The Swi5 Transcription Factor of *Saccharomyces cerevisiae* Has a Role in Exit From Mitosis Through Induction of the cdk-Inhibitor Sic1 in Telophase

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ABSTRACT

Deactivation of the B cyclin kinase (Cdc28/Clb) drives the telophase to G1 cell cycle transition. Here we investigate one of the control pathways that contributes to kinase deactivation, involving the cell cycle-regulated production of the cdk inhibitor Sic1. We show that the cell cycle timing of *SIC1* expression depends on the transcription factor Swi5, and that Swi5-dependent *SIC1* expression begins during telophase. In contrast to Swi5, the related transcription factor Ace2, which can also induce *SIC1* expression, is not active during telophase. The functional consequence of Swi5-regulated *SIC1* expression *in vivo* is that both *sic1*Δ and *swi5*Δ strains have identical mitotic exit-related phenotypes. First, both are synthetically lethal with *dbf2*Δ, resulting in cell cycle arrest in telophase. Second, both are hypersensitive to overexpression of the B cyclin *CLB2*. Thus, Swi5-dependent activation of the *SIC1* gene contributes to the deactivation of the B cyclin kinase, and hence exit from mitosis.

EXIT from mitosis has come to mean the cell cycle events between the end of metaphase and the beginning of G1, and is controlled in part by deactivation of the B cyclin kinase activity. The B cyclin kinase consists of an activating B cyclin subunit in association with a cyclin-dependent catalytic subunit (cdk), and deactivation of this kinase involves regulated degradation of the cyclin subunit (MURRAY *et al.* 1989). However, B cyclin kinase deactivation is not required for sister chromatid separation nor for anaphase chromosome movements in either Xenopus or yeast (HOLLOWAY *et al.* 1993; SURANA *et al.* 1993), but is required for the telophase to G1 cell cycle transition, including spindle disassembly, formation of two separate nuclei, and cytokinesis (SURANA *et al.* 1993). Thus, there are at least two independently regulated steps during mitotic exit; first, the separation of sister chromatids during the metaphase to anaphase transition, and second, the deactivation of B cyclin kinase at the end of telophase.

One mechanism of B cyclin kinase deactivation is the degradation of the activating cyclin subunit by ubiquitin-mediated proteolysis, which is targeted by a conserved sequence in the cyclin protein called the "destruction box" (GLOTZER *et al.* 1991). Several genes encoding enzymes of the ubiquitin system are known to be involved in cyclin degradation. These include the ubiquitin-activating enzyme Ubc4 in yeast and Xenopus (IRNIGER *et al.* 1995; KING *et al.* 1995), and the ubiquitin-conjugating enzyme Ubc9 in yeast (SEUFERT *et al.* 1995). Another factor that is required for cyclin degradation is the anaphase promoting complex (APC), which is conserved in organisms from yeast to human (IRNIGER *et al.* 1995; KING *et al.* 1995; TUGENDREICH *et al.* 1995). As its name suggests, APC is a protein complex required for cell cycle progression from metaphase into anaphase, playing a role in the regulation of sister chromatid separation. Like the many budding yeast genes mentioned below, APC also appears to be required for the telophase to G1 transition (IRNIGER *et al.* 1995), presumably because of its role in B cyclin degradation. However, APC is active throughout the interval from the end of metaphase until some time into G1, and therefore it alone cannot account for the cell cycle timing of B cyclin disappearance at the telophase to G1 transition.

In budding yeast there are many genes that, in contrast to APC, are more specifically required at the end of telophase, and that may therefore be regulating B cyclin degradation at this stage. These include *DBF2, DBF20, CDC5* and *CDC15,* which encode protein kinases, *CDC14,* which encodes a phosphatase, *TEM1* and *LTE1,* which encode a monomeric GTP-binding protein and G-nucleotide exchange factor, respectively, and the oncogene homologues *RAS1, RAS2* and *RSR1* (JOHNSTON *et al.* 1990; SCHWEITZER and PHILIPPSEN 1991; TOYN *et al.* 1991; WAN *et al.* 1992; KITADA *et al.* 1993; SHIRAYAMA *et al.* 1994a,b; MORISHITA *et al.* 1995). There are a great many genetic interactions between these genes, suggesting that they communicate with one another in the cell, and may be involved in a signal transduction network that controls the deactivation of the B cyclin kinase at the end of mitosis. The relationship, if any, of these genes to APC is not clear at present.

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In addition to cyclin degradation, cdk inhibitors could also play a role in deactivation of the B cyclin kinase during mitotic exit. One such cdk inhibitor in yeast is Sic1, a potent inhibitor of the B cyclin kinase *in vitro* (MENDENHALL 1993) encoded by the *SIC1/SDB25* gene (DONOVAN *et al.* 1994; NUGROHO and MENDENHALL 1994). Cells lacking Sic1 have a range of phenotypes suggesting a role during mitosis (DONOVAN *et al.* 1994; NUGROHO and MENDENHALL 1994). In addition, Sic1 is involved in regulation of the G1 to S phase transition through inhibition of the S phase kinase (SCHWOB *et al.* 1994). Previously, we showed that the *SIC1* gene interacts genetically with the *DBF2* gene, and that *SIC1* gene expression is under cell cycle control in late mitosis to early G1. The Sic1 protein is also unstable, being present in cells only around the end of mitosis and into G1 (DONOVAN *et al.* 1994; SCHWOB *et al.* 1994). The Dbf2 protein kinase is required for the telophase to G1 transition and also plays a role in the G1 to S phase transition (JOHNSTON *et al.* 1990; TOYN and JOHNSTON 1994). Thus, there is evidence that both Sic1 and Dbf2 have dual roles in control of the cell cycle; first, during entry into S phase, and second, during exit from mitosis. The current focus on the control of exit from mitosis has concerned the degradation of cyclins, but our previous data suggest that Sic1 also plays a role in mitotic exit. We therefore set out to elucidate the mechanisms that regulate the activity of Sic1 during the control of exit from mitosis. Since the gene is cell cycle regulated and the protein is unstable, we have concentrated on how *SIC1* gene expression contributes to its regulation.

In the present study, we show that the function of Sic1 in mitotic exit requires Swi5-dependent gene expression. The *SWI5* gene encodes a zinc finger motif transcription factor and is expressed under cell cycle control during G2 and M phase (LYDALL *et al.* 1991). Swi5 protein enters the nucleus during late mitosis and G1, and activates specific genes such as *HO, CDC6, RME1, ASH1* and *EGT2* (STILLMAN *et al.* 1988; NASMYTH *et al.* 1990; PIATTI *et al.* 1995; TOONE *et al.* 1995; BOBOLA *et al.* 1996; KOVACECH *et al.* 1996; SIL and HERSKOWITZ 1996). The regulation of nuclear entry of Swi5 depends on the phosphorylation state of its nuclear localization signal (NLS), dephosphorylation being required for nuclear entry. The phosphorylation is catalyzed by the B cyclin kinase, suggesting that the bulk of Swi5 enters the nucleus as a result of deactivation of the B cyclin kinase during exit from mitosis (MOLL *et al.* 1991). In G1, after Swi5 has entered the nucleus and caused gene expression, the Swi5 protein is degraded (NASMYTH *et al.* 1990; TEBB *et al.* 1993). Surprisingly, we find that Swi5 causes sufficient expression of *SIC1* during telophase, when Swi5 protein is present at a low level in the nucleus, to play a role in mitotic exit.

MATERIALS AND METHODS

Media and growth conditions: Yeast was cultured in YP medium (1% yeast extract, 2% bactopeptone) supplemented with 2% raffinose, galactose or glucose (YPD) as indicated, or minimal medium made using yeast nitrogen base (DIFCO) according to the manufacturer's instructions. For induction of galactose-dependent *CLB2* expression, a final concentration of 2% galactose was added to log phase cultures growing in YP raffinose. For methionine-dependent repression of *SIC1*, a final concentration of 0.5 mM L-methionine was added to log phase cultures growing in minimal medium. Synchronous cultures were prepared using the α-factor method; synthetic α-factor peptide mating pheromone (final concentration 3.5 μg/ml) was added to log phase cultures of *MAT***a** cells growing in YPD. When the cells had arrested in G1 (usually 3.5 hr), the α-factor was removed by filtration and the cells were resuspended in fresh medium. For *ts* mutants, cultures were incubated at either a permissive temperature of 25° or a restrictive temperature of 37°.

Strains and plasmid constructs: The *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Molecular cloning methods were as described in SAMBROOK *et al.* (1991). For amplification of DNA by PCR, Taq polymerase and buffer supplied by the manufacturer was used. (The PCR process is covered by US patents 4.683.202, 4.683.195, and 4.965.188, or their foreign counterparts, issued to Hoffman-La Roche). Yeast plasmid constructs were based on either the Yplac shuttle vectors (GIETZ and SUGINO 1988) or the pRS shuttle vectors (SIKORSKI and HIETER 1989).

For chromosomal integration of the *SIC1* gene, a 2.5-kb *Eco*RI-*Hin*dIII fragment containing the *SIC1* gene, including its entire promoter region (DONOVAN *et al.* 1994), was ligated between the *Eco*RI and *Hin*dIII sites of YIplac128 to obtain YIp*SIC1*. YIplac128 and YIp*SIC1* were linearized by digestion with *Cla*I before selection of *LEU2* transformants in yeast.

For methionine-repressible expression of *SIC1* in yeast, a 0.5-kb *Xba*I-*Eco*RV fragment containing the *MET3* promoter from pHAM8 (MOUNTAIN and KORCH 1991) was ligated between the *Xba*I and *Sma*I sites of pRS306 to obtain YIp*MET*. Subsequently, a 1-kb *Eco*RI-*Eco*RI fragment containing *SIC1* was made by PCR amplification using the oligonucleotides CCGGAATTCATGACTCCTTCCACCCCACC and TTGGAATTCTTTGCAAATAAATGTAGAA and ligated into the *Eco*RI site of YIp*MET* to obtain YIp*MET-SIC1*. This plasmid was integrated into the yeast genome after digestion with either *Nco*I or *Stu*I, which cut uniquely in the *URA3* marker. To obtain the high copy number plasmid YEp*MET-SIC1*, a 1.8-kb *Xba*I-*Hin*dIII fragment containing *MET-SIC1* from YIp*MET-SIC1* was ligated between the *Xba*I and *Hin*dIII sites of YEplac112 (a *TRP1* 2μ shuttle vector).

For overexpression of *SIC1* in yeast under the control of the phosphoglycerate kinase (PGK) promoter, a 1.5-kb *Hin*dIII-*Bgl*II fragment containing the PGK promoter from pMA91 (MELLOR *et al.* 1983) was ligated between the *Hin*dIII and *Bam*HI sites of YIplac128 to obtain YIpPGK. Subsequently, a 1-kb *Eco*RI-*Eco*RI fragment containing *SIC1* from YIp*MET-SIC1* was ligated into the *Eco*RI site of YIpPGK to obtain YIpPGK-*SIC1*. This plasmid was integrated into the yeast genome after digestion with *Eco*RV, which cuts uniquely in the *LEU2* marker.

For chromosomal integration of *SWI5* or *DBF2*, a 4-kb *Kpn*I-*Sph*I yeast genomic fragment containing *SWI5* from YEp-*SWI5* (a gift from Dr. GERALDINE BUTLER, University College, Dublin) was ligated between the *Kpn*I and *Sph*I sites of YIplac211 to obtain YIp*SWI5*, or a 2-kb *Bgl*II-*Bam*HI fragment containing the *c-myc* epitope-tagged *DBF2* gene from pRS304-*DBF2myc* (TOYN and JOHNSTON 1994) was ligated into the *Bam*HI site of YIplac211 to obtain YIp*DBF2*. These plasmids were integrated into the yeast genome after digestion with *Stu*I or *Apa*I, respectively, which cut uniquely in the *URA3* marker. The

TABLE 1

Yeast strains

Name	Genotype	Source
CG379	*MATα ura3-52 leu2-3,112 trp1-289 his7*	C. GIROUX, Detroit
DTY59	*MATα his6 ura3 leu2 ace1Δ CUP1^R*	G. BUTLER, UC Dublin
DTY87	*MATα his6 ura3 leu2 ace1Δ CUP1^R swi5Δ::LEU2*	G. BUTLER, UC Dublin
DTY91	*MATα his6 ura3 leu2 ace1Δ CUP1^R ace2Δ::URA3*	G. BUTLER, UC Dublin
DTY93	*MATα his6 ura3 leu2 ace1Δ CUP1^R swi5Δ::LEU2 ace2Δ::URA3*	G. BUTLER, UC Dublin
JD100	*MATα ura3-52 leu2-3,112 trp1-289 his7 sic1Δ::TRP1*	DONOVAN *et al.* (1994)
J227-24C	*MATa ura3-52 trp1 his3Δ leu2-3,112 can1^R dbf2Δ::LEU2*	This study
J250-16A	*MATa his3Δ ura3-52 trp1 leu2-3,112 dbf2Δ::LEU2 sic1Δ::TRP1* YIp*MET-SIC1(::URA3)*	J227-24C + YIp*MET-SIC1* × JD100 + YIp*MET-SIC1*
J252-1B	*MATα ura3-52 trp1-289 ade5 leu2-3,112 swi5Δ::LEU2*	DTY87 × S7-4A
J252-9A	*MATa ura3-52 trp1-289 his6/7 leu2-3,112 swi5Δ::LEU2*	DTY87 × S7-4A
J252-17C	*MATα ura3-52 his6/7 leu2-3,112 swi5Δ::LEU2*	DTY87 × S7-4A
J252-18D	*MATa ura3-52 leu2-3,112 dbf2::URA3*	DTY87 × S7-4A
J254-2D	*MATa ura3-52 leu2-3,112 trp1 CLB2-HA dbf2-2*	This study
J261-2D	*MATα ura3-52 leu2-3,112 ade5 swi5Δ::LEU2 dbf2Δ::LEU2* YEp*MET-SIC1(::URA3)*	J227-24C + YEp*MET-SIC1* × J252-1B
J263-2D	*MATa ura3-52 leu2-3,112 trp1 his3 dbf2-2 sic1Δ::TRP1*	This study
KKY021	*MATa ura3-52 leu2-3,112 trp1-289 cdc5^{ts}::URA3*	KITADA *et al.* (1993)
L207-R12	*MATa ura3-52 leu2-3,112 tyr1 ade cdc15*	This study
S7-4A	*MATa ura3-52 leu2-3,112 trp1-289 his7 ade5 dbf2Δ::URA3*	TOYN *et al.* (1991)

YIplac211 plasmid was used as the empty vector control in experiments with these plasmids.

For galactose-inducible overexpression of *CLB2* we used the plasmid YIp*G7CLB2* (SHIRAYAMA *et al.* 1994b). This plasmid was digested with *Stu*I, which cuts uniquely in the *URA3* marker, before integration into the yeast genome. Ura$^+$ transformants were then tested by Southern analysis of *Eco*RI-digested genomic DNA using a *CLB2* probe. We found that the transformants had a variable number of stably integrated copies of the plasmid, allowing the selection of yeast strains bearing one, two, or more, copies of the galactose-inducible *CLB2* allele.

For detection of Swi5-*SIC1* promoter complexes *in vitro*, and for indirect immunofluorescence studies, *SWI5* bearing three tandem copies of the *c-myc* epitope tag at its C-terminus was expressed under the control of the PGK promoter on plasmid YIpPGK-*SWI5-3myc*. This plasmid was linearized by partial digestion using *Bst*XI, which cuts in the *LEU2* marker and in the PGK promoter, before integration into the yeast genome. In all Leu$^+$ transformants tested, Western blotting using the 9E10 anti-*c-myc* monoclonal antibody (EVAN *et al.* 1985) detected the Swi5-3myc protein with a molecular weight of *ca.* 100 kD, as expected. The details of the plasmid construction are as follows. A 0.6-kb *Kpn*I-*Kpn*I fragment containing the 3′ end of *SWI5* was made by PCR amplification using the oligonucleotides CGGGGTACCATGGGATCCTCGCCTTCT-CCCGTTCTT and CGGGGTACCCTATCTAGACCTTTG-ATTAGTTTTCATTGGCG and ligated into the *Kpn*I site of YIpPGK. This introduced a unique *Xba*I site immediately upstream of the *SWI5* stop codon. Subsequently, a 120-bp *Xba*I-*Xba*I fragment encoding the triple *myc* epitope tag from pUC119-*3myc* (Dr. S. KRON, Whitehead Institute, Cambridge, MA) was ligated into the introduced *Xba*I site at the 3′ end of *SWI5* to obtain the plasmid YIp*5myc*. The rest of the *SWI5* sequence was cloned as a 2-kb *Eco*RI-*Hin*dIII fragment by PCR amplification using the oligonucleotides TTTGAATTCGAT-GGATACATCAAACT and GTTGATCTTCAAGCTTG, in which an *Eco*RI site was introduced immediately upstream of the start codon, and the *Hin*dIII site was in the natural coding sequence of *SWI5*. This *SWI5* PCR fragment was ligated together with two other fragments: a 3.5-kb *Aat*II-*Eco*RI fragment containing the PGK promoter and bacterial sequences from YIpPGK, and a 2.2-kb *Hin*dIII-*Aat*II fragment containing the 3′ coding region of *SWI5* encoding the triple *myc* epitope tag and the *LEU2* marker from Yip*5myc*. Plasmids were introduced into yeast treated with Lithium acetate (ITO *et al.* 1983).

Determination of mRNA levels and *B* cyclin kinase activity: The detection of mRNA transcripts from yeast by Northern blotting was carried out as previously described (JOHNSTON *et al.* 1990; KITADA *et al.* 1993) using radiolabelled probes corresponding to the gene coding sequences. Assay of B cyclin kinase was carried out by determination of total H1 kinase activity in crude protein extracts (SURANA *et al.* 1991). Radioactive label on blots or gels was quantitated using phosphorimage analysis (Molecular Dynamics).

Determination of cell numbers and the proportion with divided chromatin: The concentration of cells in growing cultures was determined using a particle counter (Coulter Electronics). Yeast culture samples were lightly sonicated to disperse clumps before dilution and counting. To determine the proportion of cells with divided chromatin, culture samples were sonicated to disperse clumps and fixed by addition of an equal volume of ethanol. The cells were resuspended in a solution of 0.1 μg/ml DAPI in 150 mM NaCl, then observed by fluorescence microscopy at 1000× magnification. At least 100 cells were observed in each sample to determine the percentage of cells with divided chromatin.

RESULTS

Swi5 controls the cell cycle expression of the *SIC1* gene during telophase and G1: We have shown that the *SIC1* gene is expressed under cell cycle control at around the end of M phase (DONOVAN *et al.* 1994). Two transcription factors known to regulate gene expression

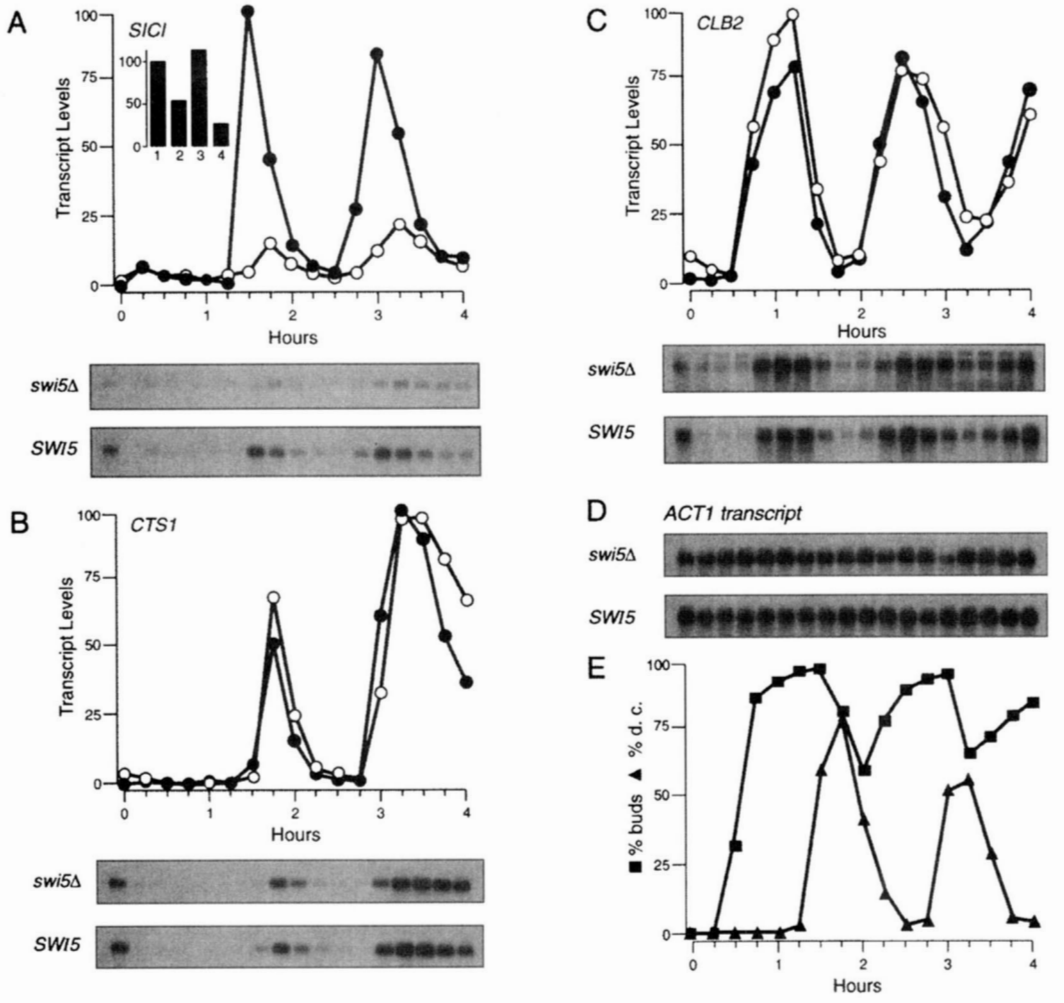


FIGURE 1.—Swi5 controls the cell cycle timing of *SIC1* gene expression. Cultures of strain J252-9A (*swi5*Δ) containing either the plasmid YIp*SWI5* (●) or control plasmid YIplac211 (○) were synchronized by the α-factor method and samples were taken for Northern blot analysis. The Northern blot was quantitated by phosphorimage analysis and normalized to the level of actin mRNA detected on the same blot. The results of quantitation are shown in graphical form above the corresponding northern blots. (A) *SIC1* mRNA, (B) *CTS1* (chitinase) mRNA, (C) *CLB2* mRNA, (D) *ACT1* (actin) mRNA, and (E) percentages of cells with buds (■) and divided chromatin (▲) in the J252-9A + YIp*SWI5* culture. The inset in A shows the levels of *SIC1* mRNA in log phase cultures of DTY59 (lane 1, wild-type), DTY87 (lane 2, *swi5*Δ), DTY91 (lane 3, *ace2*Δ) and DTY93 (lane 4, *swi5*Δ *ace2*Δ).

at approximately this stage of the cell cycle are Swi5 and Ace2 (DOHRMANN *et al.* 1992). The expression of *SIC1* mRNA in *swi5*Δ, *ace2*Δ, *swi5*Δ*ace2*Δ, and isogenic wild-type strains during log phase growth was therefore examined by Northern analysis (Figure 1A inset). The level of transcript was reduced in the *swi5*Δ strain, and further reduced in the *swi5*Δ*ace2*Δ double mutant, as recently shown by JANSEN *et al.* (1996). This suggests that in wild-type cells, Swi5 is responsible for most, but not all, of the *SIC1* expression, and that in the absence of Swi5, Ace2 is responsible for part of the residual *SIC1* expression. To determine the role of Swi5 in the cell cycle timing of *SIC1* expression, synchronous cultures of *swi5*Δ and an isogenic wild-type strain were prepared by the α-factor method, and samples were taken to determine *SIC1* mRNA levels (Figure 1). As expected, in the *SWI5* control strain, *SIC1* was strongly cell cycle regulated (DONOVAN *et al.* 1994). In marked contrast, in the *swi5*Δ strain, peak levels of *SIC1* mRNA were reduced. The regulation of the *CTS1* mRNA, which is controlled exclusively by Ace2, and the *CLB2* mRNA was not affected by the absence of Swi5. As previously noted, the *CTS1* mRNA level was lower in the first cell cycle after synchronization by α-factor (DOHRMANN *et al.* 1992). Significantly, *SIC1* was expressed distinctly earlier than *CTS1* in the wild-type control strain, but, in the *swi5*Δ strain, the residual *SIC1* expression occurred later in the cell cycle, coinciding with the cell cycle timing of *CTS1*. This is consistent with the residual *SIC1* expression being dependent on Ace2 (Figure 1A inset). Thus, the precise cell cycle timing of *SIC1* expression depended on the presence of Swi5, suggesting not only that Swi5 plays the predominant role in *SIC1* expression, but also that the Swi5 and Ace2 transcription factors are differentially regulated, Swi5 activity being earlier in the cell cycle than Ace2 activity.

We proposed previously that Sic1 plays a role in the control of exit from mitosis (DONOVAN *et al.* 1994), and since the Sic1 protein is unstable it would therefore have to be produced before the end of mitosis. Therefore we examined the possibility that Swi5 may act before the end of mitosis. However, the analysis of synchronous cultures alone is not sufficient to establish whether the cell cycle regulated expression of *SIC1* actually took place before the end of mitosis or immediately afterward in early G1. We therefore analyzed telophase-arrested cells to determine whether *SIC1* expression took place, and whether expression was Swi5-depen-

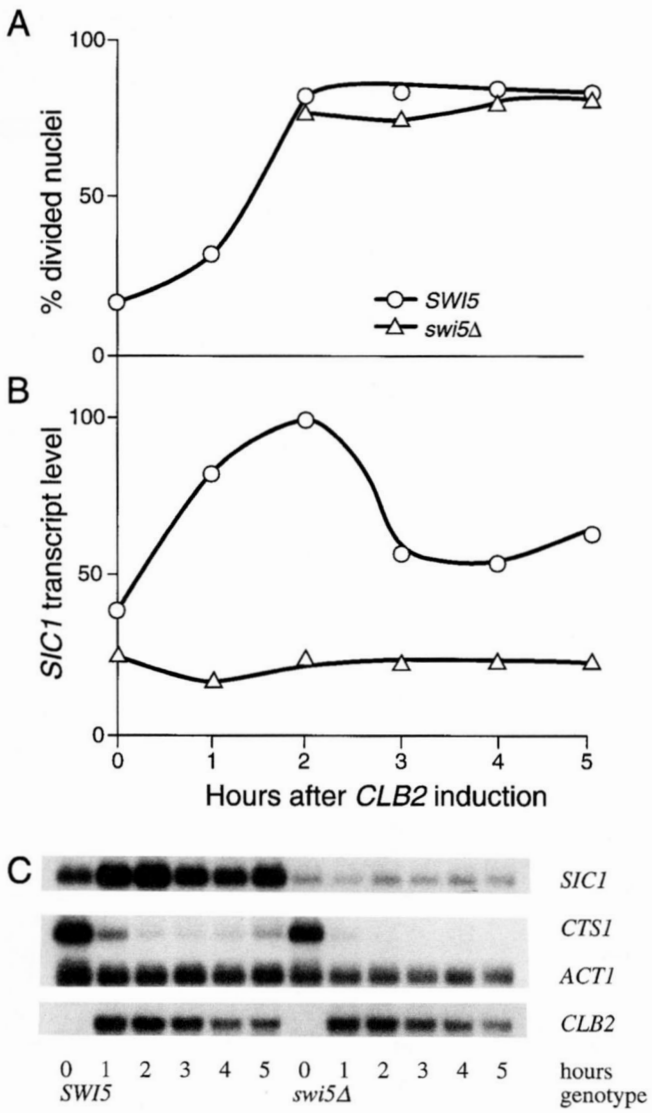


FIGURE 2.—Swi5 induces *SIC1* gene expression during telophase. Log phase cultures of strains DTY59 (*SWI5*, ○) and DTY87 (*swi5*Δ, △) both containing two integrated copies of plasmid YIp*G7CLB2* (galactose-inducible *CLB2* expression) were grown in YP raffinose medium at 30°. Galactose was added at time zero, samples were taken for Northern blot analysis, and quantitated as for Figure 1. (A) Percentage of cells with large buds and divided chromatin. (B) Relative levels of *SIC1* mRNA normalized to actin mRNA levels detected on the same blot. (C) Autoradiogram of the Northern blot. The yeast strain, the hours after galactose addition, and the mRNA detected are indicated.

dent. Cell cycle arrest in telophase can be obtained either by expression of nondegradable cyclin mutants or by very high overexpression of the B cyclin Clb2 (SURANA *et al.* 1993). Two copies of a *GAL7-CLB2* construct (SHIRAYAMA *et al.* 1994b) were therefore integrated into *swi5*Δ and isogenic wild-type strains. Log phase cultures were prepared in YP raffinose medium, and galactose was added to induce *CLB2* expression. The cells in both cultures induced *CLB2* expression to the same level (Figure 2), and arrested in telophase as judged by the proportion of cells with divided nuclei. The expression of *SIC1* mRNA in the *SWI5* control culture increased during the cell cycle arrest in telophase and was over fourfold higher in the control than in the *swi5*Δ strain. In contrast, the *CTS1* transcript, which is expressed specifically under the control of the Ace2 transcription factor, was not expressed in telophase. Two conclusions can be drawn from this experiment. First, *SIC1* is expressed in telophase under the control of the Swi5 transcription factor. Incidentally, *SIC1* mRNA expression was also found to be Swi5-dependent during a *dbf2*-mediated telophase arrest (data not shown), confirming that Swi5 activity during telophase does not depend on the method used to obtain telophase cells. Second, as suggested in Figure 1, the Swi5 and Ace2 transcription factors are clearly active at distinct stages of the cell cycle: Swi5 becomes active in telophase, and Ace2 becomes active after the transition into G1.

***SIC1* mRNA expression increases during the telophase to G1 transition:** During telophase the bulk of the Swi5 protein is retained in the cytoplasm, and yet Swi5-dependent *SIC1* expression takes place. The question therefore arises as to what effect the increased rate of nuclear entry of Swi5 has in the regulation of *SIC1* expression during the transition from telophase to G1. To analyze this, a synchronous culture of a *dbf2* strain was prepared at the permissive temperature by the α-factor method. Part of this culture was then shifted to the restrictive temperature after 1 hr to synchronously arrest the cell cycle in telophase, and then shifted back to the permissive temperature to allow a synchronous transition into G1. Samples were taken to determine the level of *SIC1* and *SWI5* mRNA by Northern blotting (Figure 3). In the control culture, which was incubated throughout at the permissive temperature, *SIC1* and *SWI5* expression were cell cycle regulated as expected. At the restrictive temperature the cells arrested in telophase, *SWI5* expression was maintained at a constant level, and a low level of *SIC1* expression took place. After shifting back to the permissive temperature *SWI5* expression went down and *SIC1* expression rapidly increased, presumably due to the rapid influx of the Swi5 protein into the nucleus as the cells exited mitosis (NASMYTH *et al.* 1990). Thus, the level of *SIC1* expression rapidly changes from a low level during telophase to a high level during the telophase to G1 transition.

Deletion of *SIC1* causes a delay at the telophase to G1 cell cycle transition: Sic1 has multiple roles in the cell cycle and is nonessential for growth in culture. As a result, deletion of the *SIC1* gene alone has partial effects on the timings of different stages of the cell cycle. To investigate specifically the effect of the *sic1*Δ allele on the telophase to G1 transition, we made highly synchronized *sic1*Δ and *SIC1* control cultures by a combination of the α-factor method and release from a *dbf2*-induced cell cycle arrest (Figure 4). This produced a synchronous population of telophase cells in both cul-

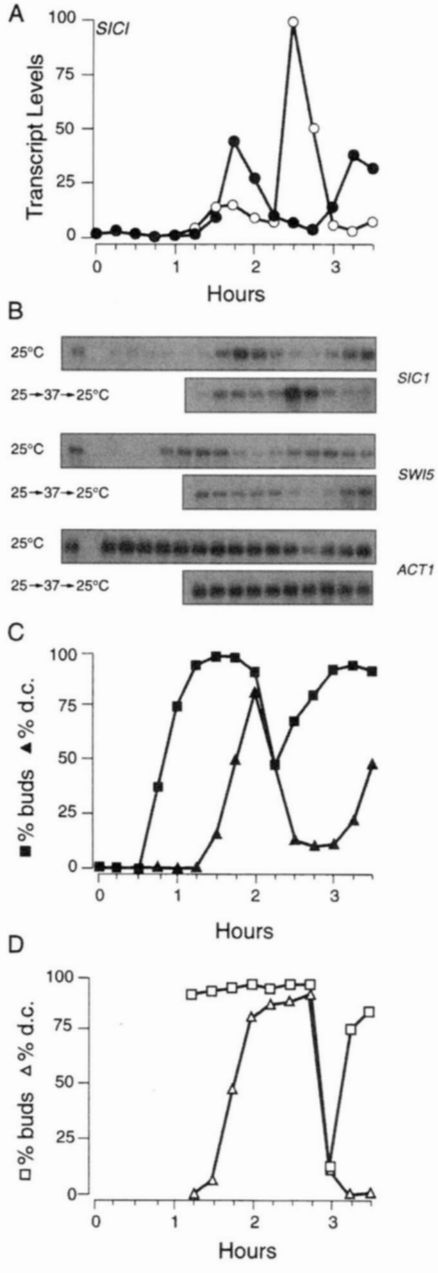


FIGURE 3.—*SIC1* expression increases during the telophase to G1 transition. Strain J254-2D (*dbf2*) was synchronized at 25° by the α-factor method. After 1 hr part of the culture was shifted to 37° and incubated for 75 min before shifting back to 25° for the remainder of the experiment. Samples were taken for Northern blot analysis. (A) The level of *SIC1* transcript in the temperature shifted culture (○) and the culture at 25° (●) was normalized to the level of *ACT1* transcript on the same blot. (B) Autoradiograms of hybridized *SIC1*, *SWI5*, and *ACT1* probes on the Northern blot; the samples from the 25° culture and the temperature shifted culture are indicated. The times at which the samples were taken correspond to the axis of A. The sample shown on the extreme left of the 25° samples was taken from the log phase culture before synchronization. (C) The percentage of cells with buds (■) and with divided chromatin (▲) in the 25° culture. (D) The percentage of cells with buds (□) and with divided chromatin (△) in the temperature-shifted culture.

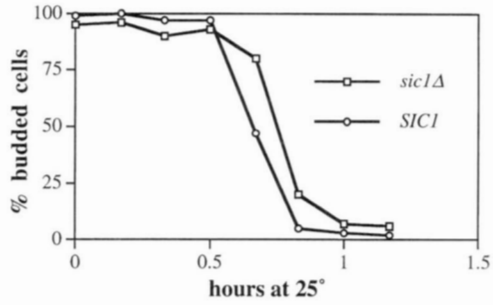


FIGURE 4.—Lack of the *SIC1* gene causes a delay in telophase. YIp*SIC1* and the control plasmid YIplac128 were introduced into strain J263-2D (*dbf2 sic1*Δ). The resulting *SIC1* and *sic1*Δ strains (see legend) were then synchronized by a combination of the α-factor method and temperature shifts. First, the culture was synchronized by the α-factor method at 25°, Second, after 60 min at 25°, the synchronous cultures were shifted to 37° for 80 min, resulting a uniform population of telophase cells (>95% with divided chromatin). The cultures were then shifted back to 25° (time 0), and samples were taken to determine the percentage of budded cells in the culture.

tures, allowing the rates of progression from telophase into G1 to be compared. After synchronization, greater than 95% of the cells in both cultures had large buds and divided chromatin, indicative of telophase, and after 30 min incubation the cells began to divide, as judged by the number of large budded cells remaining in the culture. The *sic1*Δ strain was slower to divide by nearly 10 min, some 10% of the normal cell cycle time. This result indicates that Sic1 plays a role in the telophase to G1 transition in cycling cells.

Overexpression of *SIC1*, but not *SWI5*, overcomes telophase arrest caused by *dbf2*, *cdc5* and *cdc15*: Overexpression of *SIC1* is sufficient to suppress the telophase defect of *dbf2* mutants (DONOVAN *et al.* 1994). We have now found that this is a more general effect since *SIC1* overexpression also drives *cdc5* and *cdc15* cells out of a telophase arrest (Figure 5). A construct containing *SIC1* under the control of the yeast *PGK* promoter was introduced into *cdc5* and *cdc15* mutants, and growth was tested at the restrictive temperature. Both mutants were able to grow at the restrictive temperature only when they contained the *PGK-SIC1* construct. The *PGK-SIC1* construct also rescued *dbf2* strains (not shown). In contrast, overexpression of *SWI5* under the control of the *PGK* promoter did not suppress any of the mitotic exit mutants. Two conclusions can be drawn from these experiments. First, overexpression of *SIC1* provides a general means of getting cells out of telophase, suggesting that Sic1 activity is regulated primarily by the amount of Sic1 protein produced in cells. Second, overexpression of *SWI5* clearly does not lead to high levels of Sic1, consistent with there being a mechanism actively preventing accumulation of high levels of Swi5 in the telophase nucleus.

Yeast strains lacking Swi5 have the same telophase phenotypes as strains lacking Sic1: Swi5 appears to be

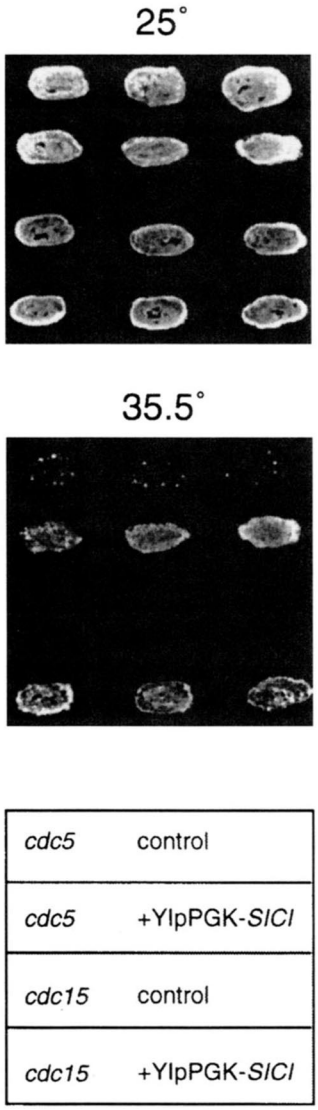


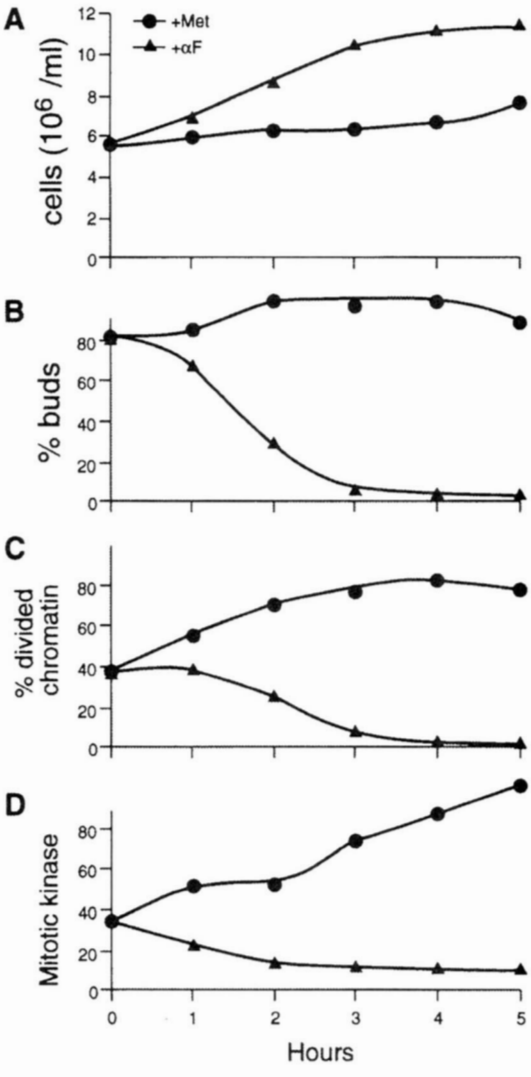
FIGURE 5.—*cdc5* and *cdc15* mutants are rescued by overexpression of *SIC1*. The integrating plasmids YIpPGK-*SIC1* (*SIC1* overexpression) or YIpPGK (empty vector control) were introduced into strains KKY021 (*cdc5*) and L207-R12 (*cdc15*). Three transformants of each were patched out, replica-plated onto YPD, and incubated at 25° or 35.5° for 1 day, as indicated.

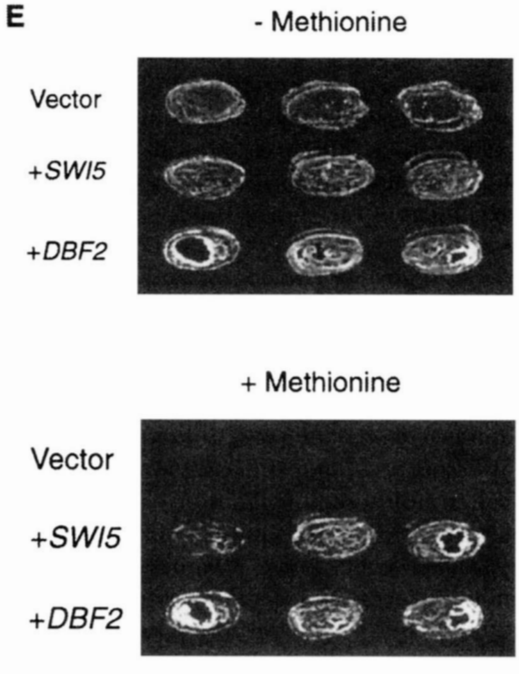
the key cell cycle regulator of *SIC1* expression. Since Sic1 has a role in ending mitosis, this implies that Swi5 too must have a role in the control of mitotic exit. To determine if this is the case, we investigated whether *swi5*Δ has phenotypes in common with *sic1*Δ. Two mitotic exit phenotypes were tested; synthetic lethality with *dbf2*Δ and increased sensitivity to overexpression of B cyclin.

Both Sic1 and Dbf2 have roles in the initiation of DNA synthesis, as well as roles in exit from mitosis (see Introduction). Simultaneous deletion of *SIC1* and *DBF2* is lethal (DONOVAN *et al.* 1994), and in principle this could be due to either an arrest of mitosis or an arrest of S phase. Therefore, a *sic1*Δ*dbf2*Δ double delete strain was constructed, containing an integrated copy of *SIC1* under the control of the yeast *MET3* promoter. On medium lacking methionine, the *MET3-SIC1* construct sustained growth of the strain. Addition of methionine shuts off expression of the *SIC1* gene in this strain, which can then be used to determine which stage of the cell cycle is affected by the *sic1*Δ*dbf2*Δ double deletion. Methionine was therefore added to a log phase culture, and samples were taken to determine cell number, buds, divided nuclei, and B cyclin kinase activity (Figure 6,A–D). After addition of the methionine, cell division stopped almost immediately, resulting in large budded cells with divided nuclei and high B cyclin kinase activity, indicative of a telophase arrest. The immediate cessation of division indicates a late execution point for the function of Sic1, that is, a cell cycle timing of Sic1 function consistent with telophase. As a control, in a parallel experiment α-factor was added, instead of methionine, since this leads to a late G1 arrest and prevents initiation of DNA synthesis. In this culture, there was a doubling in cell numbers before the cells arrested in G1 in an unbudded state with low B cyclin kinase activity. Thus, in the absence of Dbf2, *sic1*Δ cells clearly arrest in late M phase, and Sic1 is therefore essential under these conditions for the telophase to G1 transition.

To determine if *swi5*Δ and *dbf2*Δ were also synthetically lethal, strain J252-17C (*swi5*Δ) and strain J252-18D (*dbf2*Δ) were mated. The resulting diploid was then sporulated for tetrad analysis. Of 18 tetrads, three were parental ditype, three were nonparental ditype, and 12 were tetratype. Of the 18 predicted *swi5*Δ*dbf2*Δ double delete strains none formed normal colonies. All 18 germinated and 15 produced one or two buds before they died. Another two grew into small clumps of up to five budded cells, and one formed a tiny colony, which we presume had reverted from the lethal phenotype. Other combinations of deletions were not synthetically lethal, most notably *swi5*Δ*sic1*Δ, which formed normal-sized colonies. The *dbf2*Δ*ace2*Δ double mutant also formed normal colonies, as did combinations of deletions with the *DBF2* homologue *DBF20*; *dbf20*Δ*swi5*Δ, and even a *dbf20*Δ*swi5*Δ*ace2*Δ triple mutant.

If the synthetic lethality of *swi5*Δ with *dbf2*Δ results from the lack of *SIC1* expression, then ectopic expression of *SIC1* should be able to rescue the *swi5*Δ*dbf2*Δ double mutant. A high copy number *TRP1* vector containing *SIC1* under the control of the *MET3* promoter was introduced into strain S7-4A (*dbf2*Δ*::URA3*) and the transformed strain was mated with J252-1B (*swi5*Δ *::LEU2*) for tetrad analysis. In the 12 tetrads dissected, there were five viable spore clones containing all three of the markers, *TRP1 URA3 LEU2*, corresponding to *swi5*Δ*dbf2*Δ double delete strains containing the *MET3-SIC1* construct. In a further genetic cross, the *MET3-SIC1* construct was introduced into strain J227-24C (*dbf2*Δ*::LEU2*), and the transformed strain was crossed with strain J252-1B (*swi5*Δ*::LEU2*) for tetrad analysis. In the 12 tetrads dissected there were three identifiable





tetratypes, and these contained four viable spore clones, of which three were Leu^+ and one was Leu^-. In each case one of the Leu^+ clones was unable to grow in the presence of methionine, indicating that growth in the absence of methionine was *SIC1*-dependent. To demonstrate that the *SIC1*-dependent growth was itself dependent on the simultaneous deletion of *SWI5* and *DBF2*, one of these three strains, J261-2D (*swi5*Δ*::LEU2 dbf2*Δ*::LEU2* YEp*MET3-SIC1::TRP1*), was transformed with integrating plasmids containing wild-type copies of *SWI5* or *DBF2*. Growth in the presence of methionine was tested by replica-plating (Figure 6E). Either *SWI5* or *DBF2* was able to restore growth of this strain in the presence of methionine. In other genetic experiments (not shown) we found that the *swi5*Δ*dbf2*Δ double mutant could not be rescued by an integrated *MET3-SIC1* construct (which leads to a lower level of *SIC1* expression than the high copy number constructs described above), in contrast to the *sic1*Δ*dbf2*Δ double mutant, which can be rescued by the integrated *MET3-SIC1* construct. This suggests that Swi5 might have other roles besides *SIC1* expression in the control of mitotic exit.

The other mitotic exit phenotype that *swi5*Δ and *sic1*Δ share is based on the sensitivity of yeast to interference with B cyclin kinase deactivation. It has been shown that overproduction of the yeast B cyclin Clb2, which prevents B cyclin kinase deactivation, causes cell cycle arrest in telophase (SURANA *et al.* 1993), and that yeast mutants that are defective for the telophase to G1 transition are hypersensitive to Clb2 overproduction (SHIRAYAMA *et al.* 1994b). Thus, if Sic1 plays an active role in exit from telophase, a *sic1*Δ yeast strain should also be hypersensitive. A construct containing *CLB2* controlled by the yeast *GAL7* promoter was introduced into *sic1*Δ and isogenic control strain, which allows inducible overexpression of the *CLB2* gene in the presence of galatose. Upon transfer to medium containing galactose only the *sic1*Δ strain containing the *GAL7-CLB2* plasmid was unable to grow (Figure 7A).

To determine if *swi5*Δ was also hypersensitive to *CLB2* overexpression, the *GAL7-CLB2* construct was integrated into *swi5*Δ and isogenic wild-type strains. The

FIGURE 6.—The synthetic lethality of *sic1*Δ with *dbf2*Δ is caused by a telophase arrest; *swi5*Δ is synthetically lethal with *dbf2*Δ too. (A–D) A log phase culture of strain J250-16A (*dbf2*Δ *sic1*Δ YIp*MET-SIC1*) was grown in minimal medium at 25° in the absence of methionine. The culture was split in two; then either L-methionine (●) or α-factor (▲) was added at time zero. Samples were taken to determine the concentration of cells (A), the percentage of cells with buds (B), the percentage of cells with divided chromatin (C), and the level of B cyclin kinase activity (D). (E) Synthetic lethality of *swi5*Δ with *dbf2*Δ results from defective expression of *SIC1*. Plasmids YIp*SWI5*, YIp*DBF2*, or control vector YIplac211 were introduced into strain J261-2D (*swi5*Δ *dbf2*Δ YEp*MET-SIC1*) as indicated. Yeast patches were replica-plated onto minimal medium in the absence, or the presence, of methionine as indicated and incubated for 1 day at 30°.

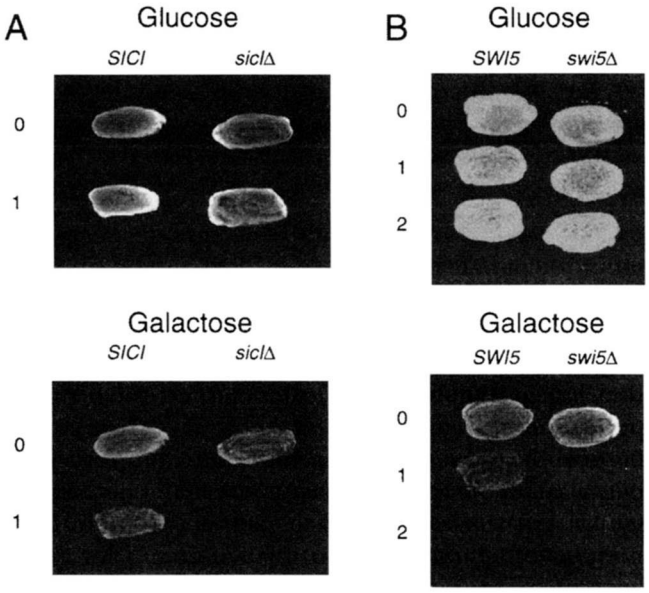


FIGURE 7.—Both *sic1*Δ and *swi5*Δ are hypersensitive to overexpression of *CLB2*. (A) One copy of the integrating plasmid YIp*G7CLB2* (galactose-inducible *CLB2* overexpression) was introduced into the isogenic strains CG379 (wild type) and JD100 (*sic1*Δ). Yeast containing or lacking the plasmid was replica-plated onto YP medium containing glucose or galactose, as indicated, and incubated for 2 days at 30°. (B) Zero, one or two copies of plasmid YIp*G7CLB2* were introduced into strains DTY59 (*SWI5*) or DTY87 (*swi5*Δ), as indicated. Yeast patches were replica-plated to YP medium containing glucose or galactose, as indicated, and incubated for 1 day at 30°.

number of integrated copies was determined by Southern analysis, and transformants with one or two copies were selected for further analysis. Wild-type and *swi5*Δ strains containing zero, one, or two copies of *GAL7-CLB2* were then replica-plated onto medium containing galactose or glucose (Figure 7B). A single copy of *GAL7-CLB2* was sufficient to prevent growth of the *swi5*Δ strain on galactose, whereas two copies were necessary to prevent growth of the wild-type strain. Thus, the *swi5*Δ exhibits increased sensitivity to interference with deactivation of the B cyclin kinase. In both *sic1*Δ and *swi5*Δ strains arrested by overexpression of *CLB2,* the majority of the cells had divided chromatin (not shown), confirming that the arrest was in telophase.

Based on the similarity in phenotype of *swi5*Δ and *sic1*Δ, we conclude that Swi5 contributes to the control of the telophase to G1 cell cycle transition through its role in the control of *SIC1* expression.

DISCUSSION

Swi5 regulates *SIC1* expression during telophase: The Sic1 protein is unstable (DONOVAN *et al.* 1994; SCHWOB *et al.* 1994), and therefore new Sic1 protein has to be produced every cell cycle before the end of mitosis to account for its role in the control of mitotic exit. We showed previously that the *SIC1* gene is expressed under cell cycle control around late mitosis and early G1 (DONOVAN *et al.* 1994). This suggested that gene expression might be rate-limiting for Sic1 protein production, and raised the possibility that a transcription factor might be involved in the control of mitotic exit. Here we have shown that the precise cell cycle timing of *SIC1* expression depends on Swi5, and that Swi5 begins to activate *SIC1* during telophase. Thus, the Swi5-dependent timing of *SIC1* expression is consistent with Swi5 having a role in the control of mitotic exit. Swi5 activates *SIC1* by binding specifically to the *SIC1* promoter (data not shown). It has been found independently that both Swi5 and Ace2 can bind to the *SIC1* promoter at the sequence GCCAGC between positions −166 and −161 (D. L. BHOITE and Dr. D. STILLMAN, personal communication). Similar sequence elements are found in the promoters of other Swi5 regulated genes, such as *CDC6, RME1, ASH1* and *EGT2* (PIATTI *et al.* 1995; TOONE *et al.* 1995; BOBOLA *et al.* 1996; KOVACECH *et al.* 1996; SIL and HERSKOWITZ 1996), but binding of Swi5 to a promoter has been previously demonstrated only in the case of the *HO* gene (STILLMAN *et al.* 1988; TEBB *et al.* 1993).

The bulk of Swi5 protein is retained in the cytoplasm during telophase arrest (NASMYTH *et al.* 1990), and we confirmed this for telophase arrests caused by *CLB2* overexpression and by *dbf2* temperature shift (data not shown). It may therefore seem strange that Swi5 causes gene expression during telophase. However, the cytoplasmic retention of Swi5 is not absolute, and sufficient Swi5 enters the nucleus to allow transcription of a Swi4/Swi6-independent *HO* reporter construct during *cdc15*-induced telophase arrest (NASMYTH *et al.* 1990). This is consistent with our experiments showing *SIC1* expression during the *CLB2*-induced arrest, and during *dbf2*-, *cdc5*-, and *cdc15*-mediated telophase arrest (data not shown). In addition, Swi5-dependent expression of *HO* from the wild-type promoter takes place during late G1, when cells contain no detectable Swi5 at all (TEBB *et al.* 1993). Hence, small amounts of Swi5 protein are active in the nucleus in late G1 and in telophase, although not detectable using current immunofluorescent techniques. However, the low level of Swi5 entering the nucleus during telophase arrest can in fact be observed directly by using degradation-resistant Swi5 mutants, such as the Δ11 and Δ59 constructs (NASMYTH *et al.* 1990; MOLL *et al.* 1991; TEBB *et al.* 1993), which partially accumulate in the nucleus under these conditions. We obtained the same result with an *N*-terminally truncated Swi5 protein (data not shown). Thus, Swi5 clearly enters the nucleus during telophase but cannot accumulate to observable levels because it is degraded there. The amount of Swi5 in the nucleus seems to depend on the relative rates of nuclear entry and of Swi5 protein degradation. During the telophase to G1 transition, the rate of nuclear entry greatly increases (NASMYTH *et al.* 1990), allowing it to accumulate to observable levels in the nucleus. Thereafter, degradation removes the Swi5, and the process begins again in

the next cell cycle when Swi5 is produced under cell cycle control during G2 and M phase (LYDALL *et al.* 1991).

The Swi5 and Ace2 transcription factors are differentially regulated: Swi5 and Ace2 are known to drive gene expression at about the same stage of the cell cycle (DOHRMANN *et al.* 1992). However, we observed a clear difference in the cell cycle timing of two transcripts; the Swi5-dependent transcript *SIC1* and the Ace2-dependent transcript *CTS1*. In a synchronous wild-type culture, *SIC1* was expressed distinctly in advance of *CTS1*. In a synchronous culture of the isogenic *swi5*Δ strain, there was a reduced level of *SIC1* expression, and the cell cycle timing of this reduced level of expression coincided with the cell cycle timing of *CTS1* expression. Thus, the timing of *SIC1* expression depends largely on the presence of Swi5. The residual expression in the *swi5*Δ is partly dependent on Ace2 and takes place with the expected cell cycle timing of Ace2 activity, indicating a difference in the cell cycle timing between Swi5-induced and Ace2-induced gene expression.

Expression of *SIC1* was readily detected in telophase-arrested cells. In marked contrast, the Ace2-dependent transcript *CTS1* was not expressed in telophase cells. If these results can be generalized to other genes regulated by these two transcription factors, then this demonstrates a fundamental difference in the regulation of Swi5 and Ace2; Swi5 becomes active in telophase, whereas Ace2 remains inactive until early G1. The apparently slight difference in timing of their function is particularly important because it affects whether or not an unstable protein can contribute to regulation of the exit from mitosis. In the case of *SIC1*, which is expressed under the control of both Swi5 and Ace2, this means that it is specifically Swi5-dependent transcription that allows Sic1 to be produced in time to contribute to the control of the telophase to G1 transition.

Swi5-dependent expression of *SIC1* contributes to the control of the telophase to G1 cell cycle transition: Swi5 is the predominant transcription factor involved in *SIC1* gene expression. However, in the absence of Swi5, part of the residual gene expression clearly depends on the related transcription factor Ace2. Therefore, to determine if Swi5-dependent *SIC1* expression is required for the function of Sic1 in mitotic exit, we tested *swi5*Δ strains for phenotypes related to control of mitotic exit that they might share with *sic1*Δ strains. Previously we showed that *sic1*Δ is synthetically lethal with *dbf2*Δ (DONOVAN *et al.* 1994), but it was not determined how this was related to the cell cycle roles of Sic1 and Dbf2. A *sic1*Δ*dbf2*Δ double mutant was therefore made containing a methionine-repressible *SIC1* construct. Upon addition of methionine to cultures of this strain the cells arrested in telophase, showing that the double mutant was defective for mitotic exit. We then constructed *swi5*Δ *dbf2*Δ double mutants and found that they too were inviable, but could be sustained by ectopic expression of *SIC1*, indicating that *swi5*Δ*dbf2*Δ is inviable at least partly because it is unable to express *SIC1* to sufficient levels during telophase. In contrast, the related transcription factor Ace2 showed no such genetic interactions. Thus, in spite of residual *SIC1* expression in *swi5*Δ strains, Swi5-dependent expression is essential for the activity of Sic1 in mitotic exit.

A second mutant phenotype indicative of defective mitotic exit depends on overexpression of the B cyclin gene *CLB2* (SHIRAYAMA *et al.* 1994b). Increased production of the activating cyclin subunit interferes with deactivation of the B cyclin kinase. We found that both *sic1*Δ and *swi5*Δ strains were hypersensitive to overexpression of *CLB2*. Moreover, log phase *sic1*Δ cells arrested within one cell cycle in telophase upon induction of *CLB2* expression (data not shown). Thus, under these conditions both *SIC1* and *SWI5* were essential for the telophase to G1 transition.

Finally, we showed that highly synchronized cells recovering from a telophase arrest underwent the transition into G1 more slowly in the absence of Sic1, further confirming the role of Sic1 during exit from telophase in cycling cells.

The telophase signal transduction network: We have shown that Swi5 controls the cell cycle expression of the *SIC1* gene during the telophase to G1 cell cycle transition, and that both Swi5 and Sic1 play active roles in this cell cycle transition (see above). We therefore propose that Swi5-dependent cell cycle transcription of *SIC1* is required to produce Sic1 at a time when it can contribute to B cyclin kinase deactivation. Considering what is already known about Swi5 regulation, this suggests a potential positive feedback loop. The B cyclin kinase controls the nuclear entry of Swi5 by inhibitory phosphorylation of the nuclear localization signal in the Swi5 protein (MOLL *et al.* 1991). Thus, inhibition of the B cyclin kinase by Sic1 could potentially lead to increased production of Sic1 by allowing increased Swi5 nuclear entry and hence further *SIC1* expression, so helping to ensure that cells either remain in telophase with high kinase activity, or switch irreversibly to G1 with no B cyclin kinase activity. Positive feedback has been proposed to account for the rapid accumulation of Cln/Cdc28 kinase activity at START (CROSS and TINKELENBERG 1991; DIRICK and NASMYTH 1991). Although plausible at the time, this has recently been disproved (STUART and WITTENBERG 1995). Further studies will therefore be required to determine how the Swi5/Sic1 pathway is regulated, and whether or not positive feedback is involved.

The Swi5/Sic1 pathway is redundant with the role of Dbf2 in mitotic exit, that is, Dbf2 and Swi5/Sic1 are on parallel pathways that transmit a signal for B cyclin kinase deactivation and the telophase to G1 transition (Figure 8). Although deletion of the *DBF2* gene makes the telophase to G1 transition dependent on Swi5 and Sic1, recessive ts *dbf2* mutations cause lethal telophase

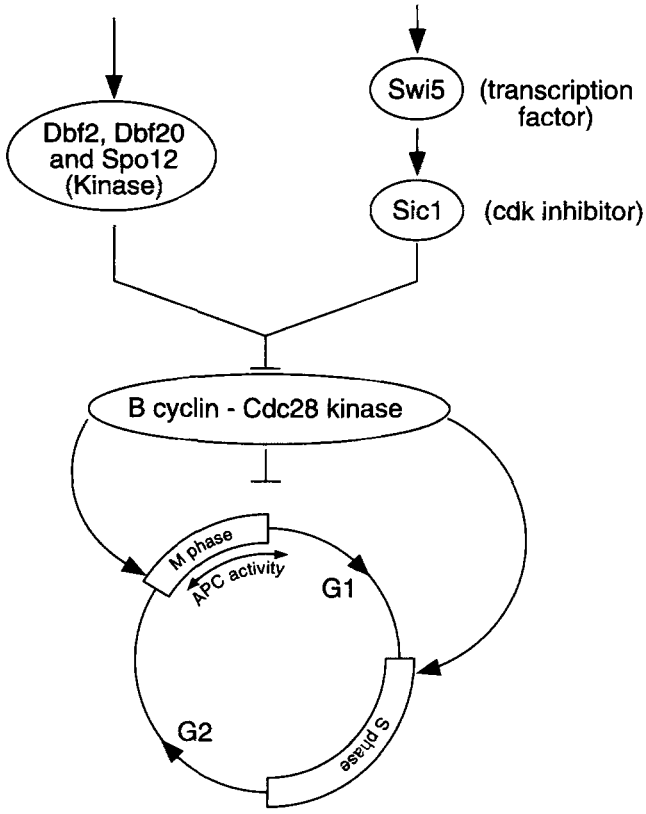


FIGURE 8.—The telophase signal transduction network. The B cyclin kinase inhibits the transition from telophase (end of M phase) into G1. The telophase signal transduction network regulates the cell cycle timing of B cyclin kinase deactivation. At present, this network appears to have two branches, either of which is sufficient to regulate B cyclin kinase deactivation. One branch involves the Swi5-dependent transcription of the Sic1 cdk inhibitor, the other involves the Dbf2 protein kinase. Also shown is the interval of the cell cycle during which the anaphase promoting complex (APC) is active, and arrows indicating other roles of B cyclin kinases in the cell cycle.

arrest. The unusual genetics of Dbf2 arise because inactive mutant Dbf2 protein blocks the function of the related Dbf20 protein by competing for the Spo12 protein, which is available in a limited cellular quantity (TOYN and JOHNSTON 1993). Thus, the ts *dbf2* mutant at the restrictive temperature is equivalent to the *dbf2*Δ *dbf20*Δ double delete, which causes lethal telophase arrest, even in the presence of Swi5 and Sic1. The pathway containing Dbf2 and Dbf20 is therefore essential, unlike the Swi5/Sic1 pathway, which is dispensable. The lethality of *swi5*Δ *dbf2*Δ and *sic1*Δ *dbf2*Δ, and viability of *swi5*Δ *dbf20*Δ and *sic1*Δ *dbf20*Δ, is most likely a reflection of the relatively higher contribution of Dbf2 than Dbf20 to the pathway; Dbf2 is responsible for the bulk of the kinase activity of these two proteins (TOYN and JOHNSTON 1994).

Many other genes, such as *RAS1*, *TEM1* and *CDC15* (see Introduction), are required for the telophase to G1 transition and interact genetically with one another and with *DBF2* and *SIC1*. The proteins these genes encode therefore most likely fit together in the telophase signal transduction network, but it remains to be determined precisely how they do so at the biochemical level. In addition, the relationship between APC and this signal transduction network remains unclear. No genetic interactions are known between the genes encoding APC and the genes specifically controlling the telophase to G1 transition. Our present view is that, while APC is required for B cyclin degradation and hence B cyclin kinase deactivation, it is the signal transduction network during telophase that determines the final cell cycle timing of B cyclin kinase deactivation.

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