdk1 kinase (20, 24).

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