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Mutations in *SID2*, a Novel Gene in *Saccharomyces cerevisiae*, Cause Synthetic Lethality With *sic1* Deletion and May Cause a Defect During S Phase

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ABSTRACT

SIC1 encodes a nonessential B-type cyclin/CDK inhibitor that functions at the G1/S transition and the exit from mitosis. To understand more completely the regulation of these transitions, mutations causing synthetic lethality with *sic1*Δ were isolated. In this screen, we identified a novel gene, *SID2*, which encodes an essential protein that appears to be required for DNA replication or repair. *sid2-1 sic1*Δ strains and *sid2-21* temperature-sensitive strains arrest preanaphase as large-budded cells with a single nucleus, a short spindle, and an ~2C DNA content. *RAD9*, which is necessary for the DNA damage checkpoint, is required for the preanaphase arrest of *sid2-1 sic1*Δ cells. Analysis of chromosomes in mutant *sid2-21* cells by field inversion gel electrophoresis suggests the presence of replication forks and bubbles at the arrest. Deleting the two S phase cyclins, *CLB5* and *CLB6*, substantially suppresses the *sid2-1 sic1*Δ inviability, while stabilizing Clb5 protein exacerbates the defects of *sid2-1 sic1*Δ cells. In synchronized *sid2-1* mutant strains, the onset of replication appears normal, but completion of DNA synthesis is delayed. *sid2-1* mutants are sensitive to hydroxyurea indicating that *sid2-1* cells may suffer DNA damage that, when combined with additional insult, leads to a decrease in viability. Consistent with this hypothesis, *sid2-1 rad9* cells are dead or very slow growing even when *SIC1* is expressed.

PASSAGE through the eukaryotic cell cycle is regulated by cyclin-dependent kinases (CDKs). CDKs are active when bound to cyclins and it appears that cyclins are responsible for much of the functional specificity of the cyclin-CDK complex. The activity of CDK complexes is regulated at the level of expression of CDKs and cyclins, as well as post-translationally by phosphorylation, regulated degradation, and CDK inhibitors. In the budding yeast *Saccharomyces cerevisiae*, Cdc28p is the main CDK involved in cell cycle control, forming a complex with both the G1 cyclins (Cln1-3) and the B-type cyclins (Clb1-6). The G1 cyclins act upstream of the events controlling the G1/S phase transition, including bud formation, microtubule organizing center duplication, and DNA replication (reviewed by Lew *et al.* 1997). One essential function of the G1 cyclins is the inactivation of the B-type cyclin-Cdc28 inhibitor, Sic1p, as *sic1* deletion rescues strains containing a *cln1*Δ *cln2*Δ *cln3*Δ triple deletion that are otherwise inviable (Schneider *et al.* 1996).

As an inhibitor of Clb-Cdc28 kinase activity, Sic1p appears to function to allow cells to exit mitosis as well as to prevent premature DNA replication (Schwob *et al.* 1994; Toyn *et al.* 1997). *SIC1* transcripts are cell cycle regulated, accumulating late in M phase dependent upon the transcription factor Swi5p, and disappearing at the G1/S transition (Knapp *et al.* 1996). A decrease in Clb2p-Cdc28p activity is needed for passage into G1 and it appears that Sic1p assists in this process by inhibiting the CDK complex (Toyn *et al.* 1997). While cells can enter G1 with elevated levels of Clb2p, overexpressed Clb2ΔDBp (destruction box deleted) is lethal, causing cell cycle arrest in telophase (Surana *et al.* 1993). Clb2-Cdc28 kinase activity is also decreased by proteosome degradation of Clb2p after its ubiquitination by the anaphase-promoting complex (APC; Surana *et al.* 1993; Irniger *et al.* 1995).

At the G1/S transition, degradation of Sic1p releases inhibition of Clb5/6p-Cdc28p, which then induce DNA replication (Schwob *et al.* 1994; Schneider *et al.* 1996). Sic1p proteolysis is dependent on its Cln-dependent phosphorylation (Schneider *et al.* 1996; Verma *et al.* 1997) and upon Cdc34p, an E2 ubiquitin-conjugating enzyme (Schwob *et al.* 1994). Inducing *GAL-CLB5ΔDB* expression advances DNA replication in *sic1*Δ, but not *SIC1* cells, indicating that Sic1p has a function in regulating S phase entry (Schwob *et al.* 1994). Clb5p and Clb6p seem to have related, but not identical, roles in initiating DNA replication. *clb5*Δ cells initiate DNA replication normally, but take twice as long to complete DNA synthesis (Epstein and Cross 1992; Kühne and Linder 1993). Cells deleted for *clb6* display a normal onset and duration of replication. However, when *clb5* and *clb6* are both deleted, DNA synthesis is delayed, but the

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duration of replication, once begun, is unaffected (Schwob and Nasmyth 1993). Recent evidence suggests that Clb5p can activate early and late origins of replication while Clb6p can activate only the early origins (Donaldson *et al.* 1998). This result agrees with the phenotypes of *clb5* and *clb6* single mutants. The phenotype of the double deletion can then be explained if the remaining Clbs (Clb1-4) can trigger both early and late origins to fire (Donaldson *et al.* 1998).

In addition to timing DNA replication, inhibition of Clb5/6p-Cdc28p activity by Sic1p may function to regulate origin loading and the DNA replication machinery. Binding of the six-subunit origin recognition complex (ORC), the Mcm family (Mcm2-7), and Cdc6p is thought to make the origins competent for firing by Clb5/6p-Cdc28p kinase activity (reviewed in Diffley 1996; Stillman 1996). Origin loading is inhibited by CDK activity and this is thought to be the basis for a mechanism that allows replication to occur once per cell cycle (Dahmann *et al.* 1995). Cdc6p appears to recruit Mcm binding to the origins. In the presence, but not in the absence of *SIC1*, late G1 expression of Cdc6p (under the control of the *HO* promoter) can promote Mcm binding (Tanaka *et al.* 1997). This suggests that Sic1-induced delay of Clb-Cdc28 activity allows proper origin binding of competence factors in preparation for DNA replication. Clb-Cdc28 kinase activity and Sic1p also affect DNA replication in a Cdc6- and ORC-independent fashion, suggesting that the kinase may also have direct effects on enzymes required for DNA synthesis (Duncker *et al.* 1999). One possibility is that the Clb-Cdc28 kinase regulates the association of DNA polymerase-primase (polα) to chromatin (Desdouets *et al.* 1998).

SIC1 is not an essential gene, but *sic1* cells show a high frequency of chromosome loss and breakage (Nugroho and Mendenhall 1994). Several genetic backgrounds make *SIC1* essential, including *dbf2*Δ, *GAL-CLB2*, *cdh1*Δ/*hct1*Δ, *cdc23-1*, and *rsi1-1* (*apc2*; Schwab *et al.* 1997; Toyn *et al.* 1997; Kramer *et al.* 1998). Overexpression of Sic1p is able to rescue *cdc5*, *cdc14-1*, *cdc15*, and *cdc20-1* strains (Schwab *et al.* 1997; Toyn *et al.* 1997; Jasperson *et al.* 1998). All of these genetic interactions appear to be related to Sic1's function at the exit from mitosis. Cdc23p and Apc2p are members of the APC (Zachariae *et al.* 1998b) while Cdc20p and Cdh1p/Hct1p seem to function as APC activators (Schwab *et al.* 1997; Visintin *et al.* 1997). *dbf2*, *cdc5*, *cdc14*, and *cdc15* all have terminal arrest phenotypes late in mitosis (Byers and Goetsch 1973; Pringle and Hartwell 1981; Johnston *et al.* 1990; Kitada *et al.* 1993) and may activate the APC or dephosphorylate Cdc28 substrates or regulators (Jaspersen *et al.* 1998; Visintin *et al.* 1998). Taken together, these data indicate that *SIC1* plays an important role in late mitosis. If *SIC1* also has a significant role in regulating DNA replication, it is possible that genes exist which, when mutated in combination with *sic1*Δ, disrupt the normal regulated process of DNA replication sufficiently to render the cells inviable. To identify such genes, as well as other genes playing roles in the exit from mitosis, we screened for mutations causing synthetic lethality with *sic1*Δ. Here we report on the discovery of a novel gene, *SID2*, which appears to play a role in DNA replication.

MATERIALS AND METHODS

Yeast strains and media: YP-dextrose (YPD), YP-galactose (YPGal), and synthetic complete (SC) minimal media were made by standard techniques (Ausubel *et al.* 1987). Hydroxyurea and α-factor (both from Sigma Chemical, St. Louis) were used at 0.2 M and 0.1 μM, respectively. The strains and plasmids used in this study are listed in Tables 1 and 2, respectively. All yeast strains are isogenic with BF264-15D (*trp1-1a leu2-3,112 ura3 ade1 his2*) and are *bar1*$^{-}$ unless otherwise noted. Standard methods were used for all strain constructions, crosses, and transformations (Ausubel *et al.* 1987; Rose *et al.* 1990; Guthrie and Fink 1991). A disruption of *sic1* marked with *TRP1*, *SIC1* under the control of the inducible *GAL1* promoter (Nugroho and Mendenhall 1994; the gifts of M. Mendenhall) and a disruption of *rad9* marked with *LEU2* (Weinert and Hartwell 1990) were integrated into the BF264-15D background. The *clb5::ARG4* and *clb6::ADE1* disruptions were made in the BF264-15D background and have been previously described (Epstein and Cross 1992; Schwob and Nasmyth 1993). The *CLB2*^{HA} construct (Schwab *et al.* 1997), *CLB5*^{HA}, and *CLB5*Δ*DB*^{HA} constructs have also been described (Cross *et al.* 1999). Yeast strain L40 and plasmids pNIA, pNIAE2, and pNEAE2 have been previously described (Rhee *et al.* 2000).

Plating efficiency assays: Tenfold serial dilutions in water were made from fresh stationary-phase cultures and 5 μl from each dilution was plated. Plates were incubated for 2–4 days at 30°.

Mutagenesis and *sic1*Δ lethality screen: LY623 and MJ65 yeast cells were mutagenized using standard procedures (Rose *et al.* 1990) to ~30% viability. Mutagenized cells were plated on YPGal (~200 colonies per plate). The colonies were then screened by replica plating for mutants that were alive on YPGal and dead on YPD.

Library screening: *LEU2 CEN4* plasmids, which complemented *sid2-1*, were isolated by transforming a *sid2-1* strain (MJ163) with American Type Culture Collection library 77162 (constructed by P. Hieter in pBS32). Transformants were selected on SCGal-Leu minimal media plates and replica plated to YPD and YPGal in order to isolate colonies that could grow on YPD. The plasmids were recovered from Dex^{+} strains (Hoffman and Winston 1987) and plasmid linkage was tested after retransformation. Partial sequence was obtained by The Rockefeller University Protein/DNA Technology Center using primer pBRSB (ACCGCACCTGTGGCGCCG), which hybridizes to pBR322 sequences 31 base pairs upstream of the *Bam*HI site.

Cloning and disrupting *SID2*: All restriction enzymes and DNA modifying enzymes were used according to the manufacturer's instructions. A 3.4-kb fragment (*Pst*I to *Spe*I) containing the entire *YJR046w* open reading frame (ORF) was isolated from a *LEU2 CEN4* library plasmid that rescued *sid2-1*. This fragment was cloned into pRS405 to form pMJ01 and into pRS415 to form pMJ02 (Figure 1). pMJ01 was digested with *Nco*I (which cuts uniquely in *YJR046w*) and integrated by homologous recombination into the *sid2-1* haploid MJ193 to form MJ257. The integration was confirmed by Southern blot

TABLE 1

Strains used in this study

Strain	Relevant genotype	Source
KK1	*MAT*α *sic1::TRP1 GAL1-SIC1::URA3 rad9::LEU2*	MJ65
KK11	*MAT*α *SID2 sic1::TRP1 GAL1-SIC1*	KK1 × MJ163
KK17	*MAT***a** *sid2-1 sic1::TRP1 GAL1-SIC1*	KK1 × MJ163
KK20	*MAT***a** *SID2 sic1::TRP1 GAL1-SIC1 rad9::LEU2*	KK1 × MJ163
KK23	*MAT*α *sid2-1 sic1::TRP1 GAL1-SIC1 rad9::LEU2*	KK1 × MJ163
L40	*lexAoperator::HIS3 lexA operator::lacZ trp1*	Hollenberg *et al.* (1995) Rhee *et al.* (2000)
LY623	*MAT***a** *sic1::TRP1 GAL1-SIC1::URA3*	
LY677	*MAT*α *cdc15-2 sic1::TRP1*	K2944 (Oehlen and Cross 1994)
LY699	*MAT*α *dbf2::URA3 sic1::TRP1 GAL1-SIC1::URA3*	S7-4A (L. H. Johnston)
LY907	*MAT*α *sid2-1 sic1::TRP1 GAL1-SIC1*	
LY909	*MAT*α *sic1::TRP1 GAL1-SIC1*	
LY914	*MAT***a** *HIS2 his3*	
LY915	*MAT***a**/*MAT*α *HIS2/HIS2 his3/his3*	
LY925	*MAT***a** *HIS2 his3 SID2-PrA::his5*$^{+}$	
LY985	*arg4 sic1::TRP1 GAL1-SIC1::URA3 clb6::ADE1 clb5::ARG4*	
LY986	*MAT***a** *arg4 sic1::TRP1 GAL1-SIC1::URA3*	
LY987	*arg4 sic1::TRP1 GAL1-SIC1::URA3 sid2-1*	
LY988	*arg4 sic1::TRP1 GAL1-SIC1::URA3 clb6::ADE1 clb5::ARG4 sid2-1*	
LY989	*arg4 sic1::TRP1 GAL1-SIC1::URA3 clb5::ARG4*	
LY991	*arg4 sic1::TRP1 GAL1-SIC1::URA3 clb5::ARG4 sid2-1*	
LY1023	*MAT***a** *HIS2 his3 sid2::LEU2 pSU3[SID2-RS416]*	
LY1036	*MAT***a** *HIS2 his3 SID2*	LY914
LY1037	*MAT***a** *HIS2 his3 sid2-21*	LY914
LY1118	*MAT***a** *sid2-1*	
MJ55	*MAT***a** *sic1::TRP1 GAL1-SIC1::URA3*	
MJ58	*MAT***a** *sic1::TRP1 GAL1-SIC1::URA3*	
MJ65	*MAT*α *sic1::TRP1 GAL1-SIC1::URA3*	
MJ160	*MAT***a** *sid2-1 sic1::TRP1 GAL1-SIC1::URA3*	
MJ163	*MAT***a** *sid2-1 sic1::TRP1 GAL1-SIC1::URA3*	
MJ193	*MAT*α *sid2-1 sic1::TRP1 GAL1-SIC1::URA3*	
MJ249	*MAT*α *cdh1::LEU2 sic1::TRP1 GAL1-SIC1::URA3*	W320 (Schwab *et al.* 1997)
MJ257	*MAT*α *siD2::LEU2 sic1::TRP1 GAL1-SIC1::URA3*	
MJ282	*MAT***a** *sid2-1 CLB2*^{HA} *sic1::TRP1 GAL1-SIC1::URA3*	W9317 (Schwab *et al.* 1997)
MJ288	*MAT***a** *CLB2*^{HA} *sic1::TRP1 GAL1-SIC1::URA3*	W9317 (Schwab *et al.* 1997)
MJ292	*MAT***a** *cdh1::LEU2 CLB2*^{HA} *sic1::TRP1 GAL1-SIC1::URA3*	W320, W9317 (Schwab *et al.* 1997)
MJ316	*MAT***a** *SID2 sic1::TRP1 GAL1-SIC1 CLB5*^{HA}	
MJ317	*MAT***a** *sid2-1 sic1::TRP1 GAL1-SIC1 CLB5*^{HA}	
MJ319	*MAT***a** *SID2 sic1::TRP1 GAL1-SIC1 CLB5*Δ*DB*^{HA}	
MJ321	*MAT*α *sic1::TRP1 GAL1-SIC1::URA3 sid2-1 CLB5*Δ*DB*^{HA}	
MJ322	*MAT***a** *sic1::TRP1 GAL1-SIC1::URA3 sid2-1*	
MJ323	*MAT***a** *sic1::TRP1 GAL1-SIC1::URA3*	
MJx21-4D	*MAT*α *dbf2::URA3 GAL1-SIC1::URA3*	S7-4A (L. H. Johnston)

All strains are *ade1 trp1-1 ura3 leu2-3,112 his2 bar1* and are from this study unless otherwise noted.

analysis. MJ257 was crossed to a *SID2* strain (MJ58). The diploid was sporulated and the tetrads were dissected for analysis. If the *sid2-1* mutation was not linked to *YJR046w*, approximately half of the Leu^{-} spores should have had the Sid^{-} phenotype because the mutation would have been segregating independently of the *LEU2* integration. None of the spores (54 spores analyzed) were Sid^{-}, indicating that the *sid2-1* mutation was linked to *YJR046w*. In addition, when MJ257 was crossed to *sid2-1* strains, all Leu^{+} spores were Sid^{+} and all Leu^{-} spores were Sid^{-} (47 spores analyzed), indicating tight linkage between the integrated DNA and the *SID2* locus.

SID2 was deleted in a diploid heterozygous for *sic1*Δ and *GAL1-SIC1* using pMJ07, which was derived from pMJ01 in the following way. The *Xba*I polylinker site in pMJ01 was removed with a *Spe*I/*Not*I digest. The 5′ overhanging ends of the digested plasmid were blunted using Klenow enzyme and then ligated, to form pMJ03. pMJ03 was then digested with *Xba*I, liberating a 970-bp fragment internal to *SID2*, and the ends were blunted with Klenow enzyme. A 3.5-kb fragment containing the *LEU2* and Kan^{r} genes from pJA51-ΔP digested with *Sma*I (Cross 1997) was ligated to the digested MJ03 to form MJ07. pMJ07 was digested with *Hin*dIII and the resulting 5-kb fragment was gel purified and used to disrupt *SID2* in a diploid by homologous recombination. A diploid containing the deletion (confirmed by Southern blot) was sporulated and tetrads were dissected.

Construction of the temperature-sensitive *SID2* allele, *sid2-21*: *SID2-HIS3* (pSH6) and *SID2-URA3* (pSU3) plasmids were made by gap repair by first isolating a 5.6-kb fragment (*Sca*I to *Dra*III) containing all of *SID2* and flanking vector sequences

TABLE 2

Plasmids used in this study

Name	Vector	Relevant genes	Reference
pMJ01	RS405	*SID2, LEU2*	This study
pMJ02	RS415	*SID2, LEU2*	This study
pMJ03	RS405	*SID2, LEU2* (*Xba*I in polylinker removed)	This study
pMJ07	RS405	*sid2::LEU2*-Kan^r	This study
pSIC1	RS315	*SIC1, LEU2*	M. Tyers
pSH6	RS413	*SID2, HIS3*	This study
pSU3	RS416	*SID2, URA3*	This study

from pMJ02 and 3-kb *Pvu*I vector fragments from both pRS413 and pRS416. The pMJ02 *SID2* fragment and either the pRS413 or pRS416 fragment were cotransformed into LY914 and the resultant plasmids were isolated. pSH6 was then mutagenized with hydroxylamine according to standard procedures (Rose *et al.* 1990).

Haploid strains containing pSU3 (*SID2-URA3*) were deleted for *SID2* using pMJ07 as described above. The *sid2::LEU2* deletion was confirmed in the resulting Leu$^+$ FOA^S strains by Southern blot analysis. One such strain, LY1023, was transformed with pSH6 (*SID2-HIS3*) and became FOA^R as expected. LY1023 was then transformed with hydroxylamine-mutagenized pSH6 and plated on SCDex-His at room temperature (~200 colonies per plate). The colonies were replica plated to SCDex-His and SCDex + FOA at both room temperature and 37° and then screened for mutants that were dead only on SCDex + FOA at 37°. The plasmids were recovered from these strains and transformed back into LY1023 to verify the phenotype. Five *sid2-ts* plasmids were isolated from ~7700 colonies screened with a 1% frequency of *sid2* null mutations (FOA^S at room temperature and 37°).

The 3.5-kb *Spe*I to *Sal*I fragments containing the *sid2-ts* alleles were isolated from the five plasmids and cloned into pRS406 digested with *Xho*I and *Spe*I. These new *sid2-ts* integrating plasmids were digested with *Xho*I (which cuts uniquely in *SID2*) and integrated by homologous recombination into LY914. Purified Ura$^+$ transformants were patched onto YPD at room temperature and then streaked on SCDex + FOA at room temperature. The resulting FOA^R colonies were screened for temperature-sensitive growth. Temperature-sensitive strains were recovered from only one of the original five alleles (*sid2-21*) although at least 48 FOA^R colonies from 12 independent transformants were screened for each allele. Integration after digestion of the remaining four plasmids with *Bst*EII, which cuts upstream of *SID2*, was also tried, but again, no temperature-sensitive recombinants were recovered after passage on FOA. One of the resulting *sid2-21* temperature-sensitive strains, LY1037, was transformed with SU3 and found to become temperature resistant, confirming that the temperature-sensitive growth of LY1037 was due to *sid2-21*. Furthermore, when LY1037 was crossed to MJ257 (*SID2::LEU2*), temperature sensitivity and Leu$^+$ segregated in repulsion in all 12 tetrads analyzed.

Tagging Sid2 with protein A: A Sid2-protein A fusion protein was constructed by PCR amplification of the protein A gene and an adjacent *his5*$^+$ marker (the *Schizosaccharomyces pombe* homolog of the *S. cerevisiae HIS3* gene) from pBXAHis5 (M. Rout, The Rockefeller University) using primers with homology to *SID2* at their 5′ ends and to protein A or *his5*$^+$ at their 3′ ends. The pBXAHis5 plasmid was derived from pFA6a-HIS3MX6 (Wach *et al.* 1997). The following oligonucleotides were used to amplify the protein A-*his5*$^+$ fragment for C-terminal addition of protein A to Sid2: 046-PROTA 5′, GGATAAAACAGATTTTCTAAGCTGTTGCAAATCCACAAATCAAAACAACAAGATGGTGAAGCTCAAAAACTTAAT; 046-HIS3′, CGTACATACACAATGCACAGTCTTCAAAGTAAAATACCAACGTATGTATCAAGATCGTCGACGGTATCGATAAGCTT where the underlined sequences correspond to those from *SID2*. Twenty-five cycles of PCR consisting of 1 min at 95°, 1 min at 55°, and 4 min (+5-sec increase/cycle) at 72° were performed. The PCR products were transformed into *his3/his3* diploid cells (LY915) and His$^+$ transformants were selected. Homologous recombination resulted in *SID2-ProA* fusions linked to *his5*$^+$. Putative protein A-tagged strains were analyzed by Western and Southern blotting to verify tagging of the Sid2 protein.

Construction of *lexA-GAL4-SID2* fusions and "one-hybrid" assay: The *SID2* gene was amplified with forward primer 5′-*AAAGAGATCGAATACCCGGGGATCC*TTATGAGTGGCACAGCCTAAT-3′ and reverse primer 5′-*TCGCCCGGAATTAGCTTGGCTGCAG*TTCAATCTTGTTGTTTTGAT-3′ using the Expand High Fidelity PCR system (Roche, Indianapolis). The underlined sequences correspond to those from *SID2* and the italicized sequences correspond to sequences in the pNIA plasmid. Thirty-three cycles of PCR each consisting of of 1 min at 94°, 1 min at 50°, and 2 min at 68° were performed. The PCR fragment was recombined into plasmid pNIA (Rhee *et al.* 2000) by cotransformation into yeast. Plasmids were recovered from yeast (Hoffman and Winston 1987), electroporated into *Escherichia coli*, and analyzed by restriction analysis. Six independently derived NIA-SID2 plasmids were transformed into strain L40 (Rhee *et al.* 2000) and liquid β-galactosidase assays were performed on log phase cultures grown under selective conditions as described (Ausubel *et al.* 1987).

Yeast fractionation: Fractionation was performed (Rout and Kilmartin 1998) using the modifications for *S. cerevisiae* described with a 1-liter culture of Wickerham's media grown overnight to an optical density (660 nm) of 0.8. The volumes loaded for Western blot analysis were adjusted to compensate for varying total volumes of each collected fraction.

Phenotypic analysis of *sid2-1 sic1*Δ and *sid2-21* strains: *SID2* and *sid2-1* strains (both *sic1*Δ *GAL1-SIC1*) were grown overnight to early log phase in liquid YPGal media. The cultures were then split and dextrose was added to one-half of each to a final concentration of 2%. YPD cultures of *sid2-21* and *SID2* strains were grown overnight at 25° to early log phase and then shifted to 37°. For both experiments, samples were removed at 2-hr intervals and processed for FACS analysis, cell counting, or immunofluorescence staining as described below. For the synchronization experiments, early log phase cultures of *SID2, sid2-1, SID2 sic1*Δ *GAL1-SIC1*, and *sid2-1 sic1*Δ *GAL1-SIC1* were grown in YPGal at 30°. α-factor was added and incubation continued for 3 hr. Cells were centrifuged, washed in YP lacking sugar, and resuspended in fresh 30° YPD. Samples were removed at 12-min intervals.

FACS analysis and cell counting: Flow cytometric DNA quantitation was performed as described elsewhere (Epstein and Cross 1992). Growth curve samples were fixed with 3 ml of 0.74% formaldehyde in 1× PBS. The samples were sonicated for 12 sec and cell number was analyzed using a Coulter counter. Microscopic analysis was used to determine the percentage of cells that were unbudded, small budded, or large budded. At each time point, at least 200 cells were counted. Small-budded cells were those where the daughter bud was less than two-thirds the size of the mother bud. Large-budded cells had a daughter bud greater than two-thirds the size of the mother bud.

Field inversion gel electrophoresis assay: Yeast strains 1036 (*SID2*) and 1037 (*sid2-21)* were grown to early log phase in YPD at 23°. Samples of the wild type and mutant were removed for

processing, the cultures were shifted to 37°, and samples were then removed at 2-hr intervals. For the hydroxyurea-treated control sample, hydroxyurea was added directly to an aliquot of the log phase culture of wild-type cells (final concentration, 0.2 M) and incubation continued for 3 hr at 30°. Chromosomal DNA samples were prepared in agarose plugs as described (Schwartz and Cantor 1984; Rose *et al.* 1990). Samples containing equivalent OD_{600} units of cells were applied to a 1% agarose gel, electrophoresed in 0.5× Tris-borate-EDTA buffer at 4–8° at 8.3 V/cm, stained with ethidium bromide overnight, and then destained for 1 hr. Field inversion was controlled by a PC500 SwitchBack pulse controller (Hoefer, Amersham Pharmacia Biotech, Piscataway, NJ) with a run time of 32 hr, a pulse time of 1–50 sec and an F/R ratio of 3.0:1.

Immunofluorescence staining: Immunofluorescence microscopy was done essentially as described previously (Wente *et al.* 1992). Tubulin was visualized using anti-tubulin antibody (Wente *et al.* 1992; 1:200 dilution) followed by Cy-3 donkey conjugated anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). The fluorescent DNA-specific dye 4′,6-diamidino-2-phenylindole (DAPI) was used to visualize yeast nuclei.

Immunoprecipitation and detection of HA-tagged proteins: The immunoprecipitation protocol is based on methods described previously (Levine *et al.* 1996). Yeast cultures (100 ml) were grown overnight to an optical density (600 nm) of 1.0. Cells were collected and washed in TNN buffer (50 mM Tris pH 7.5, 250 mM NaCl, 5 mM EDTA, 10% glycerol, 0.1% Nonidet P-40). TNN extraction buffer [TNN + 5% aprotinin (Sigma), 0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 mM NaPPi (pH 7.4), 10 mM NaF] was used for cell breaking, antibody incubation, and immunoprecipitation with the protein A-agarose slurry. The extract was incubated for 1 hr on ice with 1 μl of the monoclonal HA11 antibody (ascitis; Babco). Following incubation with the antibody, the extract was added to 30 μl of a protein A-agarose slurry (Sigma) prewashed with TNN and rotated at 4° for 1 hr.

SDS-polyacrylamide gel electrophoresis (10%) and transfer to Immobilon were done as previously described (Cross and Blake 1993). Following the transfer, the immunoblots were blocked overnight [PBS with 0.5% Nonidet P-40, 0.1% Tween 20, 0.5% BSA, and 15% milk (Carnation)]. The blot was incubated with antibodies (1.5 hr for the primary, 1 hr for the secondary) in PBS-Tween 20 (0.2%) with 1% milk at room temperature. The primary antibody, polyclonal rabbit anti-HA (Covance Research Products, Richmond, CA), was diluted 1:7500. The secondary antibody was a 1:1500 dilution of polyclonal donkey anti-rabbit conjugated to horseradish peroxidase (Amersham). The samples were washed three times for 10 min each with PBS-Tween 20 following each antibody incubation. The proteins were then detected using an enhanced chemiluminescence kit (Amersham).

Other Western blots were processed as described above except for the following modifications. The blots were blocked for 1 hr at room temperature in PBS-Tween 20 with 2.5% milk and incubated with the antibodies in PBS-Tween 20 containing 5% milk. A 1:1000 dilution of rabbit anti-mouse IgG (Organon Teknika, Durham, NC) was used to detect protein A tags. Mouse monoclonal anti-Nop1 (Aris and Blobel 1988) was diluted 1:2000 and mouse monoclonal anti-PGK (Molecular Probes, Eugene, OR) was diluted 1:10,000. The secondary antibody was a 1:1000 dilution of polyclonal donkey anti-rabbit (sheep anti-mouse for Nop1 and PGK) conjugated to horseradish peroxidase (Amersham).

RESULTS

Isolation of mutants synthetically lethal with *sic1* deletion: To identify factors that assist *SIC1* at the G1/S transition or at the exit from mitosis, we isolated mutations that caused synthetic lethality with *sic1*Δ. *sic1*Δ *GAL1-SIC1* strains (LY623 and MJ65) were mutagenized and screened for mutants alive on YPGal (*SIC1* expressed) and dead on YPD (*SIC1* repressed). Approximately 28,000 colonies were screened by replica plating and 21 recessive mutants were isolated. The mutants were named *SID* for ***SIC1* Indispensable**.

By complementation testing, the following groups were established: *sid1* (seven alleles), *sid2* (one allele), *sid3/dbf2* (six alleles), and *sid4/cdc15* (one allele). The remaining six mutants do not fall into the four complementation groups described above and define at least two more complementation groups. They remain unsorted because of difficulties with backcrossing. There appears to have been spontaneous diploidization of many of the mutants, resulting in minimal spore viability following tetrad dissection after crosses to wild-type haploid strains. Attempts to sporulate the diploidized strains after transformation with *MAT*-containing plasmids were unsuccessful. *sid1* has yet to be cloned, though two *LEU2* CEN libraries have been thoroughly screened for rescue plasmids (39,000 transformants). Preliminary analysis suggests that *sid1 sic1*Δ strains arrest as large-budded cells with segregated nuclei (data not shown). *sid3 sic1*Δ mutants failed to complement a *dbf2*Δ *sic1*Δ *GAL1-SIC1* strain (LY699) on YPD and *sid3* was meiotically linked to *dbf2*Δ (MJX21-4D) in tetrad analysis. The *sid4 sic1*Δ mutant failed to complement a *cdc15-2 sic1*Δ strain (LY677). Linkage could not be established, however, as the *sid4* mutant had spontaneously diploidized. Although *cdh1*Δ *sic1*Δ spores are inviable (Schwab *et al.* 1997), no *cdh1* mutants resulted from this screen as determined by complementation testing to MJ249 and MJ242. *cdh1*Δ *sic1*Δ spores were isolated in our strain background using *GAL1-SIC1* to suppress the lethality. *cdh1*Δ *sic1*Δ spores that did not contain *GAL1-SIC1* were inviable as previously reported. *cdh1*Δ *sic1*Δ *GAL-SIC1* strains were able to grow when replica plated to YP-dextrose, though they clearly grew more slowly than *sic1*Δ *GAL1-SIC1* strains (data not shown). Thus the very low level of expression from *GAL1-SIC1* on glucose is probably sufficient to partially rescue *cdh1*Δ *sic1*Δ *GAL-SIC1* strains on glucose medium, which may account for our failure to isolate *cdh1* mutations in our screen. The isolated *sid* mutants were all found to complement *cdc5* and *cdc14* strains.

***SID2* is *YJR046w*, a novel gene:** To clone *SID2*, a *LEU2 CEN4* library was screened for plasmids that could rescue the lethality of *sid2-1 sic1*Δ *GAL1-SIC1* cells on YPD. Four plasmids containing the yeast ORF *YJR046w/TAH11* and flanking regions were isolated after screening 8000 transformants. An insert containing only *YJR046w* intact was subcloned into RS415 and the resulting plasmid (pMJ02) was able to rescue the lethality of *sid2-1 sic1*Δ *GAL1-SIC1* cells on YPD (Figure 1A). *LEU2* was integrated adjacent to *YJR046w* in a *sid2-1* strain and was found to be meiotically linked to *SID2* (materials and methods). *SID2* encodes

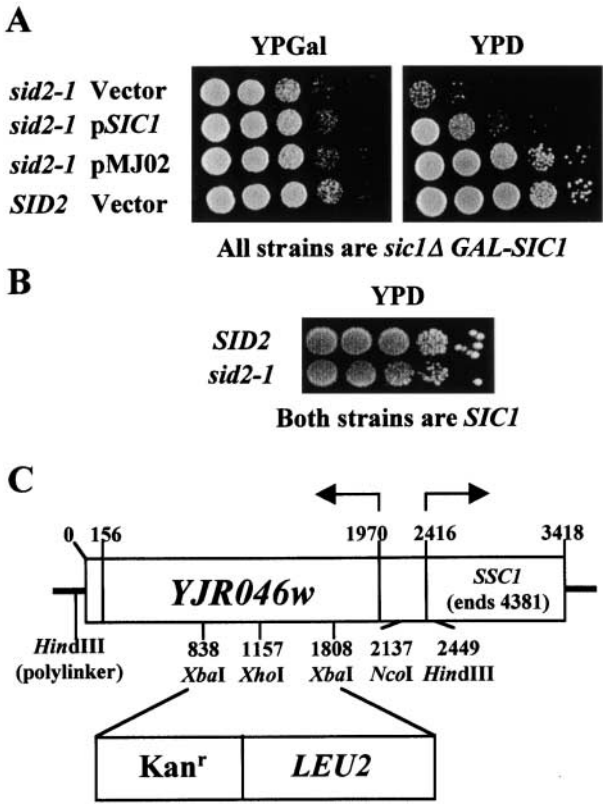


FIGURE 1.—(A) A *CEN*-based plasmid carrying *YJR046w* (pMJ02) rescues *sid2-1 sic1* lethality. Strain MJ193 (*sid2-1 sic1*Δ *GAL1-SIC1*) was transformed with the *YJR046w*-containing plasmid pMJ02, the RS415 vector, and a RS415 *CEN*-based plasmid containing *SIC1* under the control of its own promoter. MJ58 (*sic1*Δ *GAL1-SIC1*) was transformed with RS415. The strains were grown overnight to stationary phase in SCGal-Leu and 5 μl of 10-fold serial dilutions were plated on YPGal and YPD and incubated for 3–4 days at 30°. The *SIC1* plasmid-containing *sid2-1 sic1*Δ strain shows a slight rescue compared to vector but did not grow nearly as well as the *YJR046w*-containing *sid2-1 sic1*Δ strain on YPD. All transformants grew equally well on YPGal where *GAL1-SIC1* was expressed. (B) *sid2-1 SIC1* and *SID2 SIC1* strains have similar plating efficiencies. Strains 914 (*SID2 SIC1*) and LY1118 (*sid2-1 SIC1*) were grown overnight to stationary phase in YPD and plated as described above. (C) The cloned region contained in pMJ01 (RS405) and pMJ02 (RS415) that rescues *sid2-1* and the *SID2* disruption construct (pMJ07) where a Kan^r/*LEU2* fragment replaced ~1 kb of *YJR046w* coding sequence.

a 604-amino-acid protein lacking significant homology to any known genes. Since *SID2* was known to be essential in wild-type cells (HUANG *et al.* 1997), a *sid2*Δ *GAL1-SIC1* strain was constructed to determine if the *sid2* null mutation could be rescued by elevated Sic1 levels. *SID2* was deleted in a diploid heterozygous for *GAL1-SIC1* and *sic1*Δ using a construct that removed 1 kb internal to *SID2* and inserted a 3.5-kb fragment containing *LEU2*-Kan^r (Figure 1C). The diploid was sporulated and the tetrads were dissected on galactose-containing media. All viable spores were Leu$^-$; the spores predicted to contain *sid2::LEU2* were inviable even when they were predicted to contain *GAL1-SIC1*. Therefore, overexpression of *SIC1* does not suppress the lethality caused by deletion of *SID2*. Most of the further analysis of *SID2* was performed using *sid2-1* strains. The *sid2-1* mutation was backcrossed from the original mutagenized strain into the *sic1*Δ *GAL1-SIC1* background at least four times before further analysis.

SID2 on a centromere-based plasmid completely rescued the lack of growth of the *sid2-1 sic1*Δ cells (Figure 1A). Similarly, *GAL1-SIC1* overexpression permitted growth of *sid2-1 sic1*Δ strains on YPGal that was comparable to wild type (Figure 1A). To demonstrate that growth of *sid2-1 sic1*Δ strains on YPGal was dependent on the presence of *GAL-SIC1*, we dissected diploids heterozygous for *sid2-1*, *sic1*Δ, and *GAL-SIC1* on YPGal. *SID2::LEU2* was also segregating in the cross, allowing unambiguous determination of *sid2-1* spores. Dissection of 65 tetrads on YPGal gave 23 spores that could unequivocally be predicted to be *sid2-1 sic1*Δ and lacking *GAL-SIC1*; all were dead. In contrast, the viability of *SID2 sic1*Δ cells lacking *GAL-SIC1* was 86% ($n = 29$).

The screen that isolated *sid2-1* identified mutants that were viable in the presence of high levels of *SIC1* and inviable in the absence of *SIC1*. To determine whether *sid2-1* mutant cells required the high levels of *SIC1* expressed from the *GAL1* promoter throughout the cell cycle, we assayed the growth of *sid2-1* cells in the presence of lower levels of *SIC1*. In contrast to the complete suppression by *GAL-SIC1*, *sid2-1 sic1*Δ strains were only partially rescued by *SIC1* on a centromere-based plasmid when *SIC1* was expressed under the control of its own promoter (Figure 1A). However, a more complete rescue of *sid2-1* strains by endogenous *SIC1* was observed in backcrosses where the wild-type *SIC1* gene was segregating against *sic1*Δ. When diploids heterozygous for *sid2-1* and *sic1*Δ were sporulated and tetrads were dissected, colonies that were *sid2-1 SIC1* were similar in size to those that were *SID2 SIC1* (data not shown). When *sid2-1 SIC1* spores were analyzed, they gave plating efficiencies more similar to wild-type *SID2 SIC1* strains (Figure 1B), although a slight decrease in both the number of colony forming units and colony size could still be observed in the *sid2-1 SIC1* strain. The observed difference between the ability of plasmid and chromosomal *SIC1* to rescue may be due to a lack of regulatory regions in the *SIC1*-containing plasmid. The suppression of *sid2-1* by wild-type levels of *SIC1* demonstrates that overexpression of *SIC1* is not absolutely required for the viability of *sid2-1* mutant cells. Although the *sid2-1 SIC1* mutant cells appear to grow fairly similarly to wild type, they do have an increase in the number of cells with a 2C DNA content and large buds compared to *SID2 SIC1* cells (Figure 7, E and F). Based on the other phenotypes of *sid2* mutants, it is likely that this phenotype is due to a defect in DNA replication or induction of a DNA damage checkpoint. It is less likely that the *sid2* mutant cells replicate their DNA prematurely compared to wild type because *sid2* mutant cells are actually delayed

in DNA replication compared to wild-type cells (see below).

Sid2p, while predominantly cytoplasmic, has a functional nuclear import signal: Determining the timing of Sid2p expression or its intracellular localization might aid our understanding of how and when Sid2p functions. To detect Sid2p, we tagged the genomic locus of *SID2* with protein A and detected it by Western blot. *SID2-PrA* strains do not show a growth defect and have a wild-type FACS profile (data not shown). In addition, *SID2-PrA sic1*Δ strains are viable. This indicates that the protein A tag does not interfere with Sid2p function. A strain containing the tagged *SID2* gene was arrested with α-factor and released into YPD. Protein extracts were made at 12-min intervals following the α-factor release and Sid2p levels were found to remain constant throughout the cell cycle (data not shown).

We could not detect Sid2-PrA by immunofluorescent staining of either logarithmically growing or hydroxyurea (HU)-arrested cells, perhaps because the level of expression is low or the localization is diffuse. Following fractionation of the Sid2-protein A-containing strain, Sid2p protein was visualized by Western blot and found to be predominately cytoplasmic, although a small amount of nuclear Sid2p could be detected (Figure 2A). Interestingly, analysis of Sid2p's localization using a one-hybrid assay (Rhee *et al.* 2000) suggests that Sid2p has a functional nuclear localization signal (NLS; Figure 2B). A fusion protein containing a modified *lexA* DNA-binding domain, the *GAL4* transcriptional activation domain, and Sid2p (NIASID2) could enter the nucleus and activate transcription from a *lexA*-operator-driven *lacZ* reporter gene. In contrast, a control fusion containing the cytoplasmic protein VirE2, which is of similar size to Sid2p, could not activate transcription from the reporter gene (NIAE2, Figure 2B; Rhee *et al.* 2000). Levels of transcriptional activation from the Sid2p-containing construct were lower than that from a control construct containing the NLS from SV40 T antigen (NEAE2). Taken together with the fractionation experiments, these data suggest that Sid2p has a functional NLS, although it may be weak or subject to regulation.

***sid2-1 sic1*Δ and *sid2-21* cells have a preanaphase arrest:** To characterize the effect of the *sid2-1* mutation, we analyzed the morphology of *sid2-1* and *SID2* cells (both *sic1*Δ *GAL1-SIC1*) in the absence of *SIC1* expression by shifting cultures from YPGal (*SIC1* expressed) to YPD (*SIC1* repressed). *sid2-1 sic1*Δ cells slowed proliferation and then arrested 6 hr after repression of *GAL1-SIC1*, showing only minimal increases in cell number at later time points (Figure 3A). There was no decrease in viability up to 5 hr after the shift to dextrose-containing media and a slight decrease in viability (less than fourfold) by 10 hr after the shift (data not shown). *sid2-1 sic1*Δ cells accumulated with large buds at the arrest point (Figure 3, B and D). Repression of *SIC1* slightly decreased the number of unbudded cells in the *SID2* strain but otherwise had little effect on the distribution of cell morphologies (Figure 3B).

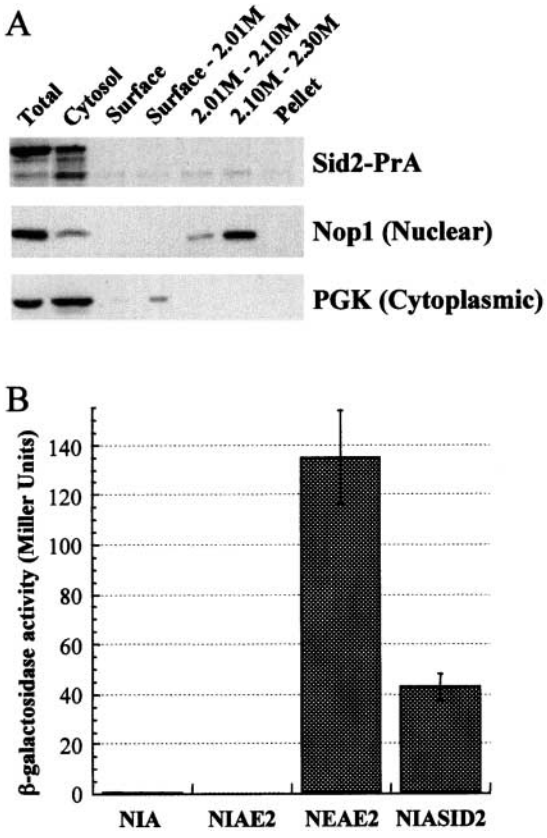


Figure 2.—Sid2p is predominantly cytoplasmic, but has a functional nuclear localization signal. (A) Strain LY925, in which *SID2* was genomically tagged at its carboxyl terminus with protein A, was fractionated using a sucrose gradient. Cytosolic, surface, surface to 2.01 M sucrose, 2.01–2.10 M sucrose, 2.10–2.30 M sucrose, and pellet fractions were collected and appropriate amounts were loaded to adjust for varying collection volumes. The fractions were examined by Western blot probing for Sid2-PrA, Nop1 (a nuclear control), and PGK (3-phosphoglycerate kinase, a cytoplasmic control). (B) β-Galactosidase assays were performed on strain L40, which contains *lacZ* under the control of the lexA operator, after transformation with plasmids encoding fusion proteins. All the plasmids contain a modified *lexA* DNA-binding domain and the *GAL4* transcriptional activation domain. Control plasmid NIA contains the *lexA-GAL4* fusion, while the remaining plasmids contain fusions between that sequence and other coding regions. Plasmid NIAE2 carries the sequence encoding the cytoplasmic VirE2 protein from *Agrobacterium tumefaciens*, plasmid NEAE2 carries the sequence encoding the SV40 large T Ag NLS and VirE2, and NIASID2 carries the entire *SID2* coding sequence. Error bars represent the standard error of two experiments; the data for NIASID2 is the average of the results of two experiments performed with six independently isolated NIASID2 plasmids. Activity levels and standard error for the NIA and NIAE2 transformants were 0.43 ± 0.04 units and 0.31 ± 0.03 units, respectively.

Similarly, we compared the morphology of wild-type *SID2* cells and cells containing a temperature-sensitive allele, *sid2-21*, after incubation at the nonpermissive temperature. *sid2-21* cells began to die by 3 hr after shift to 37° (Figure 4A). After 4 hr of incubation at 37°, the *sid2-21*

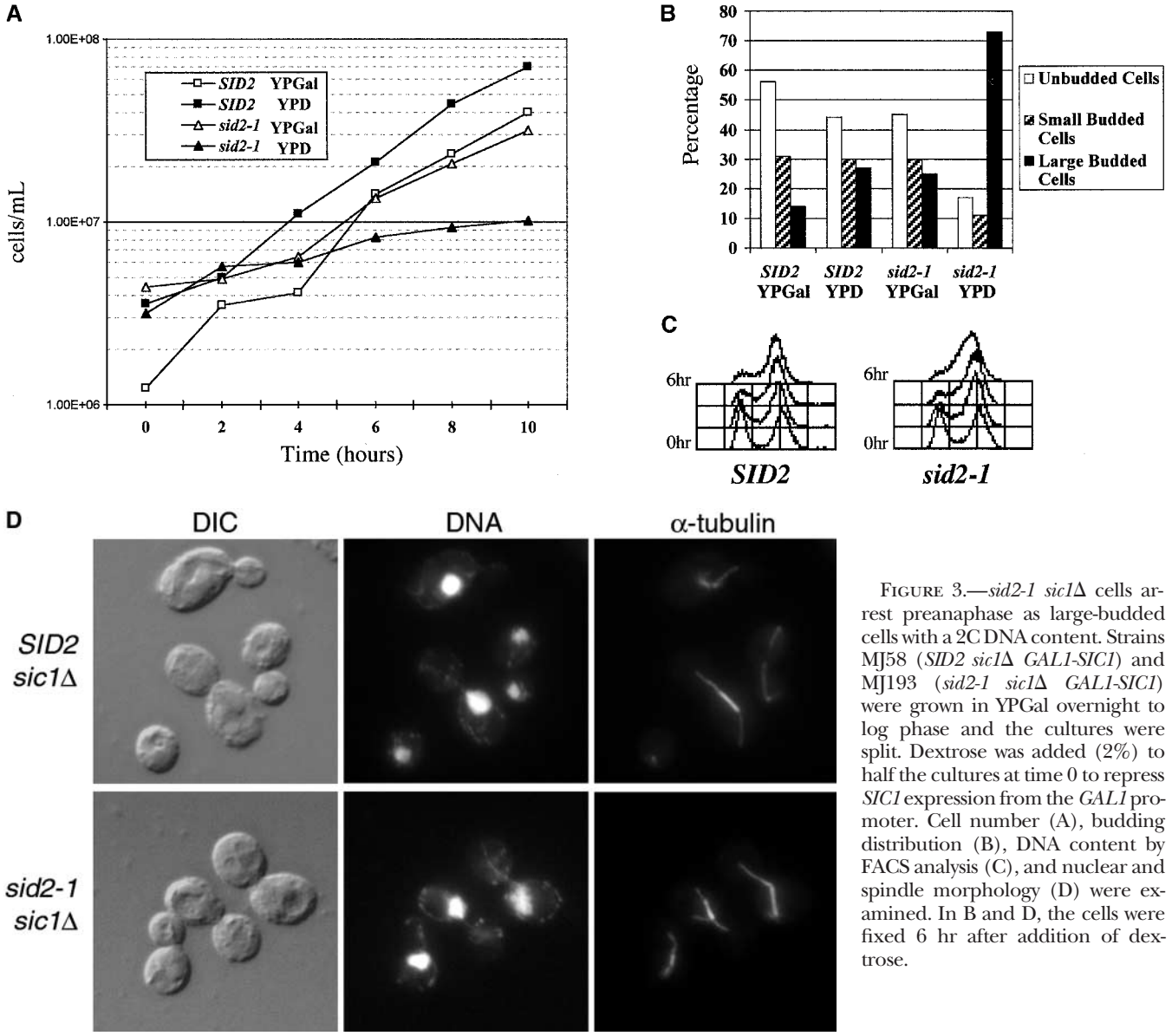


FIGURE 3.—*sid2-1 sic1*Δ cells arrest preanaphase as large-budded cells with a 2C DNA content. Strains MJ58 (*SID2 sic1*Δ *GAL1-SIC1*) and MJ193 (*sid2-1 sic1*Δ *GAL1-SIC1*) were grown in YPGal overnight to log phase and the cultures were split. Dextrose was added (2%) to half the cultures at time 0 to repress *SIC1* expression from the *GAL1* promoter. Cell number (A), budding distribution (B), DNA content by FACS analysis (C), and nuclear and spindle morphology (D) were examined. In B and D, the cells were fixed 6 hr after addition of dextrose.

cells accumulated with large buds similar to the *sid2-1 sic1*Δ-arrested cells (Figure 4, B and D). The distribution of cell morphologies of *SID2* cells, in contrast, was unaffected by the temperature shift (Figure 4B).

The arrest of *sid2-1 sic1*Δ and *sid2-21* cells was further examined using FACS to analyze DNA content. By 6 hr after repression of *GAL1-SIC1*, the DNA content of *SID2 sic1*Δ cells was predominately 2C, though a small 1C peak was also observed (Figure 3C). Cells deleted for *sic1* have a short G1 phase probably due to the lack of S phase cyclin/CDK (Clb5/Clb6p-Cdc28p) inhibition (SCHWOB *et al.* 1994). The DNA content of the mutant *sid2-1 sic1*Δ cells also shifted primarily to an ~2C peak. However, in contrast to the *SID2 sic1*Δ cells, the *sid2-1 sic1*Δ population at 6 hr after shift to YPD had a less distinct 1C peak, and the 2C peak was much broader, having a shoulder of cells with DNA content between 1C and 2C (Figure 3C). These data indicate that, although the mutant cells were able to replicate at least most of their DNA in the absence of *SIC1* expression, they may have some defect associated with DNA synthesis.

The DNA contents of the *SID2* and *sid2-21* populations of cells were similar at 25°, with cells approximately equally distributed between two distinct peaks at 1C and 2C (Figure 4C). For the *SID2* cells, this profile remained constant after the temperature shift to 37°. In contrast, 2 hr after the shift to the nonpermissive temperature, the *sid2-21* cells accumulated with a DNA content intermediate between the 1C and 2C peaks. By 4 hr at the nonpermissive temperature, the DNA content of the *sid2-21* cells shifted to a broad, ~2C peak (Figure 4C). These data indicate that, like the *sid2-1 sic1*Δ cells, the *sid2-21* mutant cells appear to replicate most or all of their DNA under nonpermissive conditions. However, the accumulation of cells in S phase at 2 hr suggests that *sid2* mutants may have a defect in DNA replication (see also below).

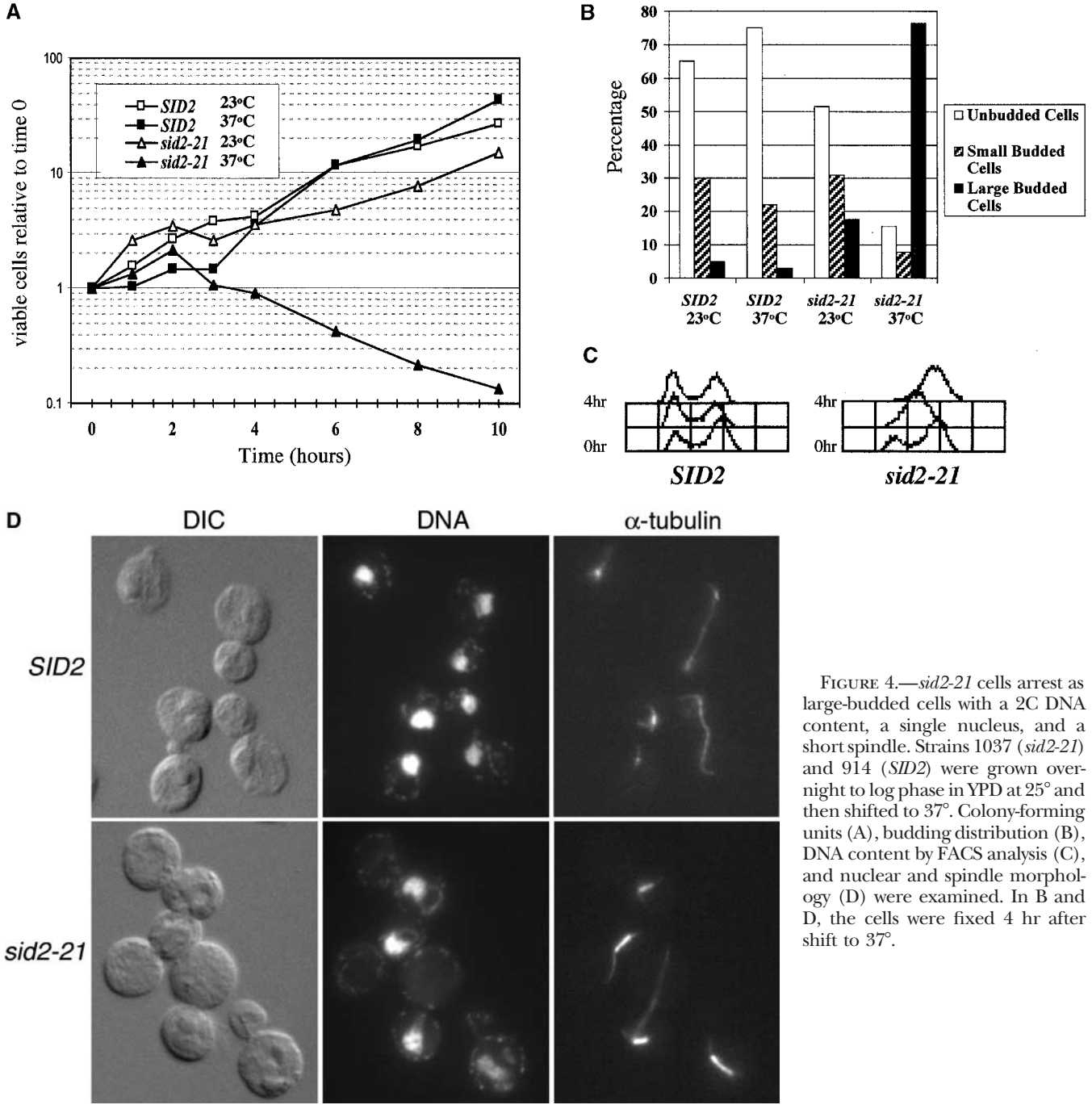


FIGURE 4.—*sid2-21* cells arrest as large-budded cells with a 2C DNA content, a single nucleus, and a short spindle. Strains 1037 (*sid2-21*) and 914 (*SID2*) were grown overnight to log phase in YPD at 25° and then shifted to 37°. Colony-forming units (A), budding distribution (B), DNA content by FACS analysis (C), and nuclear and spindle morphology (D) were examined. In B and D, the cells were fixed 4 hr after shift to 37°.

To characterize the defect in the *sid2* mutants more completely, *sid2-1 sic1*Δ, *SID2 sic1*Δ, *sid2-21*, and *SID2* cells were stained with DAPI and tubulin was visualized by indirect immunofluorescence. In contrast to the *SID2 sic1*Δ and *SID2* cells that were at various cell cycle stages, the *sid2-1 sic1*Δ and *sid2-21*-arrested cells appeared to be preanaphase with a single nucleus and a short spindle (Figures 3D and 4D). The arrest morphology of *sid2 sic1*Δ and *sid2-21* cells suggests that, unlike other mutations that are lethal in combination with *sic1*Δ, the primary defect of the *sid2* mutants is not in the reduction of Clb2p-CDK activity. Mutants that fail to inactivate Clb2p-CDK activity arrest primarily in telophase with elongated spindles and DNA segregated between the mother and bud (SURANA *et al.* 1993). The arrest phenotype demonstrated by the *sid2-1 sic1*Δ and *sid2-21* cells has some similarities to phenotypes demonstrated by mutants that affect DNA replication or APC activation. Of these, the slow S phase observed in the *sid2-21* mutant strains is most consistent with a defect in DNA replication.

Both S and M phase B-type cyclins are only slightly stabilized in *sid2-1* mutants: One possibility, based on the morphology of *sid2-1 sic1*Δ cells, was that *sid2-1* affected APC activity. It has previously been demonstrated that a

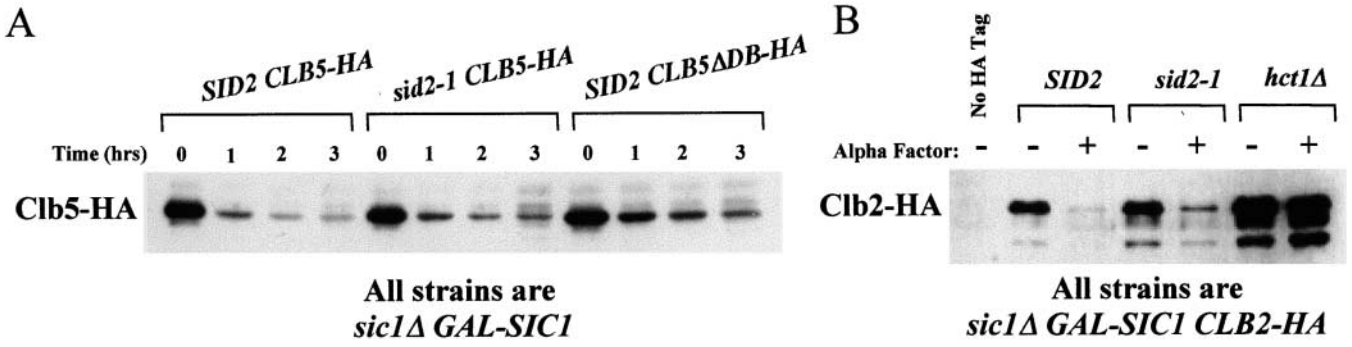


FIGURE 5.—Clb5p and Clb2p are only slightly stabilized in *sid2-1* α-factor-arrested cells. Strains (A) MJ316 (*SID2 sic1*Δ *GAL1-SIC1 CLB5*^{HA}), MJ317 (*sid2-1 sic1*Δ *GAL1-SIC1 CLB5*^{HA}), and MJ319 (*SID2 sic1*Δ *GAL1-SIC1 CLB5*Δ*DB*^{HA}) and (B) MJ288 (*SID2 sic1*Δ *GAL1-SIC1 CLB2*^{HA}), MJ282 (*sid2-1 sic1*Δ *GAL1-SIC1 CLB2*^{HA}), and MJ292 (*cdh1*Δ/*hct1*Δ *sic1*Δ *GAL1-SIC1 CLB2*^{HA}) were grown overnight to log phase and arrested with 0.1 μM α-factor for 3 hr. (A) Clb5p levels were examined at 1-hr intervals after the addition of α-factor and (B) Clb2p levels were examined before and after the arrest. The samples were processed by immunoprecipitation followed by immunoblotting.

number of mutants that affect APC activity are lethal in combination with deletion of *sic1* (TOYN *et al.* 1997; SCHWAB *et al.* 1997; KRAMER *et al.* 1998). Mutants that fail to activate the APC have defects in the degradation of Clb2p (IRNIGER *et al.* 1995; ZACHARIAE *et al.* 1996; KRAMER *et al.* 1998). In addition, Clb5p degradation appears, at least in part, also to be regulated by the APC (IRNIGER and NASMYTH 1997; SHIRAYAMA *et al.* 1999). Failure to degrade the B-type cyclins (caused by a mutation in an APC component), coupled with lack of inhibition of the CDK kinase (caused by deletion of *sic1*), appears to result in levels of CDK kinase that are too high to allow progression through mitosis. We therefore analyzed Clb5p and Clb2p stability in *sid2-1 sic1*Δ cells to determine whether increased B-type cyclin levels could contribute to the observed phenotypes.

An asynchronous culture of a genomically tagged *CLB5*^{HA} strain was treated with α-factor, which blocks *CLB5* expression (EPSTEIN 1992). Clb5p turnover was examined by following protein levels during the arrest. A strain in which Clb5-HAp's destruction box was removed was used as a positive control (*CLB5*Δ*DB*^{HA}). The levels of Clb5-HAp resulting from the deletion of the destruction box do not affect the viability of cells even in the absence of *sic1* (Figure 6). The percentage of unbudded cells at each hourly time point was comparable for all three strains, indicating that they arrested with similar kinetics (data not shown). Clb5-HAp levels in the *sid2-1* strain were slightly higher than in the *SID2* strain, though Clb5-HAp was not stabilized to the degree that it was upon removal of the destruction box (Figure 5A), suggesting that this defect is not sufficient to explain the arrest of *sid2-1* cells.

To assay the stability of Clb2p in *sid2-1* cells, *CLB2*^{HA} cells were arrested with α-factor as described above. In wild-type strains, Clb2p is degraded at this G1 arrest point (AMON *et al.* 1994). While the *sid2-1* cells did not uniformly arrest at 1C as determined by FACS analysis, the profile was comparable to the *SID2* strain (data not shown). Clb2-HAp was present at a slightly higher level in *sid2-1* α-factor-arrested cells than in *SID2* α-factor-arrested cells (Figure 5B). Cdh1p/Hct1p targets Clb2p for degradation by the APC in late mitosis, and G1 cells deleted for *CDH1/HCT1* show greatly increased levels of Clb2p compared to wild type (SCHWAB *et al.* 1997; see also Figure 5B). Clb2-HAp was not stabilized in *sid2-1* cells to the degree that it was in *cdh1*Δ/*hct1*Δ cells. Taken together, these results suggest that while *SID2* may have some role in decreasing Clb5p and Clb2p-CDK kinase activity, it is most likely very minor as Clb stability is not affected to the degree that it is by removing either the destruction box (Clb5p) or *CDH1/HCT1* (Clb2p). This suggests that Sid2p is not a component of the APC and that the arrest of *sid2-1 sic1*Δ cells is not likely to be due primarily to a defect in APC activity (see DISCUSSION).

***sid2-1* interacts genetically with S phase cyclins:** As *sid2-1 sic1*Δ cells had a preanaphase arrest and *sid2-1* did not appear to affect APC function, we thought it likely that *sid2-1* was causing a defect in DNA replication. We therefore analyzed the effects of deleting the S phase cyclins, *CLB5* and *CLB6*, on the growth of *sid2-1 sic1*Δ cells. If Sic1p rescues *sid2-1* by inhibiting the kinase activity of S phase cyclin-CDK complexes, then deleting these genes should mimic Sic1p expression. A deletion of *CLB5* partially suppressed the growth defect of *sid2-1 sic1* cells, and the *clb5 clb6* double deletion almost completely rescued *sid2-1 sic1* cells (Figure 6, top). Deleting *CLB6* alone did not have a detectable effect (data not shown). Since the removal of these S phase activators rescued the arrest caused by *sid2-1* in *sic1* cells, we hypothesized that increasing S phase cyclin levels would have the opposite effect. The destruction box of the more potent of these two cyclins, *CLB5*, was removed (CROSS *et al.* 1999) and *sid2-1 sic1*Δ *GAL1-SIC1 CLB5*Δ*DB*^{HA} strains were constructed. The *CLB5*Δ*DB*^{HA} construct results in partial stabilization of Clb5-HAp (Figure 5A). *sid2-1* cells that contained *CLB5*Δ*DB*^{HA} showed at least a 10-fold decrease in plating

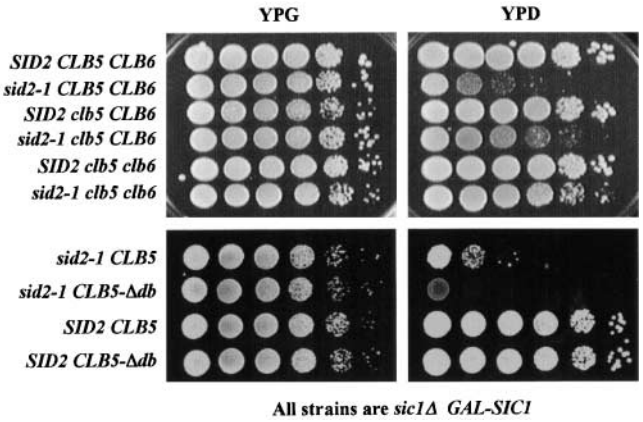


FIGURE 6.—*sid2-1* interacts genetically with S phase cyclins. (Top) Strains LY986 (*SID2 sic1*Δ *GAL1-SIC1*), LY987 (*sid2-1 sic1*Δ *GAL1-SIC1*), LY989 (*SID2 sic1*Δ *GAL1-SIC1 clb5*Δ), LY991 (*sid2-1 sic1*Δ *GAL1-SIC1 clb5*Δ), LY985 (*SID2 sic1*Δ *GAL1-SIC1 clb5*Δ *clb6*Δ), and LY988 (*sid2-1 sic1*Δ *GAL1-SIC clb5*Δ *clb6*Δ) were grown overnight to stationary phase in YPGal and 5 μl of 10-fold serial dilutions were plated on YPGal and YPD and incubated for 3–4 days at 30°. (Bottom) Strains MJ322 (*sid2-1 sic1*Δ *GAL1-SIC1*), MJ321 (*sid2-1 sic1*Δ *GAL1-SIC1 CLB5*Δ*DB*^{HA}), MJ323 (*SID2 sic1*Δ *GAL1-SIC1*), and MJ319 (*SID2 sic1*Δ *GAL1-SIC1 CLB5*Δ*DB*^{HA}) were grown and plated as described for the top.

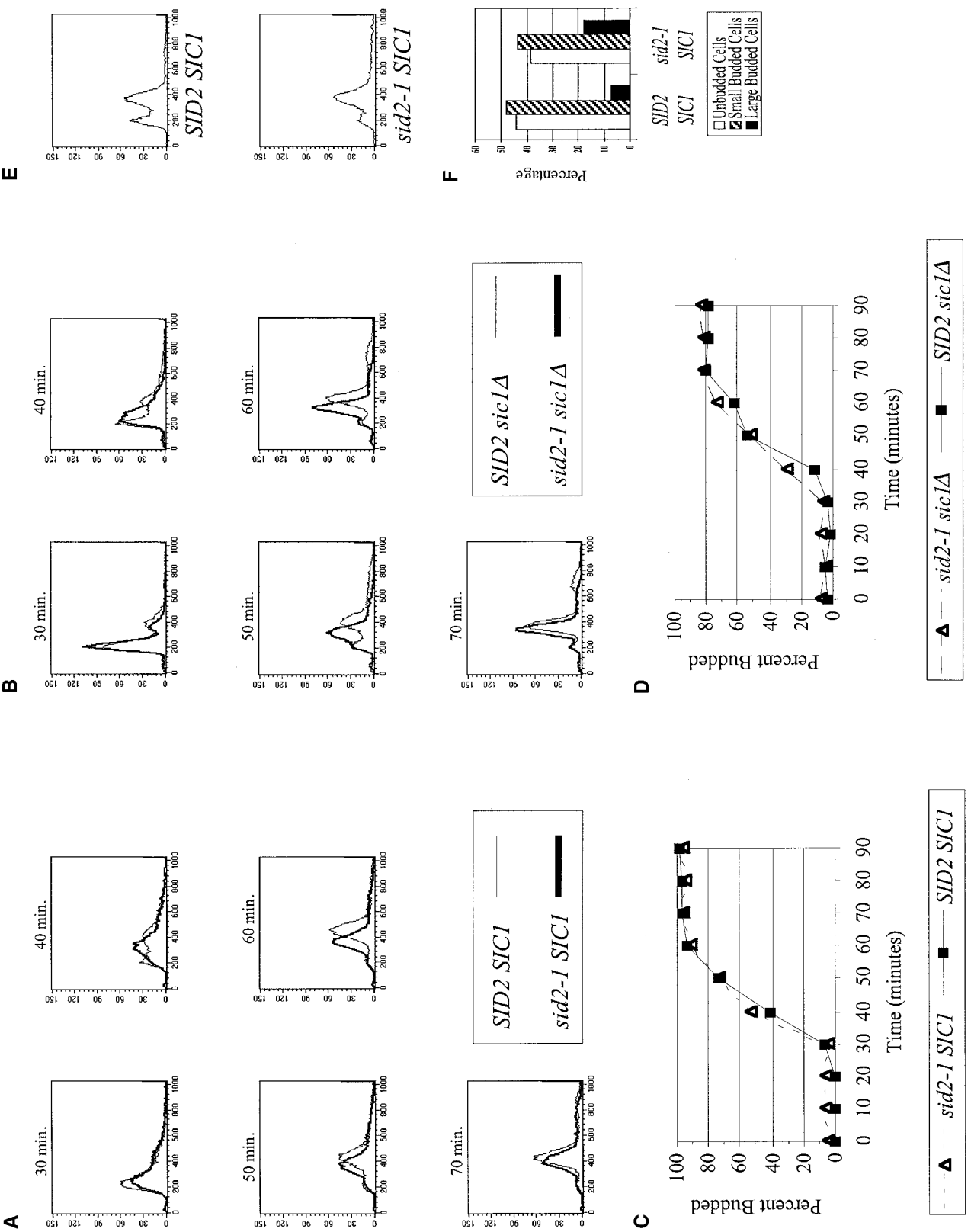
efficiency compared to strains with wild-type Clb5 levels when grown in the absence of *SIC1* (Figure 6, bottom). The *CLB5*Δ*DB*^{HA} construct did not appear to have an effect on *SID2 sic1*Δ cells. Taken together, these interactions suggest that Sid2p's major function is related to DNA replication, since the main (but not the only) biological function of *CLB5* and *CLB6* is to trigger replication (SCHWOB and NASMYTH 1993; SEGAL *et al.* 1998).

***sid2* mutants may accumulate DNA damage during replication:** *sic1*Δ cells exhibit accelerated entry into DNA replication presumably resulting from premature Clb5p- and Clb6p-associated Cdc28 kinase activity (SCHWOB *et al.* 1994). It is possible, therefore, that *sid2 sic1*Δ synthetic lethality could be the result of DNA damage or synthesis defects occurring because of unregulated replication. This would be consistent with the preanaphase arrest observed for the *sid2-1 sic1* mutants, since DNA damage results in a checkpoint-dependent preanaphase arrest. To analyze the progression of the mutant cells in S phase, we used α-factor to synchronize the cells in G1 and monitored DNA synthesis by FACS analysis. *SID2*, *sid2-1*, *SID2 sic1*Δ *GAL1-SIC1*, and *sid2-1 sic1*Δ *GAL1-SIC1* strains were released from the α-factor arrest into YPD. All cells began budding and replicating their DNA at approximately the same time, by about 40 min after the α-factor release (Figure 7). Due to the speed and partial asynchrony of DNA replication, the *SID2* strains do not accumulate detectably at an intermediate stage between 1C and 2C while replicating their DNA. Only a decrease in the 1C peak and a commensurate increase in the 2C peak could be observed. At the same time intervals, however, *sid2-1* cells, regardless of their *SIC1* genotype, do accumulate at a point between 1C and 2C and are delayed in reaching a completed 2C state (Figure 7). Consistent with the profiles for the *sid2-1 SIC1* strain, the replication delay for *sid2-1 sic1*Δ *GAL1-SIC1* cells is also observed when strains are released into YPGal where *GAL1-SIC1* is expressed (data not shown). It may be that errors or DNA damage occur during DNA replication due to the *sid2-1* mutation, since damage slows the rate of S phase progression due to a Mec1- and Rad53-dependent checkpoint (PAULOVICH and HARTWELL 1995), and sufficient DNA damage can cause arrest with a nearly 2C DNA content.

We were interested in determining whether replication was completed in *sid2* strains and used field inversion gel electrophoresis to probe the structure of chromosomes in *sid2* mutant strains. Chromosomes isolated from cells blocked in replication fail to band properly on similar gel systems (HENNESSY *et al.* 1991). This is most likely due to the presence of replication forks and bubbles, which make the chromosomes heterogeneous in size and alter their migration properties. As expected, DNA isolated from cells blocked in S phase by treatment with hydroxyurea failed to band (Figure 8, lane 9), while DNA from a wild-type strain in log phase demonstrated a characteristic chromosome banding pattern (Figure 8, lanes 1–4). Under permissive conditions, DNA isolated from a *sid2-21* mutant strain migrated similarly to wild type (lane 5). In contrast, under nonpermissive conditions, the DNA isolated from the *sid2-21* mutant showed much less banding than the wild-type strain (Figure 8, lanes 6–8). When taken together with the FACS analysis, these data suggest that, although the *sid2-21* mutant cells may replicate most of their DNA, they still have some replication forks or bubbles present at the time of arrest. In some experiments, there was slighly more banding in the mutant at 37° than in the hydroxyurea-treated sample. It is likely that this is either the result of a less complete arrest or the presence of fewer forks and bubbles in the *sid2-21*-arrested cells.

If DNA replication is slow and fails to be completed in the *sid2* mutant strains because of the accumulation of damage, it is possible that *sid2-1* mutants would be sensitive to DNA damaging agents or compounds that affect DNA replication, since then damage would be occurring for two independent reasons. *sid2-1 sic1*Δ *GAL1-SIC1* strains were ~100-fold more sensitive to HU treatment than *SID2 sic1*Δ *GAL1-SIC1* strains (Figure 9A). A similar effect of HU was found when *sid2-1 SIC1* strains were grown on YPD, where *GAL1-SIC1* was repressed (data not shown). Following UV treatment, a less dramatic decrease in plating efficiency, of ~5- to 10-fold, was found when comparing *sid2-1* to *SID2* strains (data not shown). In contrast, neither *sid2-1* nor *sid2-21* strains showed any sensitivity to the microtubule depolymerizing drug, benomyl, when assayed at a range of drug concentrations (data not shown).

It may be that the *sid2-1* mutation is directly or indirectly (by affecting DNA repair) causing DNA damage, which is then exacerbated by UV or HU to a point of decreased colony formation even with *GAL1-SIC1* expression. A de-


A
B
C
D
E
F
30 min.
40 min.
50 min.
60 min.
70 min.
SID2 SIC1
sid2-1 SIC1
SID2 sic1Δ
sid2-1 sic1Δ
SID2 SIC1
sid2-1 SIC1
Percent Budded
Time (minutes)
Percentage
Unbudded Cells
Small Budded Cells
Large Budded Cells
SID2
SIC1
sid2-1
SIC1

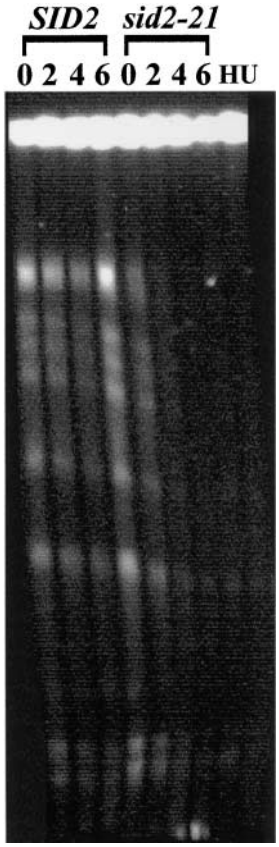


FIGURE 8.—Chromosomes isolated from *sid2-21* strains show decreased banding on an inverted field gel. Strains 1036 (*SID2*) and 1037 (*sid2-21*) were grown to early log phase in YPD. Aliquots of the cultures were removed, the cultures shifted to 37°, and incubation continued for 6 hr. The DNA replication inhibitor HU was added to an aliquot of strain 1036 and incubated for 3 hr at 30°. Chromosomal DNA was isolated from each sample, separated on a field inversion gel, and stained with ethidium bromide. Numbers represent the hours the sample was incubated at 37° before chromosome isolation.

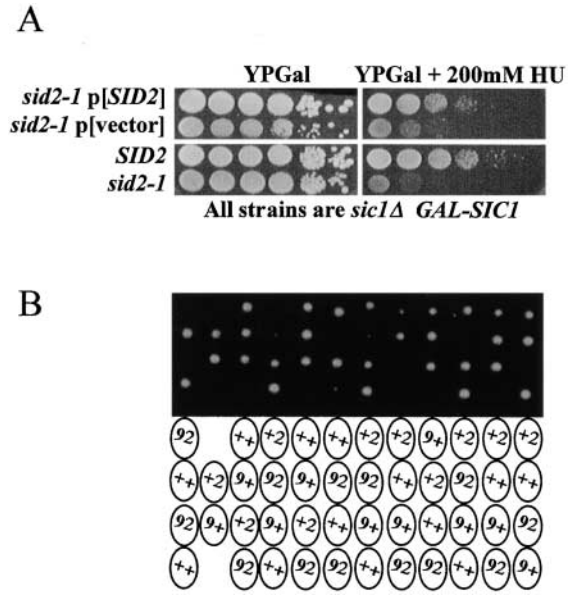


FIGURE 9.—(A) *sid2-1* cells are sensitive to hydroxyurea. Strain MJ163 (*sid2-1 sic1*Δ *GAL1-SIC1*) was transformed with either a plasmid containing *SID2* (pMJ02) or vector (RS415). The strains were grown overnight to stationary phase in SCGal-Leu. Strains LY909 (*SID2 sic1*Δ *GAL1-SIC1*) and LY907 (*sid2-1 sic1*Δ *GAL1-SIC1*) were grown overnight to stationary phase in YPGal. Five microliters of 10-fold serial dilutions were plated on YPGal and YPGal with 200 mM hydroxyurea. (B) *sid2-1 rad9* cells are slow growing or dead. Spores from a diploid strain formed by crossing KK1 (*MAT*α *sic1*Δ *GAL1-SIC1 rad9*Δ) and MJ163 (*MAT***a** *sid2-1 sic1*Δ *GAL1-SIC1*) were dissected on YPGal and incubated at 30° for 3–4 days. The observed or predicted genotype of each spore colony is noted below the tetrad plate.

fect of *sid2-1* cells in a checkpoint pathway could also cause HU and UV sensitivity, but this is less likely because of the delay observed during DNA replication in *sid2-1* strains and the cell cycle arrest phenotype observed with the *sid2-1 sic1*Δ strains.

To determine whether *sid2* mutant cells were accumulating DNA damage and to test the hypothesis that *sid2-1 sic1* cells arrest because of defects in DNA replication or repair, we analyzed *sid2 rad9* cells. *RAD9* is required for the G2 cell cycle arrest caused by DNA damage or incomplete replication (WEINERT and HARTWELL 1988, 1993). We constructed diploid strains heterozygous for *sid2-1* and *rad9*Δ and homozygous for *sic1*Δ and *GAL-SIC1*. Tetrad analysis showed that *sid2-1 rad9*Δ spore colonies were either dead or extremely slow growing, even when *GAL1-SIC1* was expressed (Figure 9B and Table 3). Furthermore, analysis of the *sid2-1 rad9*Δ cells showed that the preanaphase Cdc$^-$ arrest of *sid2 sic1*Δ cells depends on *RAD9* (Table 4). The demonstration that *RAD9* is required both for the full viability of *sid2-1* cells and for the cell cycle arrest of *sid2 sic1*Δ is consistent with the hypothesis that *sid2-1* results in defects in DNA replication or repair.

DISCUSSION

Identification of genes synthetically lethal with *sic1*Δ: *SIC1* encodes a nonessential B-type cyclin/CDK inhibitor that functions at both the G1/S transition and the exit from mitosis. Sic1p decreases Clb5/6p-associated kinase activity at the G1/S stage in the cell cycle, delaying initiation of DNA replication. This is thought to provide the cell time to prepare properly for DNA replication (load origins, synthesize nucleotides, etc.; TANAKA *et al.* 1997;

FIGURE 7.—DNA replication is delayed in *sid2-1* mutant strains. Strains MJ55 (*SID2 sic1*Δ *GAL1-SIC1*), MJ160 (*sid2-1 sic1*Δ *GAL1-SIC1*), LY914 (*SID2 SIC1*), and LY1118 (*sid2-1 SIC1*) were grown overnight to log phase and arrested with 0.1 μM α-factor for 3 hr. The cultures were then released into YPD and progression into S phase was followed by FACS analysis (A and B) and onset of budding (C and D). Asynchronous cultures of strains LY914 and LY1118 were analyzed by FACS analysis and budding index (E and F).

TABLE 3

***rad9 sid2-1* double mutants have a defect in growth and viability**

Genotype (predicted or observed)	% slow growing/dead	% fast growing
RAD9 SID2	17.9 (n = 12)	82.1 (n = 55)
RAD9 sid2-1	20.0 (n = 11)	80.0 (n = 44)
rad9::LEU2 SID2	7.3 (n = 4)	92.7 (n = 51)
rad9::LEU2 sid2-1	98.5 (n = 66)	1.5 (n = 1)

Spores from a diploid strain formed by crossing *rad9::LEU2 SID2 sic1::TRP1 GAL1-SIC1* and *RAD9 sid2-1 sic1::TRP1 GAL1-SIC1* were dissected on YPGal and incubated at 30° for 4 days. Fast growing and slow growing phenotypes refer to colony size as can be seen in Figure 8B and were assigned before scoring *sid2* or *rad9*. The *sid2-1* genotype was assigned to viable spores on the basis of their failure to grow on YPD media.

TABLE 4

***rad9 sid2-1* double mutants fail to demonstrate the preanaphase arrest observed in *RAD9 sid2-1* strains**

Genotype	% large-budded cells with a single nucleus	% anucleate cells
RAD9 SID2	10	0
rad9::LEU2 SID2	10	0
RAD9 sid2-1	80	5
rad9::LEU2 sid2-1	27	1

Strains KK11 (*SID2 sic1*Δ *GAL1-SIC1*), KK17 (*sid2-1 sic1*Δ *GAL1-SIC1*), KK20 (*SID2 sic1*Δ *GAL1-SIC1 rad9::LEU2*), and KK23 (*sid2-1 sic1*Δ *GAL1-SIC1 rad9::LEU2*) were grown overnight to log phase in YPGal and dextrose was added (2%) to repress the galactose-inducible promoter making the strains *sic1*. The cells were fixed and processed for immunofluorescence 6 hr after the addition of dextrose. At least 200 cells were scored for each genotype. The numbers represent the percentage of cells with a given morphology. The remaining cells were those that did not have a large bud with a single nucleus. This class included unbudded cells with a single nucleus, cells with small or medium buds and a single nucleus, and cells with large buds in anaphase or telophase. Large-budded cells had buds at least two-thirds the size of the mother.

Vallen and Cross 1999). As Clb5/6p-Cdc28p may affect the activity of the DNA replication machinery (Duncker *et al.* 1999), Sic1p might also act during S phase to alter the rate of elongation. A major event in the exit from mitosis is the degradation of Clb2p. Sic1p most likely works in parallel to the destruction of Clb2p by inhibiting the kinase activity of any remaining Clb2p associated with Cdc28p. To understand more completely the regulation of these two cell cycle transitions, mutations synthetically lethal with *sic1*Δ were isolated. Mutations in two genes already known to interact genetically with *SIC1*, *DBF2* and *CDC15*, were recovered. The products of both of these genes are thought to assist Sic1p in regulating the exit from mitosis, although their mechanisms of action are not yet completely established (Donovan *et al.* 1994; Jaspersen *et al.* 1998).

We found a novel gene, *SID2*, in this screen. We show that *SID2* is an essential gene (see also Huang *et al.* 1997) encoding a protein stable throughout the cell cycle. *sid2-1 sic1*Δ and *sid2-21^{ts}* strains arrest as large-budded cells with a single nucleus, a short spindle, and DNA content that is close to 2C. This is indicative of a preanaphase arrest and is likely to be due to a defect in the preceding S phase. In contrast, cells arrested because of a failure to exit from mitosis due to high Cdc28p/Clb2p kinase activity have two separated nuclei and an extended spindle (Surana *et al.* 1993; Jaspersen *et al.* 1998). This phenotype is indeed observed with *dbf2 sic1* double mutants (Toyn *et al.* 1997). *sid2-1* is the only mutation currently known to be synthetically lethal with *sic1*Δ that causes an earlier cell cycle arrest phenotype.

Both S and M phase B-type cyclins (Clb5p and Clb2p) appear to be slightly stabilized in cells containing the *sid2-1* mutation. This minor effect is unlikely to account for the *sid2-1 sic1* lethal phenotype, although we cannot fully rule this out. The complete viability of *sic1 CLB5*Δ*DB^{HA}* strains (Figure 6B) argues against the possibility that the minor effect on Clb5p stability is by itself sufficient to account for *sid2-1 sic1* lethality. Similarly, the effect on Clb2p levels is minimal compared to the effect of deleting *CDH1*. Cdh1p targets Clb2p to the APC (Schwab *et al.* 1997; Visintin *et al.* 1997; Zachariae *et al.* 1998a; Figure 5) but even the significant stabilization of Clb2p in *cdh1* cells is not lethal. One likely possibility is that the observed stabilization of the B-type cyclins is due to DNA damage in the *sid2-1* cells (Germain *et al.* 1997).

***SID2* affects DNA replication or repair:** Several arguments suggest that Sid2p acts during DNA synthesis or repair. First, the delay in completion of S phase in *sid2-1* mutants and the terminal arrest phenotype of *sid2-1 sic1*Δ and *sid2-21^{ts}* cells suggest that lack of Sid2p causes defects in DNA replication or damage that blocks cell cycle progression. Second are the observed genetic interactions between *sid2-1* and *CLB5/6*, which function primarily to regulate DNA replication. Third, an allele of *SID2* has previously been isolated as *tah11-1*, which causes a temperature-sensitive growth defect in the presence of a DNA topoisomerase I mutant (top1T722A) that mimics the cytotoxic action of camptothecin (Fiorani and Bjornsti 2000; R. J. D. Reid, P. Fiorani, M. Sugawara and M.-A. Bjornsti, unpublished results). The *tah11-1* mutant is also hypersensitive to hydroxyurea and to camptothecin when *TOP1* is overexpressed. Other mutations that result in similar phenotypes include alleles of *CDC45* and *DPB11* (Reid *et al.* 1999). Both of these genes function in DNA replication (Araki *et al.* 1995; Owens *et al.* 1997; Zou *et al.* 1997; Zou and Stillman 1998) and appear to affect Okazaki fragment maturation (Reid *et al.* 1999). Fourth, the preanaphase arrest observed in *sid2-1 sic1*Δ cells is dependent on *RAD9*, suggesting that arrest results from induction of a checkpoint due to DNA damage and/or

incomplete replication. Fifth, *sid2-1* mutant strains are sensitive to the ribonucleotide reductase inhibitor, HU, which blocks replication progression.

One possibility is that, in *sid2-1 sic1*Δ cells, *sid2-1* may lead to defects during S phase that are enhanced by the precocious onset of replication caused by lack of *sic1*. Removing the *CLB5* destruction box acts to antagonize this process further by placing additional stress in what has become a critical point in the cell cycle of *sid2-1 sic1* strains. Apparently, delaying either the initiation or completion of replication by *GAL1-SIC1* expression or by *clb5*Δ *clb6*Δ provides time for *sid2-1* cells to correct mistakes or to synthesize precursors required for DNA replication.

An alternative model for the role of *SID2* is that it acts during the preceding G1 phase, as the cell is preparing to replicate its DNA. If *sid2-1* results in the cells having not prepared properly for the forthcoming replication process, then high Clb kinase activity late in G1 may lead to lethality. High Clb kinase activity can prevent origins from becoming competent for DNA replication (Dahmann *et al.* 1995). However, we find that *sid2-1 sic1*Δ and *sid2-21* cells accumulate with close to a 2C complement of DNA, making it unlikely that the arrest we observe is due to a severe defect in origin firing. It is also unlikely that the early entry into DNA replication caused by *sic1*Δ in a *sid2-1* background is lethal due to insufficient nucleotide levels. We recently demonstrated that *MEC1* is required for a prereplication delay that allows the accumulation of sufficient deoxyribonucleotides for DNA synthesis (Vallen and Cross 1999). Overexpression of *RNR1*, the gene encoding the limiting subunit of ribonucleotide reductase, suppresses the lethality of *mec1*Δ. However, overexpression of *RNR1* does not suppress the lethality of *sid2-1 sic1*Δ strains (E. A. Vallen, unpublished results).

We also think it is unlikely that *sid2-1 sic1*Δ is lethal because of nonspecific damage to DNA. If any insult to DNA were lethal to *sic1*Δ cells, we would have expected to isolate many more mutations causing this phenotype. Although our screen was not saturated, there are a large number of genes whose null alleles cause increased DNA damage or faulty repair (Friedberg *et al.* 1991). A similar screen for mutations synthetically lethal with *sic1*Δ (Kramer *et al.* 1998) also failed to recover mutations affecting DNA repair. Furthermore, we and others have found that *sic1*Δ strains are not noticeably sensitive to UV or HU (M. D. Jacobson, unpublished observations; Nugroho and Mendenhall 1994) and that *CLB5*Δ*DB* and *sic1*Δ *CLB5*Δ*DB* strains are also not UV sensitive (M. D. Jacobson, unpublished observations). The spontaneous rate of point mutations as assayed by the frequency of canavanine-resistant colonies is also not affected by *sic1*Δ (E. A. Vallen, unpublished observations; Nugroho and Mendenhall 1994) or *sid2-1* (K. Knox and E. A. Vallen, unpublished observations). Perhaps *sid2-1* causes a very specific type of damage that is lethal in combination with *sic1*Δ. Whatever the defect, it is not entirely suppressed by *SIC1* expression as DNA synthesis is still slowed in *sid2-1 GAL-SIC1* and *sid2-1 SIC1* cells and *sid2-1 GAL-SIC1* cells are dependent on *RAD9* for full viability.

Although fractionation experiments show the majority of Sid2p is cytoplasmic, a more indirect assay suggests the protein does contain a functional NLS, which is capable of targeting a fusion protein to the nucleus. Although we can not unequivocally rule out the possibility that the fusion protein is diffusing into the nucleus, a control fusion with the known cytoplasmic protein VirE2, which is similar in size to Sid2, failed to enter the nucleus. In addition, diffusion into the nucleus is known to occur generally only for proteins less than ~40 kD (reviewed in Kaffman and O'Shea 1999). While diffusion limits likely depend on the tertiary structure of the protein as well as its molecular weight, the Sid2-containing fusion protein is predicted to have a molecular weight of ~100 kD, making it quite unlikely that the protein can diffuse into the nucleus. Interestingly, the levels of transcription activated by the Sid2 fusion were lower than those observed for the control containing the T-antigen NLS. Consistent with the fractionation experiment, it may be that only a portion of the protein is constitutively nuclear or, alternatively, that it is transported into the nucleus during a brief period in the cell cycle. The presence of a functional NLS in Sid2 suggests that the role of the protein in DNA replication or repair, while unclear at this point, could be direct. The characterization of more *sid2* alleles as well as the identification of Sid2p-interacting proteins may help illuminate the role of *SID2* in DNA replication.

Cyclin-dependent kinase inhibitors have previously been demonstrated to have an important role in the maintenance of genome integrity. In mammalian cells, CDK inhibitors are induced by irradiation and are frequently mutated in human cancers, suggesting that they have a role in ensuring genome stability. Cells deleted for *sic1* have an increased frequency of chromosome loss and breakage or recombination (Nugroho and Mendenhall 1994). It is likely that these defects are due in some way to advancing origin firing or DNA replication in *sic1*Δ cells. Identifying loci such as *SID2*, which genetically interact with CDK inhibitors and affect DNA replication or repair, will help elucidate the specific roles of CDK inhibitors in ensuring genome stability.

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