MOLECULAR AND CELLULAR BIOLOGY, Jan. 1994, p. 768–776
0270-7306/94/$04.00+0

Vol. 14, No. 1

Interaction between the Cig1 and Cig2 B-Type Cyclins in the Fission Yeast Cell Cycle

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Received 27 July 1993/Returned for modification 23 August 1993/Accepted 29 September 1993

In this report, we describe the cloning and characterization of a B-type cyclin, Cig2 from the fission yeast *Schizosaccharomyces pombe*. The *cig2* gene encodes a 45-kDa protein that is most similar to a previously identified B-type cyclin in *S. pombe*, Cdc13. Deletion of *cig2* had no observable effect on cell viability or progression through the cell cycle. Strains carrying the *cig2* null allele do, however, exhibit an enhanced ability to undergo conjugation relative to a wild-type strain. The *cig2* transcript was found to undergo periodic oscillation during the cell cycle, peaking at the G_1/S-phase boundary. We have investigated the relationship between Cig2 and the other B-type cyclins, Cig1 and Cdc13, in the fission yeast. We found that cells carrying disruptions of both the *cig1* and *cig2* genes contain multiple nuclei with a 1C DNA content, suggesting that they are delayed in progression through the G_1 phase of the cell cycle. The phenotype of this double mutant suggests that there is a delay in septum formation, possibly as a result of defective nuclear separation.

The eukaryotic cell cycle consists of a series of temporally ordered events which ensure that DNA replication and mitosis occur at discrete, nonoverlapping stages during the cell cycle. The mechanisms by which proliferating cells determine the timing and order of cell cycle events is therefore of fundamental interest. The *cdc2* gene encodes a serine threonine protein kinase that has been shown to play a pivotal role in this process since it appears to be a universal regulator of mitosis in all eukaryotic cells (for a review, see reference 27). Analysis of temperature-sensitive alleles of *cdc2* in ascomycete yeasts has established that the $p34^{cdc2}$ protein performs essential functions in both the G_1 and G_2 phases of the cell cycle. Mutant cells carrying certain temperature-sensitive alleles of *cdc2* arrest in either the G_1 phase, prior to DNA replication, or in the G_2 phase, prior to mitosis (28). Those that arrest in G_1 are then capable of sexual conjugation under appropriate conditions, indicating that cells are not committed to cell cycle progression until after the execution of $p34^{cdc2}$ function. The $p34^{cdc2}$ protein kinase activity is thought to be rate limiting for the initiation of mitosis. Dominant alleles of *cdc2* have been identified that advance the initiation of mitosis so that cells divide at a reduced size relative to wild type (30). These *cdc2* alleles bypass *cdc2* regulatory pathways, thereby shortening the G_2 phase of the cell cycle.

Although the overall amount of $p34^{cdc2}$ does not vary during the cell cycle, the activity of the Cdc2 protein kinase has been shown to oscillate (3, 11). It is becoming increasingly apparent that the oscillations in kinase activity are controlled in part by the ability of $p34^{cdc2}$ to interact with regulatory subunits known as cyclins (3, 10, 31). The cyclins make up a family of closely related proteins which function at discrete stages of the cell cycle (for a review, see reference 9). Mitotic cyclins were first identified in sea urchin embryos on the basis of their accumulation during cleavage (13, 34). It has been subsequently shown that the association between $p34^{cdc2}$ and B-type cyclins is a prerequisite for the activation of $p34^{cdc2}$ kinase activity at the onset of mitosis (10). It now appears that different forms of the Cdc2 kinase exist in the G_1 and G_2 phases of the cell cycle and that they can be distinguished on the basis of the type of cyclin in the complex (for a review, see reference 9).

The biochemical analysis of cyclins has been accompanied by genetic analysis, particularly in ascomycete yeasts. In *Saccharomyces cerevisiae*, three "CLN"-type cyclin genes act in G_1 to promote the commitment of cells to a subsequent round of cell division (8, 19, 26). These genes, *cln1*, *cln2*, and *cln3*, exhibit functional redundancy in that inactivation of any pair of them results in a cell cycle delay in G_1 whereas inactivation of all three results in a G_1 cell cycle arrest (19). In addition to the *cln* genes, five B-type cyclin genes, *CLB1* through *CLB5*, have been identified and implicated in cell cycle control in *S. cerevisiae* (12, 14, 32). *CLB1* and *CLB2* encode very similar proteins, whose transcripts appear transiently in G_2 and whose function remains unclear. *CLB3*, *CLB4*, and *CLB5* transcripts accumulate in late G_1 and are therefore thought to promote the initiation and progression of the S phase. Deletion of any one B-type cyclin allele produces only a slight phenotype, which suggests that, like the CLN cyclins, the CLB proteins exhibit some degree of functional redundancy. Deletion of *CLB2* alone is thought to delay the onset of mitosis, whereas mutants lacking *CLB1*, *CLB3*, and *CLB4* enter mitosis with almost normal kinetics (14, 32). Cells deleted for *CLB5* require more time to complete DNA synthesis, suggesting a role for *CLB5* in DNA replication (12).

In the fission yeast *Schizosaccharomyces pombe*, two B-type cyclin genes have been identified. One, *cdc13*, has been shown to be essential for the entry into mitosis (3, 20). Cells carrying either a null or temperature-sensitive allele of *cdc13* are inviable and arrest cell division in G_2. The other, *cig1*, has been shown to be dispensable for the mitotic cycle (4).

In this report, we describe the isolation and characterization of another B-type cyclin, Cig2, in *S. pombe*. Recently, Bueno and Russell reported the identification and characterization the Cig2 B-type cyclin (5). We find discrepancies between our data and certain results reported in that communication which markedly affects the role ascribed to the *cig2* gene in the cell cycle. We find that (i) the amino acid sequence of Cig2 is different from that previously published;

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TABLE 1. List of *S. pombe* strains used in this study

Strain	Genotype
SP530	*h^{-S} cdc25-22 leu1-32*
SP826	*h^{+N}/h^{+N} ura4-D18/ura4-D18 leu1-32/leu1-32 ade6-210/ade6-216*
SP976	*h^{+N}/h^{+N} leu1-32/leu1-32 ade6-704/ade6-704*
SP1051	*h^{+N} ura4 leu1-32 ade6-704*
SP1190	*h^{-S} ura4 leu1-32 ade6-704*
SP1191	*h^{-S} ura4 leu1-32 ade6-704*
SP1192	*h^{+N} cig1::ura4 ura4 leu1-32 ade6-704*
SP1193	*h^{+N} cig1::ura4 cig2::sup3-5 ura4 leu1-32 ade6-704*
SP1194	*h^{-S} cdc13-117 ura4 leu1-32 ade6-704*
SP1195	*h^{+N} cig1::ura4 cdc13-117 ura4 leu1-32 ade6-704*
SP1196	*h^{-S} cig2::sup3-5 cdc13-117 ura4 leu1-32 ade6-704*
SP1197	*h^{-S} cig1::ura4 cig2::sup3-5 cdc13-117 ura4 leu1-32 ade6-704*
SP1198	*h^{+} cig2::sup3-5 ura4 leu1-32 ade6-704*
SP1199	*h^{+N} ura4 leu1-32 ade6-704 sup3-5*
SP1200	*h^{-S} ura4 leu1-32 ade6-704 sup3-5*

(ii) the *cig2* disruption is not sufficient to rescue a *mik1 wee1-50*-induced mitotic catastrophe phenotype; (iii) a *cig2* disruption does not exhibit a synthetic lethal interaction with the temperature-sensitive *cdc25-22* mutation, and (iv) strains carrying a *cig2* disruption in combination with a disruption of *cig1* do, in fact, exhibit a synthetic phenotype. Here, we present data that suggests that Cig2 in combination with the previously identified B-type cyclin, Cig1, acts to promote progression through the G_1 phase of the cell cycle.

MATERIALS AND METHODS

Yeast strains and media. Wild-type yeast strains were an isogenic pair derived from strains originally described by Leupold (23). The genotype of the *S. cerevisiae* strain used in this study is the same as that reported by Xiong et al. (37). *S. pombe* was grown in standard yeast extract medium (YEA), yeast extract medium minus adenine (YE), and pombe minimal medium (PM) containing additional amino acids at 150 μg/ml as described. (1). The *S. pombe* strains used in this study are listed in Table 1.

Isolation and cloning of *cig2*. *cig1* and *cig2* were cloned by their ability to rescue the Cln deficiency of *S. cerevisiae* 305-15d. An *S. pombe* cDNA library was inserted into an expression vector by using the *ADH1* promoter and *Ura4* as a selectable marker. The cDNA library was used to transform the *S. cerevisiae* strain. Transformants were selected for their ability to rescue Cln1 to Cln3 deficiency by monitoring their growth on minimal medium containing glucose. Plasmids containing partial cDNAs were recovered from yeast cells by a method described by Holm et al. (22). The cDNA were sequenced by using a semiautomatic sequencer (ABI 373 DNA sequencer). The genomic clones of *cig1* and *cig2* genes were isolated from a partial *Sau*3A genomic library by hybridization with the cDNA coding sequence as probes.

Disruption of *cig1* and *cig2*. The *cig1* gene was inactivated by insertional mutagenesis with a 2.4-kb *Xho*I genomic fragment that was cloned into pBluescript to form plasmid p3-10. The plasmid contained a unique *Nco*I site in the *cig1* coding sequence. It was digested with *Nco*I, and a 1.8-kb fragment containing *ura4*$^{+}$ was blunt ended with Klenow DNA polymerase and deoxynucleoside triphosphates and ligated. A 4.2-kb *Xho*I fragment containing *ura4*$^{+}$ was used to transform strain SP826. Stable Ura4^{+} transformants were selected by replica plating onto plates containing 0.1% 5 fluoroorotic acid and onto rich medium. Approximately 60% of the stable transformants as analyzed by Southern blot carried one disrupted and one wild-type *cig1* allele. After the *h^{+N}* allele was converted to *h^{90}*, tetrads were dissected and viable progeny were segregated with a ratio of two Ura4^{+} and two Ura4^{-}. The strain carrying the *ade6-216* allele was crossed with SP1051 to yield SP1192 (*cig1::ura4*$^{+}$ ura4^{-} *leu1-32 ade6-704*).

The *cig2* gene was inactivated by using a 3-kb *Sac*I genomic fragment that was cloned into pBluescript. A 0.6-kb *Nde*I-*Nhe*I fragment was replaced with a 0.5-kb fragment containing the *sup3-5* tRNA gene. The *sup3-5* tRNA gene rescues the auxotrophy of strains containing *ade6-704*. The resulting 2.9-kb *Sac*I fragment was used to transform strain SP976. About 50% of the stable diploid Ade^{+} transformants contained a disrupted *cig2* allele. Strain SP1191, when crossed to strains carrying the *ade6-704* allele, segregated into two Ade^{+} and two Ade^{-} spores. A second disruption of *cig2* was used to construct strains carrying temperature-sensitive alleles of *cdc13-117*. In this disruption, SP1196, the *cig2* gene was inactivated by insertion of the *sup3-5* gene into the unique *Nhe*I site within the *cig2* coding sequence. When this *cig2::sup3-5* strain was crossed to SP1192 carrying the *cig1::ura4* disruption, the *cig1::ura4 cig2::sup3-5* segregant was indistinguishable from that obtained from SP1193, which is described below.

Northern analysis. Strain SP530 carrying *cdc25-22* was grown to mid-exponential phase in rich medium (YEA) at 25°C, and the cells were collected by centrifugation and reinoculated into fresh medium at 36°C for 4 h. The culture was shifted back to 25°C, and 50-ml aliquots of cells were harvested at 20-min intervals. Northern (RNA) analysis was performed essentially as recommended by Amersham protocols for nucleic acid blotting and hybridization with Hybond N^{+} membranes. A 30-μg sample of RNA was loaded in each lane, separated by formaldehyde-agarose gel electrophoresis, and transferred to Hybond N^{+} membranes in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). RNA was fixed on Hybond membranes by treatment with 0.05 M NaOH. The desired RNAs were visualized with [^{32}P]DNA probes by random-priming and standard hybridization techniques.

Conjugation assay. Strains SP1198 (*h^{+N} ura4 leu1-32 ade6-704 cig2::sup3-5*), SP1191 (*h^{-S} ura4 leu1-32 ade6-704 cig2::sup3-5*), SP1199 (*h^{+N} ura4 leu1-32 ade6-704 sup3-5*), and SP1200 (*h^{-S} ura4 leu1-32 ade6-704 sup3-5*) were grown separately in minimal medium to mid-log phase, washed, sonicated, and then induced to conjugate by plating of an equal number of cells on minimal nitrogen-rich medium at 30°C. At 48 h the number of conjugates was counted by microscopy. The mating efficiency quoted here is expressed as the number of zygotes produced per 100 cells. The efficiencies presented are representative of the results obtained from three independent experiments. In each experiment at least 500 cells or zygotes were counted.

FACS and DAPI analysis. The procedure for fluorescence-activated cell sorter (FACS) analysis is described by Costello et al. (7). *S. pombe* strains were grown to mid-exponential phase (10^7 cells) in minimal medium (PM). The cells were collected, washed once with distilled water, resuspended in 3 ml of distilled water, and then slowly added to 7 ml of ethanol with vortexing. The cells were incubated for 12 h at 4°C. They were washed and resuspended in 1 ml of 50 mM sodium citrate, RNase A was added to a final concentration of 0.5 mg/ml, and the mixture was incubated

at 37°C. The cells were stained with propidium iodide at a final concentration of 12.5 μg/ml. 4′,6-Diamidino-2-phenylindole (DAPI) analysis was performed with cells cultured in YEA liquid medium at 26°C and then shifted to the restrictive temperature (36°C). After a 4-h incubation, the cells were harvested and fixed in 2.5% glutaraldehyde for 10 min on ice. Fixed cells were washed with cold H_2O twice and then mixed with DAPI solution at a final concentration of 37.5 μg/ml.

RESULTS

Isolation of *cig2*. *S. cerevisiae* yeast strains deleted for all three *CLN* genes are inviable but can be maintained if supplied with a plasmid carrying an extrachromosomal copy of either *CLN1 CLN2*, or *CLN3*. In an attempt to identify novel cyclins in fission yeast cells, we constructed a strain in which *CLN1* and *CLN2* were rendered inactive by insertional mutagenesis. The remaining *CLN3* gene was placed under the control of the inducible *GAL1* promoter (37). Therefore, in medium containing galactose, *CLN3* expression is induced and the cells are viable. In medium containing glucose, *CLN3* expression is turned off and cells arrest in the G_1 phase of the cell cycle. We screened *S. pombe* cDNA expression libraries for plasmids that could rescue the *CLN* deficiency in this strain. The vector contained the *ura4*$^+$ gene as a selectable marker, the 2 μm replication origin, and the promoter and terminator sequences of the yeast alcohol dehydrogenase gene (*adh*). Approximately 10^7 transformants were screened for the ability to grow in medium containing glucose. We recovered 30 transformants whose growth on glucose was dependent on expression of an *S. pombe* cDNA. The cDNAs were recovered from yeast cells and could be grouped into four classes by sequence analysis. One class was identified as *cdc13* (2, 20), and two classes were subsequently identified as *cig1* and *puc1* (4, 15). The fourth represented a partial cDNA encoding Cig2 (Fig. 1) (5). The genomic clone was isolated by hybridization with the radiolabeled partial cDNA as a probe. The genomic DNA sequence is shown in Fig. 2. In contrast to the sequence reported by Bueno and Russell (5), our sequence analysis revealed that the gene comprises an uninterrupted open reading frame encoding a protein of 411 amino acids with a predicted molecular mass of 45 kDa. An amino acid sequence comparison between the predicted protein sequence and those present in current data bases indicated that the protein shares considerable homology to B-type cyclins (Fig. 3). For the most part, homology between members of this family resides in a central region of the protein commonly termed the cyclin box (25). A comparison of amino acid sequences over this region (starting at amino acid 138 of Cig2 versus 204 for Cdc13) with known yeast B-type cyclins indicates that this new cyclin most closely resembles Cdc13, displaying 65% amino acid identity with Cdc13 over this region (Fig. 3). This protein shares 48% homology with the other *S. pombe* B-type cyclin, Cig1, over the same region. It was found to contain a FLRR-SK(R) sequence motif starting at amino acid 272 and shared among all members of the B-type cyclin family. In the N-terminal region, Cig2 was found to contain a so called mitotic destruction box as defined by Glotzer et al. (17), suggesting that Cig2 is a metabolically unstable protein.

***cig2* mRNA levels oscillate during the cell cycle.** The B-type cyclins identified in *S. cerevisiae* have been shown to be subject to periodic transcriptional control during the cell cycle. To investigate the cell cycle transcriptional regulation

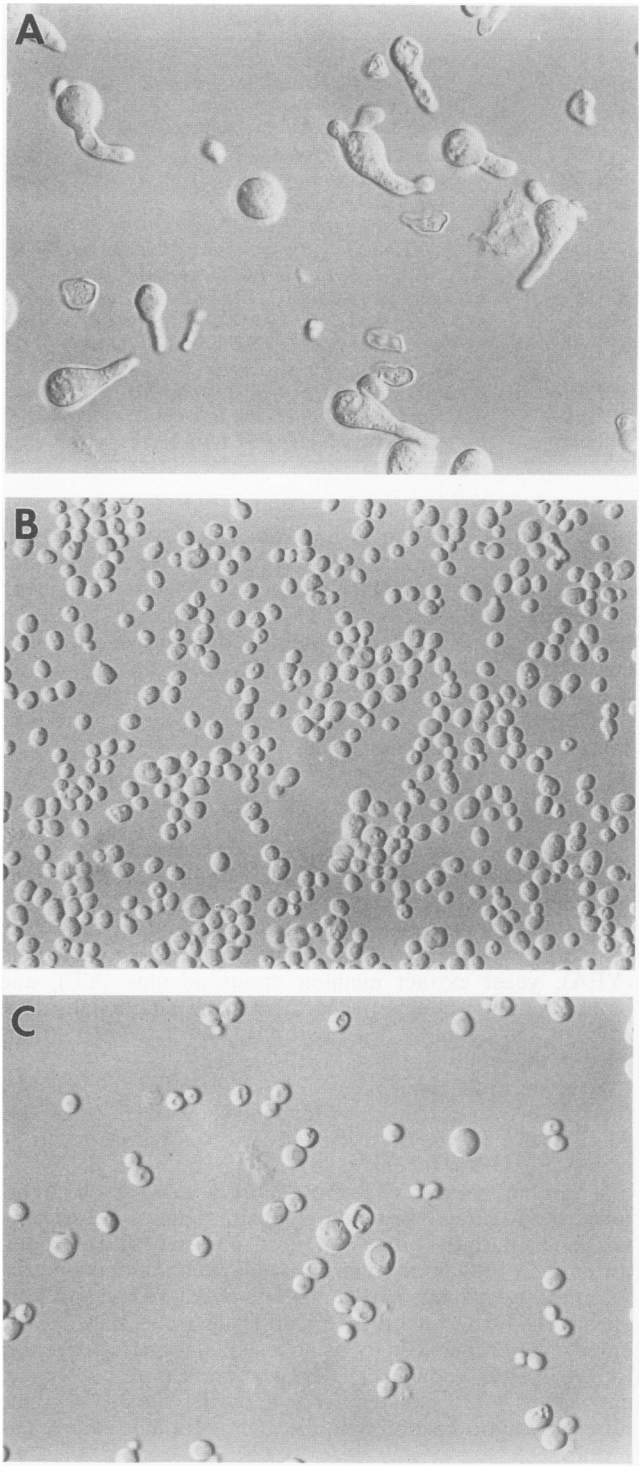

FIG. 1. Complementation of the triple *cln* deletion strain by the *S. pombe cig2* gene. The *S. cerevisiae* strain carries inactivated *cln1* and *cln2* genes. The *cln3* gene is under the transcriptional control of the *Gal-1* promoter. Differential interference contrast micrographs of the *cln1 cln2 Gal cln3* strain transformed with the expression vector alone and plated on medium containing glucose (A) or galactose (B) and with the expression vector containing the *cig2* cDNA and plated on medium containing glucose (C) are presented.

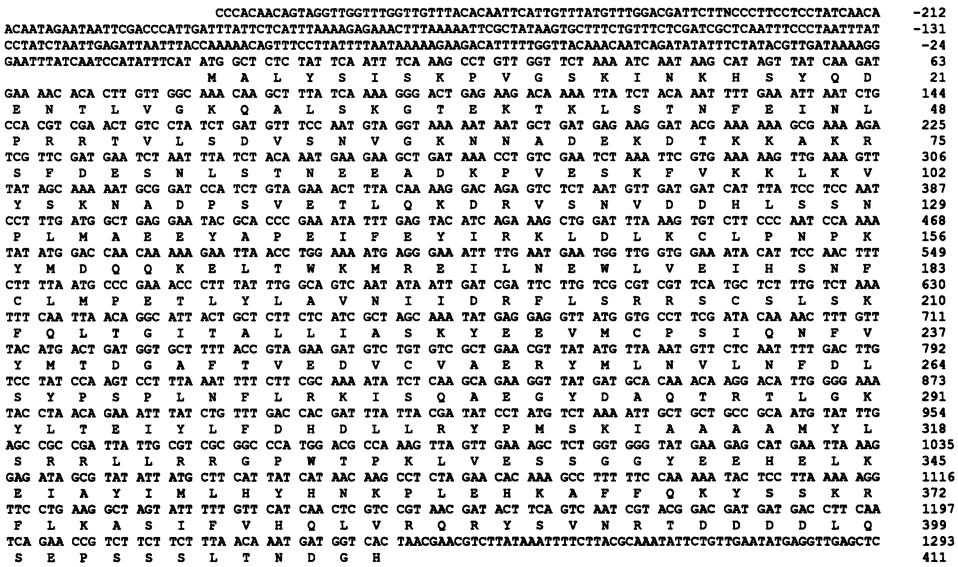

FIG. 2. The nucleotide sequence of *cig2* and the predicted amino acid sequence of the encoded protein. The deduced amino acid sequence is shown below the DNA sequence, starting with the first methionine. The consensus for the mitotic destruction box resides between residues 51 and 59. The FLRR-S(K) motif begins with residue 272.

of Cig2, Northern analysis was performed on RNA extracted from cells undergoing synchronous rounds of cell division. *S. pombe* cells carrying a temperature-sensitive allele of *cdc25* (*cdc25-22*) display a tight arrest in the G_2 phase of the cell cycle when shifted to the nonpermissive temperature of 36°C (33). The cells, however, are capable of reentering the cell cycle after being reintroduced into medium at the permissive temperature (25°C) within a certain period. This provides a convenient method for obtaining cells undergoing synchronous rounds of cell division. A culture of a strain (SP530) carrying *cdc25-22* was synchronized by restricting growth at 36°C for 4 h. The cells were then released into fresh medium at the permissive temperature of 25°C, and aliquots were removed at 20-min intervals over 6 h, corresponding to two cell cycles. Cell cycle profiles were monitored by measuring the cell count and the percentage of septated cells in the culture (Fig. 4). Northern analysis was performed on RNA extracted from these samples by using radiolabeled *cig2* cDNA as a probe. As shown in Fig. 4, the *cig2* transcript appears to oscillate in the cell cycle coincident with the onset of the S phase. To further investigate this periodic expression, the same RNA samples were hybridized with a probe containing the *S. pombe cdc22* gene, which was previously shown to be periodically expressed peaking during the S phase of the cell cycle (18). As shown in Fig. 4, the pattern of *cdc22* expression in the second cell cycle appears to be highly coincident with the pattern of *cig2* expression. Although B-type cyclins have been shown to be under periodic transcriptional control in *S. cerevisiae*, the two known B-type cyclin genes of *S. pombe*, *Cdc13* and *Cig1*, do not appear to undergo periodic oscillation, thereby distinguishing *cig2* on this basis (4, 6a). The RNA samples were hybridized with a Ura4 probe to demonstrate that Ura4 mRNA levels remained constant throughout the cell cycle and to provide an internal control.

FIG. 3. Alignment of the Cig2 amino acid sequence with yeast B-type cyclins. Identical amino acids between Cig2 and other B-type cyclins are shown in boldface type.

***cig2* is not essential for cell viability.** To investigate the function of $p45^{cig2}$, we constructed a *cig2* deletion mutant by a one-step gene replacement. The *sup3-5* tRNA gene rescues the adenine auxotrophy of a strain carrying the *ade6-704* allele. A 0.6-kb *Nde*I-*Nhe*I restriction fragment encoding the first 223 N-terminal amino acids of Cig2 was replaced with a 0.5-kb restriction fragment carrying the *sup3-5* tRNA gene. A diploid yeast strain (SP976) homozygous for the *ade6-704* allele was transformed with the construct, and Ade$^+$ transformants were obtained. Upon sporulation of a diploid heterozygous for *cig2* disruption at 30°C, haploid Ade$^+$ segregants were obtained, indicating that *cig2* is not essential for cell viability. To verify that the *cig2* sequence was deleted, genomic DNAs from a wild-type strain and a stable Ade$^+$ haploid transformant were subjected to Southern

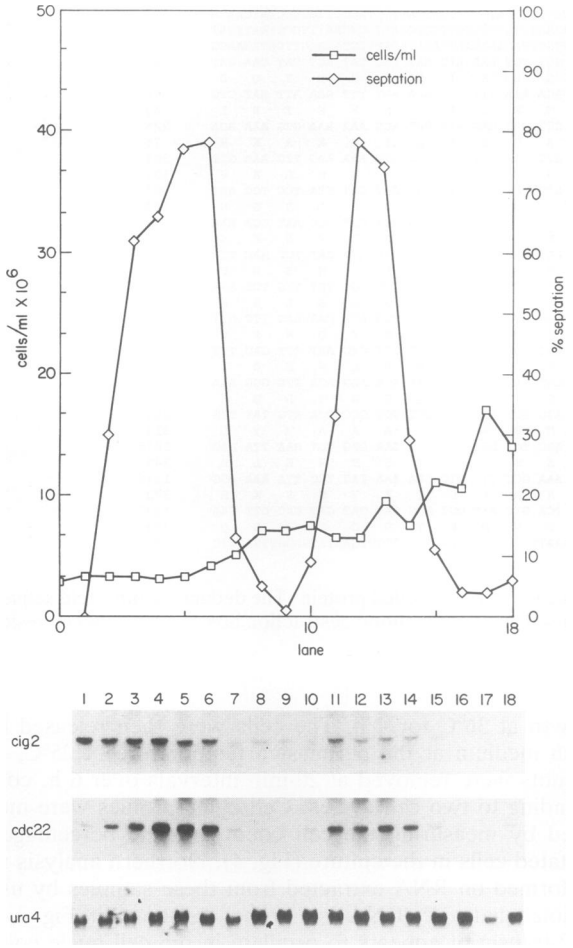

FIG. 4. Transcription profile of *cig2* mRNA during the cell cycle. Cells were synchronized by a *cdc25-22* block release. Northern analysis was performed on RNA samples prepared at 20-min intervals following release from the restrictive temperature to the permissive temperature. The lanes numbered 1 to 18 correspond to each 20-min interval, with the first lane containing RNA taken before release to the permissive temperature. The septation index is plotted with the 10^6 cells per milliliter values to give an indication of the synchrony in the cell population.

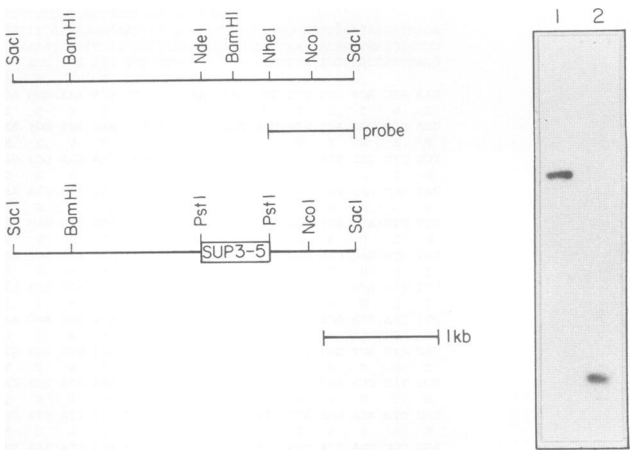

FIG. 5. Deletion of the *cig2* gene. The restriction map of a 3-kb *Sac*I genomic fragment is shown on top. The 0.6-kb *Nde*I-*Nhe*I fragment was replaced with a 0.5-kb fragment containing *sup3-5* tRNA, as shown at the bottom. Southern analysis was performed on genomic DNA isolated from the wild type (SP1051) (lane 1) or a *cig2* deletion mutant (lane 2) that was digested with *Sac*I and *Pst*I. A 0.6-kb *Nhe*I-*Sac*I fragment was used as a probe.

analysis (Fig. 5). The genomic DNA isolated from these strains was digested with *Sac*I and *Pst*I and probed with a radiolabeled 0.65-kb *Nhe*I-*Sac*I restriction fragment. This probe hybridized to a 3-kb fragment from DNA isolated from a wild-type strain and to a 0.65-kb fragment from DNA from the stable Ade^+ transformant, confirming a successful gene replacement. The growth rate of the *cig2*::*sup3-5* mutant was found to be essentially indistinguishable from that of wild-type cells. In an asynchronous culture, the *cig2*::*sup3-5* cells appeared slightly elongated relative to the wild type. In addition, a slight increase in the number of binucleate cells (10%) was observed in the *cig2*::*sup3-5* culture when compared with the wild type. FACS analysis revealed that approximately 95% of the cell population had a 2C DNA content in a *cig2*::*sup3-5* culture (Fig. 6). A similar number (10%) of binucleate cells was observed with an asynchronous *cig1*::*ura4* mutant population. Cells carrying the *cig2*::*sup3-5* disruption undergo conjugation and sporulation with high efficiency. These results demonstrate that the *cig2* gene is not essential for vegetative growth, conjugation, or sporulation.

***cig2*::*sup3-5* strains display enhanced conjugation frequencies.** In the course of our characterization of the *cig2* disruption, we noticed that cells carrying the *cig2*::*sup3-5* null mutation were moderately enhanced for conjugation. Mating-efficiency assays were performed (see Materials and Methods). Cultures of wild-type cells (SP1199 and SP1200) and cells carrying the *cig2*::*sup3-5* null mutation (SP1191 and SP1198) were grown to mid log phase and then induced to conjugate by plating of an equal number of cells carrying either $cig2^+$ or *cig2*::*sup3-5* with opposite mating types on minimal nitrogen-rich medium. Mating efficiency was assayed by calculating the number of zygotes produced over 48 h. Cells which carry the *cig2*::*sup3-5* null mutation exhibited a fourfold-enhanced conjugation frequency compared with $cig2^+$ wild-type cells with an isogenic background. The enhanced mating efficiency of the *cig2*::*sup3-5* mutant was independent of the *sup3-5* allele. The *cig2*::*sup3-5* mutant did not, however, bypass the requirement for nutritional limitation since no conjugation was observed on rich medium. These results suggest that the $p45^{cig2}$ protein may function in the control of the G_1 phase of the cell cycle.

Interactions with other B-type cyclins. The *cig1* gene, also cloned by its ability to complement the loss of CLN function in *S. cerevisiae*, was inactivated by inserting the *Ura4* gene into the *Nco*I site contained in the *cig1* coding sequence (see Materials and Methods). A *cig1* deletion mutant is viable (4). However, in contrast to results presented by Bueno et al., we observed that cells carrying a *cig1* disruption did not exhibit a marked lag in progression from the G_1 phase to the S phase of the cell cycle (4, 5a). On the other hand, we did observe that cells carrying inactivated alleles of either of the B-type cyclin genes *cig1* and *cig2* frequently contain two nuclei, suggesting that the cells might be delayed during septation. Upon close examination of the binucleate cells in the different populations, we found that the nuclei in the *cig1*::*ura4* disruptant appeared closer together (i.e., less well separated) than did those in cells with a *cig2*::*sup3-5* disruption. We therefore constructed a strain carrying disruptions

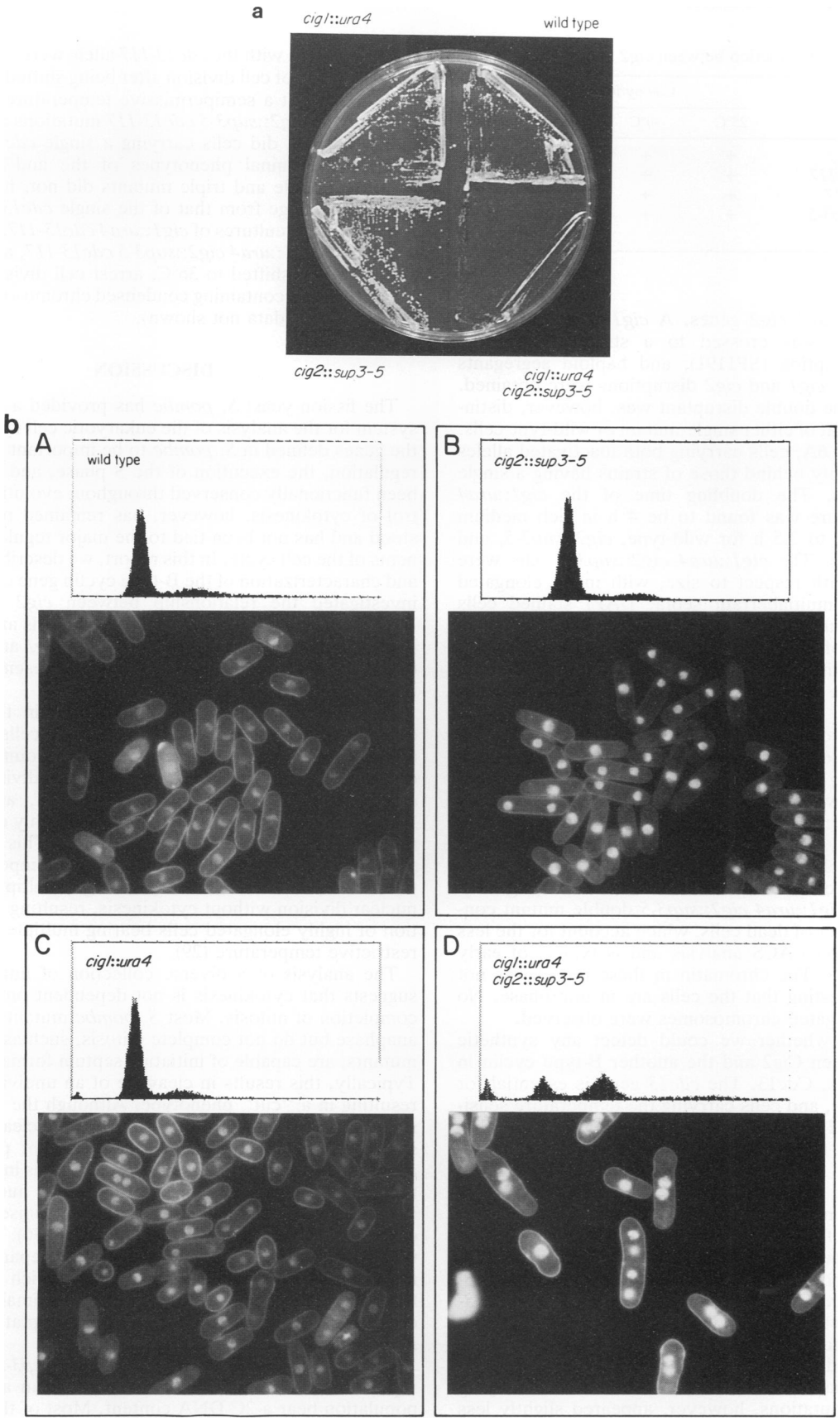

FIG. 6. Genetic interaction between *cig1* and *cig2*. (a) wild-type, *cig1*::*ura4*, *cig2*::*sup3-5*, and *cig1*::*ura4 cig2*::*sup3-5* cells were streaked onto a YEA plate and incubated at 32°C. (b) FACS analysis and fluorescent micrographs of DAPI-stained cells. Wild-type (panel A), *cig2*::*sup3-5* (panel B), *cig1*::*ura4* (panel C), and *cig1*::*ura4 cig2*::*sup3-5* (panel D) cells are shown.

TABLE 2. Interaction between *cig2* and *cdc13*

Genotype	Colony formation at:			
	25°C	30°C	33°C	36°C
cdc13-117	+	+	±	−
cig2::sup3-5 cdc13-117	+	+	+	−
cig1::ura4 cdc13-117	+	+	±	−
cig1::ura4 cig2::sup3-5 cdc13-117	+	+	±	−

in both the *cig1* and *cig2* genes. A *cig1::ura4*$^+$ *ade6-704* haploid (SP1192) was crossed to a strain bearing the *cig2::sup3-5* disruption (SP1191), and haploid segregants carrying both the *cig1* and *cig2* disruptions were obtained. Cell growth of the double disruptant was, however, distinguishable from that of either single-mutant or wild-type cells. As shown in Fig. 6A, cells carrying both inactivated alleles lagged considerably behind those of strains having a single cyclin disruption. The doubling time of the *cig1::ura4 cig2::sup3-5* culture was found to be 4 h in rich medium compared with 2 to 2.5 h for wild-type, *cig2::sup3-5*, and *cig1::ura4* strains. The *cig1::ura4 cig2::sup3-5* cells were heterogeneous with respect to size, with many elongated cells displaying multiaberrant septa. DAPI stained cells carrying the *cig1::ura4 cig2::sup3-5* disruptions are shown in Fig. 6B. Most of the cells contain two adjacent nuclei, situated on opposite sides of the longitudinal axis of the cell. In addition, some of the cells contain two nuclei on either side of the septa.

The DNA content of the strains carrying the cyclin disruptions was subjected to FACS analysis. As shown in Fig. 6B, wild-type, *cig2::sup3-5*, and *cig1::ura4* cells displayed a single 2C peak whereas exponentially growing *cig1::ura4 cig2::sup3-5* cells revealed three peaks corresponding to less than a 1C, a 2C, and a 4C DNA content. Since approximately 70% of cells are binucleate, part of the 2C peak is presumably due to nuclei with a 1C DNA content (Fig. 6B). Cultures of the *cig1::ura4 cig2::sup3-5* double mutant contain a large number of dead cells, which account for the less than <1C peak on FACS analysis and is typical of early septation mutants. The chromatin in these nuclei was not condensed, suggesting that the cells are in interphase. No abnormally segregated chromosomes were observed.

We examined whether we could detect any synthetic interaction between Cig2 and the another B-type cyclin in fission yeast cells, Cdc13. The *cdc13* gene is essential for entry into mitosis, and cells carrying the temperature sensitive allele *cdc13-117* undergo a first-cell cycle arrest with a 2C DNA content upon shift to the nonpermissive temperature (36°C) (29). The terminal phenotype of this mutant suggests that the majority of cells arrest in G_2, although partial progression into mitosis can occur (2). Strains carrying disruptions of *cig1* and *cig2* were crossed to a strain carrying the temperature-sensitive *cdc13-117* allele (see Materials and Methods). Strains carrying the *cig1::ura4 cdc13-117 cig2::sup3-5 cdc13-117* or *cig1::ura4 cig2::sup3-5 cdc13-117* mutations were isolated. The strains were streaked on YEA plates and monitored for temperature-sensitive growth. We found that all strains isolated were viable at 25 and 30°C and inviable at 36°C (Table 2). The strains carrying *cig2 cdc13-117* mutations, however, appeared slightly less temperature sensitive than did the strains carrying either *cig1::ura4 cdc13-117*, *cig1::ura4 cig2::sup3-5 cdc13-117*, or the *cdc13-117* allele alone. Cells carrying the *cig2* disruption in combination with the *cdc13-117* allele were capable of one or two rounds of cell division after being shifted to 36°C (data not shown). At a semipermissive temperature (33°C), cells carrying the *cig2::sup3-5 cdc13-117* mutations appeared less elongated than did cells carrying a single *cdc13-117* mutation. The terminal phenotypes of the and temperature-sensitive double and triple mutants did not, however, dramatically change from that of the single *cdc13-117* mutant. Asynchronous cultures of *cig1::ura4 cdc13-117*, *cig2::sup3-5 cdc13-117*, *cig1::ura4 cig2::sup3-5 cdc13-117*, and *cdc13-117* strains, when shifted to 36°C, arrest cell division as highly elongated cells containing condensed chromosomes and a 2C DNA content (data not shown).

DISCUSSION

The fission yeast *S. pombe* has provided a useful model system for the analysis of the eukaryotic cell cycle. Many of the genes defined in *S. pombe* to be important for cell cycle regulation, the execution of the S phase, and mitosis have been functionally conserved throughout evolution. The control of cytokinesis, however, has remained poorly understood and has not been tied to the major regulatory components of the cell cycle. In this report, we describe the cloning and characterization of the B-type cyclin gene *cig2*. We have investigated the relationship between *cig2* and another B-type cyclin gene *cig1*, in fission yeast cells and found that a strain carrying disruptions of both the *cig1* and *cig2* genes displayed a synthetic phenotype which resembled that of mutants defective in septum formation.

Septation is almost certainly delayed in the *cig1 cig2* double disruptant described here, since cells accumulate multiple nuclei, indicating that successive rounds of nuclear division occur in the absence of cell division. In the *cig1::ura4 cig2::sup3-5* double disruptant, approximately 70% of the cells are binucleate, with a roughly equal proportion (15%) of uni- and tetranucleate cells. This phenotype is similar to that observed in cells carrying temperature-sensitive alleles of *cdc11*, which undergo multiple rounds of nuclear division without cytokinesis, resulting in the formation of highly elongated cells bearing multiple nuclei at the restrictive temperature (29).

The analysis of a diverse collection of mitotic mutants suggests that cytokinesis is not dependent on a successful completion of mitosis. Most *S. pombe* mutants that initiate anaphase but do not complete mitosis, such as *cut1* or *top2* mutants, are capable of initiating septum formation (21, 35). Typically, this results in cleavage of an undivided nucleus, resulting in a "cut" phenotype. Although the nature of the signal that activates septum formation is unclear, it has been suggested that septum formation does not proceed until anaphase is complete (27). In this regard, it is interesting that the position of the two adjacent interphase nuclei observed in the *cig1::ura4 cig2::sup3-5* mutant is reminiscent of that of chromatin at the initiation of anaphase (16, 36). This suggests that this mutant is defective in nuclear separation. These nuclei contain decondensed chromatin, which suggests that they have exited from mitosis prior to maximal microtubule elongation. This may result in a delay in septation, accounting for the observed phenotype.

FACS analysis of the *cig1::ura4 cig2::sup3-5* double disruptant reveals that 50% of the cells in an asynchronous population bear a 2C DNA content. Most of these cells are binucleate as visualized by DAPI staining, which suggests that each individual nucleus is actually 1C in DNA content. Thus, it appears that a certain percentage of binucleate cells

must exist in G_1 and thereby lag in the cell cycle between mitosis and S phase. The slow transit from mitosis into S phase is masked in the FACS analysis by the increased presence of binucleate cells, thereby accounting for the lack of a 1C peak. Therefore, *cig1* and *cig2* might promote efficient progression through the G_1/S-phase transition.

It is interesting that the *cig2* gene is subject to periodic expression, peaking at the G_1/S boundary coincident with the *cdc22* gene. In *S. pombe* the control of DNA synthesis occurs in part through the coordinate expression of genes required for DNA synthesis. The *cdc22* gene, encoding the large subunit of ribonucleotide reductase, and more recently the *cdt1* gene have been shown to be essential for progression through the S phase (18, 21a). Both genes are periodically expressed during the cell cycle, peaking in late G_1, and are under the control of the *cdc10/sct1* transcription complex (6, 21a, 24). The similarity in the transcription patterns of *cig2* and *cdc22* described here suggests that they might be regulated by a similar mechanism.

Cells carrying the *cig2::sup3-5* null mutation exhibit a moderately enhanced ability to undergo conjugation. A plausible explanation for this observation is that the loss of the *cig2* gene product extends the period of the cell cycle during which cells are competent to undergo conjugation. The transcription pattern, when taken together with the enhanced ability of the *cig2::sup3-5* mutant to conjugate, suggests that $p45^{cig2}$ may function as a G_1- or S-phase cyclin. It is difficult to conceptually reconcile our data with results previously published by Bueno and Russell (5). Bueno and Russell show a strong genetic interaction between the *cig2* null mutation and alleles of genes that function in the G_2 phase of the cell cycle, namely *cdc25-22*, *mik1::ura4*, and *wee1-50*. Their results suggest that the loss of *cig2* results in a delay in the G_2 phase of the cell cycle. Our data are inconsistent with this interpretation. Moreover, we do not find strong genetic interactions between the *cig2::sup3-5* null mutant and *cdc25-22* or *mik1::ura4 wee1-50* alleles.

In conclusion, we have characterized a third B-type cyclin in the fission yeast *S. pombe*. Thus it appears that in *S. pombe*, as in *S. cerevisiae*, multiple B-type cyclins are involved in cell cycle control. Considering that no single *clb* disruption nor the *cig1* disruption led to a division defect, it is perhaps not surprising that a *cig2* deletion alone did not exert any defect in cell cycle progression (14, 32). The genetic data from work with budding yeast cells suggest that the several B-type cyclins perform critical and possibly overlapping functions during mitosis. The synthetic phenotype observed in cells carrying the *cig1::ura4 cig2::sup3-5* disruptions likewise suggests that these two cyclins may have redundant functions. An important question to be pursued will be whether each B-type cyclin performs a unique and identifiable function during the cell cycle.

ACKNOWLEDGMENTS

We are grateful to John Fikes and Leonard Guarente for the *S. pombe* cDNA library and to Bob Derby for assistance with the differential interference contrast microscopy. We thank Maureen Caligiuri, Karen Lundgren, and Tomo Matsumoto for critical comments on the manuscript and Craig Gawel for technical assistance. We also thank Jim Duffy, Mike Ockler, and Phil Renna for artistic assistance.

This work was supported by National Institutes of Health grant GM34607 to D.B. T.C. is an NIH postdoctoral fellow. D.B. is an Investigator of the Howard Hughes Medical Institute.

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