Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6255–6259, August 1989
Genetics

A family of cyclin homologs that control the G_1 phase in yeast

(Saccharomyces cerevisiae/CDC28/Cdc2/cell division/cell cycle)

JEFFREY A. HADWIGER*, CURT WITTENBERG, HELENA E. RICHARDSON, MIGUEL DE BARROS LOPES, AND STEVEN I. REED

Department of Molecular Biology, MB-7, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037

Communicated by John Carbon, May 24, 1989 (received for review March 13, 1989)

ABSTRACT **Two *Saccharomyces cerevisiae* genes were isolated based upon their dosage-dependent rescue of a temperature-sensitive mutation of the gene *CDC28*, which encodes a protein kinase involved in control of cell division. *CLN1* and *CLN2* encode closely related proteins that also share homology with cyclins. Cyclins, characterized by a dramatic periodicity of abundance through the cell cycle, are thought to be involved in mitotic induction in animal cells. A dominant mutation in the *CLN2* gene, *CLN2-1*, advances the G_1- to S-phase transition in cycling cells and impairs the ability of cells to arrest in G_1 phase in response to external signals, suggesting that the encoded protein is involved in G_1 control of the cell cycle in *Saccharomyces*.**

Cyclins were initially identified in the embryos of a number of marine invertebrates as proteins that undergo dramatic fluctuations in abundance as a function of cell cycle progression (1, 2). In clams and sea urchins, where early embryonic cleavages occur with a high degree of synchrony, cyclin levels can be seen to peak at the onset of mitosis. During mitosis, cyclins are rapidly degraded but begin to accumulate during the subsequent interphase. Periodicity of cyclin levels results entirely from posttranslational regulation, as the cyclin mRNAs are abundant maternal species that are translated at a constant rate during early development. Based on the kinetics of cyclin accumulation and turnover, it was proposed that these proteins might be involved in triggering mitosis. The first evidence, however, that cyclins are rate limiting for mitotic induction was the demonstration that injection of cyclin-encoding mRNA could induce maturation of *Xenopus* oocytes (3), a process analogous to mitosis in that cells resting in meiotic prophase are induced to enter into meiotic divisions.

In this report we describe a class of proteins homologous to cyclins from the budding yeast, *Saccharomyces cerevisiae*. The genes encoding these cyclin homologs were identified by using a genetic screen in which elevated expression of a heterologous sequence was required for rescue of a temperature-sensitive mutation in the gene *CDC28* (4). *CDC28* encodes the catalytic subunit of a protein kinase complex required for cell cycle initiation in *Saccharomyces* (5–8). The observation that cyclin hyperexpression can rescue temperature-sensitive *cdc28* mutations implies a role for these yeast cyclins in the maintenance or regulation of the Cdc28 protein kinase complex. Furthermore, we show that a mutation in one of the cyclin genes confers a dominant pleiotropic phenotype characterized by advance of the G_1- to S-phase transition in cycling cells as well as by loss of G_1 control of cell division in response to nutrient limitation. These results suggest that in budding yeast, a class of cyclins is rate-limiting for G_1- to S-phase transition rather than for the induction of mitosis.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Strains and Medium. Suppressor plasmids were initially isolated and subcloned by using *S. cerevisiae* strain JF210-92: *MATa, trp1, leu2, cdc28-4* (9). All subsequent genetic analysis and manipulation were performed on *S. cerevisiae* strain BF264-15D: *MATα, ade1, leu2-3,112, trp1, his2* (6) and congenic derivatives. Mutant alleles *cdc28-4, cdc28-9, cdc28-13,* and *TRP1::GAL1p::CDC28* were introduced into BF264-15D by one-step gene replacements as described by Rothstein (10). Unless otherwise noted, cultures were grown in YEPD medium (1% yeast extract/2% Bacto-peptone/2% glucose) supplemented with 50 mg each of adenine and uracil per liter.

Recombinant DNA Manipulations. Yeast transformations were carried out either by the spheroplast method described by Tschumper and Carbon (11) or by the alkali cation method described by Ito *et al.* (12). Restriction endonucleases, T4 DNA ligase, S1 nuclease, and Klenow polymerase were purchased from New England Biolabs, Bethesda Research Laboratories, Boehringer Mannheim, or Promega Biotec and used according to the specifications of the manufacturer. One-step genomic sequence replacements were performed as described by Rothstein (10), whereas genomic integration of linearized plasmids was by the method of Orr-Weaver *et al.* (13). Plasmids pJH1-38 and pJH3-45 were isolated from a library consisting of yeast genomic *Sau*3A fragments cloned into the *Bam*HI site of YEp13 (14). Plasmid pJHB1a was constructed by inserting a *Bam*HI–*Hin*dIII fragment that contained the *CLN1* gene (Fig. 1*A*) between *Bam*HI and *Hin*dIII sites of YEp13 (15). Plasmid pJH3-45-2 was constructed by inserting a *Hin*dIII–*Sal* I fragment (Fig. 1*B*), which contained the 5′ portion of the *CLN2* gene (the *CLN2-1* truncation) from plasmid pJH3-45, between the *Sal* I and most proximal *Hin*dIII sites of plasmid YRp7 (17).

The *cln1::TRP1* insertional mutation was constructed by inserting a *Nco* I–*Bgl* II fragment that contained the *TRP1* gene into the unique *Nco* I site within the *CLN1* gene of the plasmid pJHB1a. The *cln2::LEU2* insertional mutation was constructed by inserting a *Sal* I–*Xho* I fragment, which contained the *LEU2* gene from the plasmid YEp13 (15), into the unique *Xho* I site within the *CLN2* gene of the plasmid pJH3-45-2.

The truncated allele of *CLN2* described above (*CLN2-1*) was integrated at the *trp1* locus of a CLN^+ strain by transformation with plasmid pJH3-45-2 linearized at the *Bgl* II site near the *TRP1* gene (17). Integration of this plasmid at the *trp1* locus was confirmed by hybridization of genomic DNA blots.

For DNA blot analysis, DNA was transferred to Biodyne (ICN) as described by the manufacturer. DNA probes were made by using a random primer DNA labeling kit (Boehringer Mannheim) according to the manufacturer's instructions.

*Present address: Center for Molecular Genetics, University of California–San Diego, La Jolla, CA 92037.

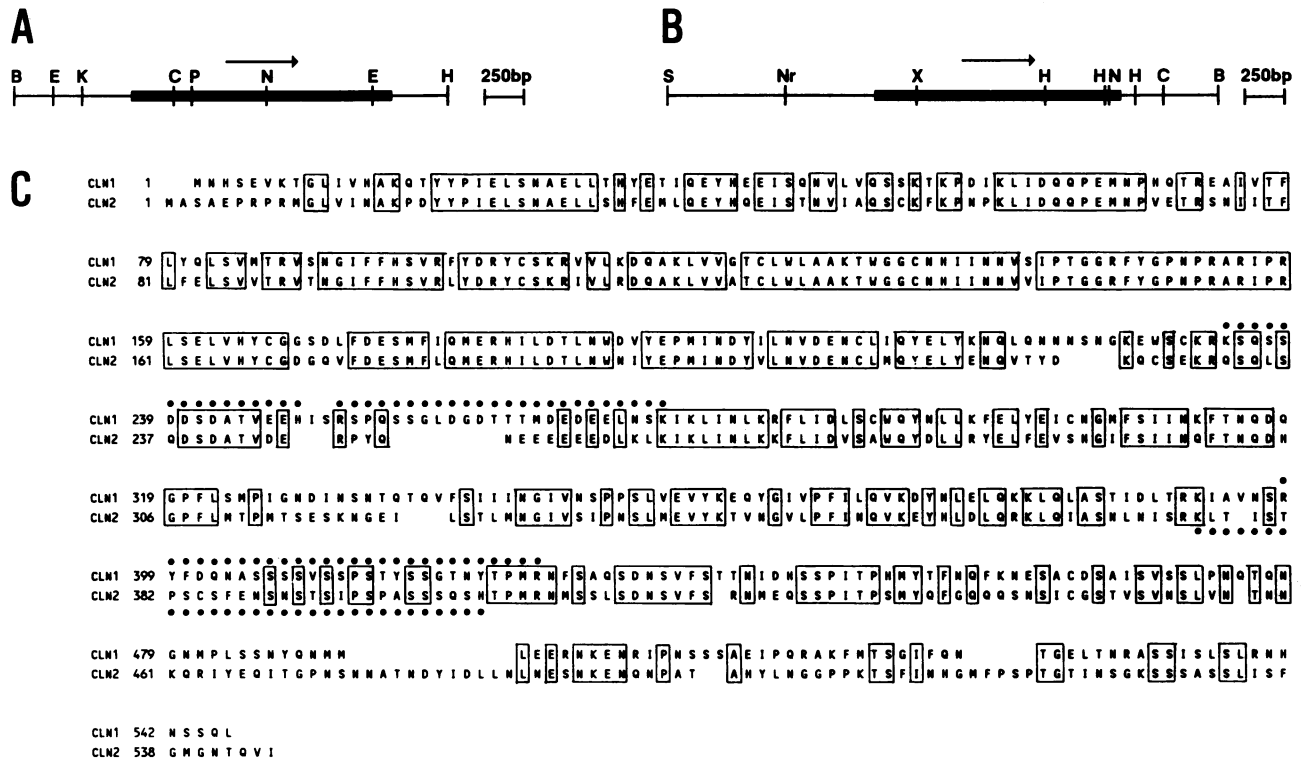

FIG. 1. Polypeptides encoded by *CLN1* and *CLN2*. (*A*) Restriction map in the region of the *CLN1* coding region. The bold line indicates the protein coding region. The *Nco* I site is the point where *TRP1* was inserted to construct the *cln1*::*TRP1* insertional mutation. The arrow indicates the direction of transcription. (*B*) Restriction map in the region of the *CLN2* coding region. The bold line indicates the protein coding region. The *Xho* I site is the point where *LEU2* was inserted to construct the *cln2*::*LEU2* insertional mutation. The most upstream *Hin*dIII site in the *CLN2* coding region is the point of truncation in the *CLN2-1* mutation. Restriction endonuclease cleavage sites are as follows: B, *Bam*HI; C, *Cla* I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; N, *Nco* I; Nr, *Nru* I; P, *Pvu* I; S, *Sal* I; X, *Xho* I. bp, Base pairs. (*C*) Predicted protein sequence of *CLN1* and *CLN2*. The single-letter amino acid code is used. Dotted lines above the *CLN1* and below the *CLN2* polypeptide sequences indicate predicted PEST sequences (16). Identical amino acids are boxed.

DNA hybridization reactions were performed as described by Reed *et al.* (9).

Photomicroscopy, Flow Cytometry, and Cell Size Determination. Unfixed yeast cells were mounted in growth medium and photographed by using a Zeiss Axiophot photomicroscope with differential interference contrast (Nomarski) optics. A 100× objective was used. For flow cytometry to determine cellular DNA content, cells were fixed in ethanol and stained with propidium iodide (18). Stained cells were analyzed for fluorescence by using a Becton Dickinson FACS IV analyzer. Size distributions of unfixed cell populations were generated by using the electrolyte displacement analysis capability of a Becton Dickinson FACS analyzer.

Starvation and Mating Pheromone Sensitivity Assays. Nitrogen starvation was performed by growing cells to midlogarithmic phase in 0.67% yeast nitrogen base without amino acids (Difco) supplemented with adenine, uracil, tryptophan, histidine, and leucine and containing 2% sucrose as a carbon source. Cells were washed once and then resuspended in the same medium containing sodium sulfate in place of ammonium sulfate. Nitrogen-starved cells were harvested at the intervals indicated, and the cell number and budding index were determined by microscopic examination following sonication.

Mating pheromone sensitivity was determined by assessing the ability of cells to form colonies on plates containing increasing concentrations of α-factor (19).

RESULTS

Isolation of *CLN1* and *CLN2*. A yeast genomic library, constructed in the multicopy vector YEp13, was screened for plasmids that could suppress the temperature-sensitive *cdc28-4* mutation. Strain JF210-92 (*cdc28-4 leu2*) was transformed with library DNA and plasmids that conferred the ability to form colonies at the restrictive temperature (36°C) were selected. Three sequences in addition to the *CDC28* gene itself were able to rescue the *cdc28-4* mutation (20). Two of these suppressor sequences, *CLN1* and *CLN2*, are the subject of this report.

Multicopy plasmids pJH1-38 (*CLN1* in YEp13) and pJH3-45 (*CLN2* in YEp13) were capable of rescuing the three temperature-sensitive *cdc28* alleles tested: *cdc28-4*, *cdc28-9*, and *cdc28-13* (4). These plasmids allowed growth at 1–2°C above the respective restrictive temperatures for the mutations. Subcloning the rescuing sequences to centromere-containing plasmids, which are maintained at low copy in transformant yeast cells, abolished the ability to rescue the temperature-sensitive phenotype (data not shown). Therefore, elevated dosage and, presumably, elevated expression of the genes in question are required. The multicopy plasmids, however, were incapable of bypassing *CDC28* function entirely. Strain MMY36, which contains a single *CDC28* gene expressed under the control of the glucose-repressible *GAL1* promoter (*pGAL1*::*CDC28*; refs. 21 and 22), is incapable of growth in medium containing glucose as a result of transcriptional repression of the *CDC28* gene. Multicopy *CLN1* and *CLN2* plasmids were unable to rescue the *cdc28* null phenotype of these cells grown in the presence of glucose.

***CLN1* and *CLN2* Encode Cyclin-Like Proteins.** The *CLN1* and *CLN2* coding regions were subcloned and subjected to DNA sequence analysis (unpublished). Single open reading frames were revealed capable of encoding polypeptides of 546 and 545 amino acids for *CLN1* and *CLN2*, respectively

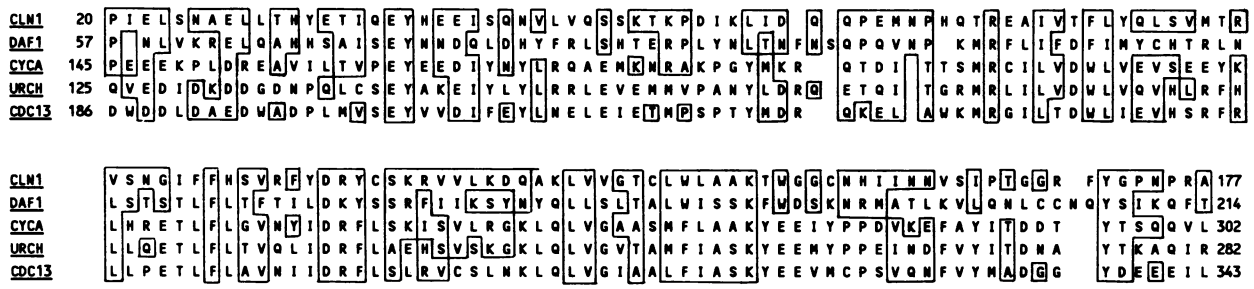

FIG. 2. Alignment of the most conserved regions of Cln1 and four members of the cyclin family. DAF1, Daf1 (yeast) (16, 23); CYCA, cyclin A (clam) (3); URCH, cyclin B (urchin) (24); CDC13, Cdc13 (fission yeast) (25). The single-letter amino acid code is used. Boxing indicates identities or conservative substitutions between Cln1 and other cyclins. Conservative substitutions are grouped as follows: D, E, N, Q; H, K, R; A, G, P, S, T; I, L, M, V; F, W, Y.

(Fig. 1*C*). Comparison of the predicted primary structures revealed that the *CLN1* and *CLN2* products are closely related. A direct alignment gives an overall identity of 57%; however, a much higher degree of homology is observed if conservative substitutions are considered. Furthermore, if analysis is restricted to the amino-terminal 50% of the respective sequences, a much higher level of identity (72%) is observed. The significance of this apparent structural divergence of carboxyl-terminal domains will be discussed below. Nevertheless, the striking homology of the predicted products of two suppressors isolated in the same manner suggests similar or overlapping function.

When the primary structures of the *CLN1* and *CLN2* products were compared to the PIR and SWISS-PROT polypeptide data bases, significant matches were found only with proteins known as cyclins. The greatest homology was localized to a domain of ≈100 residues located centrally within most cyclin sequences and close to the amino-termini of Cln1 and Cln2 (residues 20–127; Fig. 2). The latter two polypeptides are virtually identical in this region. A comparison of either Cln1 or Cln2 to cyclin A from the clam *Spisula* (3) in this region gave an identity of 24% with much greater homology (50%) if conservative substitutions are considered. A somewhat decreased, but nevertheless significant, homology is found in this region when the comparison is made to members of the cyclin B family (URCH and CDC13 in Fig. 2). This domain is contained within a 150-residue region that will be referred to as the "cyclin box," the only highly conserved region when heterologous cyclins are compared. Although the cyclins from higher eukaryotes share a higher degree of homology with each other than with Cln1 and Cln2, the structural conservation of the latter relative to other cyclins within the cyclin box region suggests a conservation of function. Hence, these genes were designated *CLN1* and *CLN2* (*CLN* for cyclin).

Insertional Mutation of *CLN1* and *CLN2*. The ability of amplified *CLN1* and *CLN2* sequences to suppress the G_1-phase defect of *cdc28* mutants raised the possibility that these genes might be important for the initiation of the cell division cycle in yeast. Therefore, a mutational analysis of *CLN1* and *CLN2* was undertaken. Diploid cells heterozygous for either *cln1*::*TRP1* or *cln2*::*LEU2* (see *Materials and Methods*), constructed by substitution of the genomic sequences with the mutant alleles by one-step gene replacement (10), were put through meiosis and sporulation to evaluate the phenotypes of haploid segregants containing the insertion mutations. Based on this analysis, it was concluded that elimination of the function of *CLN1* or of *CLN2* conferred no observable phenotype. Therefore neither of these is an essential gene. However, since the polypeptides encoded by *CLN1* and *CLN2* are highly homologous at the structural level, it is possible that they fulfill identical or overlapping functions. In that case, eliminating one would not be sufficient to bring about a loss of function. Therefore, a haploid strain containing both insertion mutations was produced first by constructing a diploid heterozyous for each and then inducing sporulation to obtain haploid segregants. Segregation of the mutant alleles could be monitored by scoring the inserted markers *TRP1* and *LEU2*. It was concluded that the double mutant was viable since spores that were prototrophic for tryptophan and leucine could form colonies. However, these grew slowly and microscopic observation indicated that the cells were extremely large and aberrantly shaped. This phenotype was accentuated at elevated temperatures, with many, but not all, cells in the population displaying a morphology that resembled that of *cdc28* mutants arrested in G_1 phase (Fig. 3 *A* and *B*). Thus, it is likely that Cln1 and Cln2 share an overlapping role in execution of G_1 functions. However, the viability of the double mutant suggests either that this role is not absolutely essential or that it is shared with the product of an additional gene or genes, which remain to be identified.

A Mutation That Suggests That *CLN2* Is a Regulatory Element of G_1-Phase Control. In the course of subcloning the *CLN2* gene, a truncated allele missing the carboxyl-terminal 30% of the coding region was produced [the 1.7-kilobase (kb) *Hin*dIII–*Sal* I subclone]. On a multicopy plasmid, this allele retained the ability to rescue temperature-sensitive *cdc28* mutations. When transformed into a wild-type cell on the same multicopy plasmid, however, it was noticed that some cells were abnormally small. When this truncated allele, designated *CLN2-1*, was integrated at an ectopic site in the yeast genome at single copy, a very distinct small size phenotype was observed. Fig. 3 *C* and *D* show micrographs of *CLN2-1* mutants and congenic wild-type cells, respectively, in logarithmic-phase growth. Fig. 4*A* shows cell size distributions of these cultures superimposed. Although the

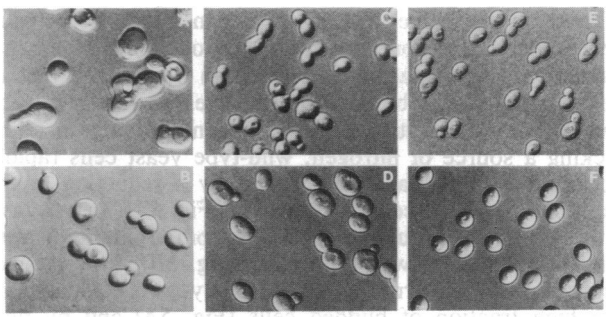

FIG. 3. Photomicrographs of recessive and dominant mutants. (*A*) Haploid cells containing the *cln1*::*TRP1* *cln2*::*LEU2* double gene disruption grown at 38°C. (*B*) Congenic CLN^+ cells (BF264-15D) grown at 38°C. (*C*)*CLN2-1* cells in logarithmic-phase growth in rich medium. (*D*) Congenic CLN^+ cells in logarithmic-phase growth in rich medium. (*E*) *CLN2-1* cells grown to stationary phase in rich medium. (*F*) Congenic CLN^+ cells grown to stationary phase in rich medium. (×550.)

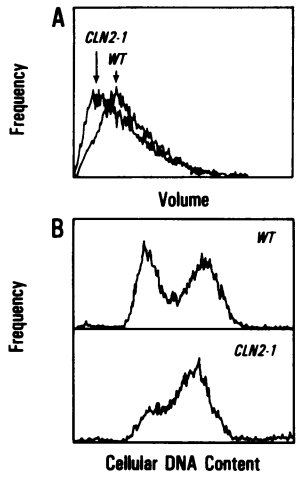

FIG. 4. Size and cell cycle parameters of *CLN2-1* mutants. (*A*) Size profiles of *CLN2-1* and wild-type (WT) cell populations. (*B*) DNA content profile of *CLN2-1* and wild-type populations as determined by flow cytometry.

populations overlap in size, it is clear that the mutant distribution, particularly at its lower end, is shifted to a smaller cell size. One possible explanation for the small cell size phenotype conferred by the *CLN2-1* mutation is that the cell cycle is advanced in the mutant. In *S. cerevisiae*, the G_1 interval is the point where the cell cycles of growing cells are restrained in order to allow sufficient growth so that an optimum average cell size is maintained (26). Cells deficient in this form of G_1 control are expected to divide at a smaller size. A population of *CLN2-1* cells was analyzed and compared to wild type by flow cytometry for DNA content per cell (Fig. 4*B*). Whereas the wild-type population contains a significant fraction of cells with a 1*n* content of DNA, presumably corresponding to the G_1 fraction, the mutant population contains virtually no G_1 cells. This result is consistent with the *CLN2-1* mutation impairing G_1 coordination between growth and division so that cells proceed directly from mitosis to the subsequent S phase.

Since loss of coordination between cellular growth and division suggests an inability to regulate passage through G_1 phase, the *CLN2-1* mutation was screened for defects in other G_1 regulatory functions. Mutant cells were tested for their sensitivity to mating pheromone, which causes wild-type haploid cells to arrest in the G_1 interval. It was found that mutant cells were slightly resistant to mating pheromone relative to wild type, requiring a 3-fold higher concentration of pheromone for cell cycle arrest than the congenic wild-type cells. Starvation is another environmental signal that causes wild-type yeast cells to arrest in G_1. *CLN2-1* mutant cells were found to be incapable of arresting in G_1 by using two different starvation regimes. When shifted to medium lacking a source of nitrogen, wild-type yeast cells rapidly accumulate in G_1, as is evidenced by a reduction in the fraction of budded cells to near zero (Fig. 5*A*). In contrast, although *CLN2-1* mutant cells cease from proliferation with similar kinetics to wild-type cells (Fig. 5*B*), they are not retained in G_1 and arrest asynchronously, as is evidenced by the high fraction of budded cells (Fig. 5*A*) and by flow cytometric analysis (data not shown). Likewise, growth of wild-type cells to stationary phase in rich medium is accompanied by accumulation of the population in the G_1 interval, as indicated by a predominance of unbudded cells (Fig. 3*F*). Under the same conditions, *CLN2-1* mutants fail to arrest homogeneously in G_1 (Fig. 3*E*). These results indicate that the *CLN2-1* mutation confers an inability to respond to nutritional signals that normally restrain cell division in G_1.

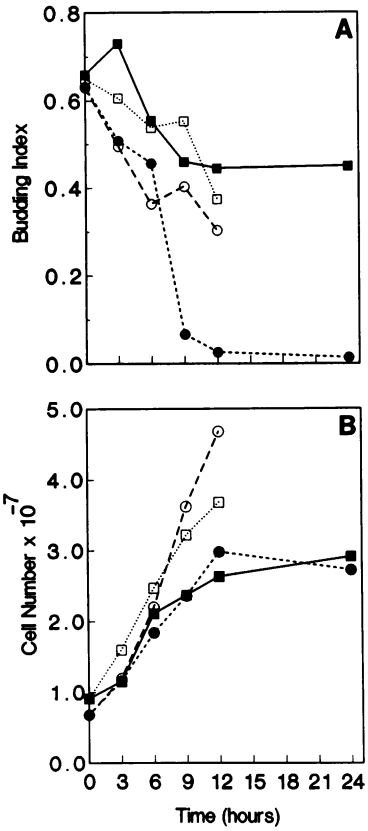

FIG. 5. Starvation characteristics of *CLN2-1* mutants. (*A*) Budding indices of *CLN2-1* and *CLN*$^+$ populations after a shift to medium without nitrogen. (*B*) Cell division after a shift of *CLN2-1* and *CLN*$^+$ populations to medium without nitrogen. ○, *CLN*$^+$ in medium with nitrogen; □, *CLN2-1* in medium with nitrogen; ●, *CLN*$^+$ in medium without nitrogen; ■, *CLN2-1* in medium without nitrogen.

DISCUSSION

We have identified two genes encoding yeast cyclin homologs by screening for suppressors of a temperature-sensitive cell division cycle mutation, *cdc28*. Investigation of cyclins in animal cells has led to the proposal that they are rate-limiting elements involved in mitotic induction. This is inferred from the kinetics of cyclin accumulation in the embryos of marine invertebrates, where peak levels immediately precede mitosis, and from experiments in which injection of cyclin mRNA caused *Xenopus* oocyctes to mature (1–3). These experiments also suggest a relationship between cyclins and maturation-promoting factors (MPFs) (27). MPF, isolated from mature eggs, when injected into oocytes causes them to complete meiosis, but MPF activity can also be shown to oscillate in embryos with similar kinetics to the accumulation of cyclins (28, 29). This is particularly intriguing since it has been shown that MPFs from frog and from starfish are homologs, in these organisms, of the Cdc28 protein kinase complex of *S. cerevisiae* (30–33). Therefore, our isolation of genes encoding cyclin-like proteins as suppressors of *cdc28* mutants is consistent with a unified model of cyclin function in which cyclins may be direct activators of the Cdc28 protein kinase and its homologs in eukaryotic organisms. Since cyclins in embryos of marine invertebrates undergo periodic accumulation and degradation through the cell cycle, cell-cycle-specific protein kinase activation may be achieved by accumulation of cyclin to a threshold level.

The properties of the *CLN2-1* mutation suggest a role for cyclins as rate-limiting regulators of the yeast cell cycle. This mutation confers a dominant pleiotropic phenotype characterized by impairment of all forms of G_1-phase regulation.

Not only are cells incapable of responding properly to environmental signals that normally lead to G_1 arrest but also cycling cells carrying the mutation proceed directly from mitosis to S phase, becoming G_1-less. The simplest explanation for these phenotypes is that cyclin accumulation and degradation are essential and rate limiting in all modes of G_1 regulation and that the stability of the mutant cyclin is altered. The distribution of PEST sequences (34) in the Cln1 and Cln2 proteins (see Fig. 1*C*) is consistent with this hypothesis. These clusters, rich in proline, glutamate, serine, threonine, and aspartate, and flanked by basic amino acids, are found in unstable proteins and have been proposed to target polypeptides for rapid turnover (34). Although the carboxyl-terminal domains of Cln1 and Cln2 share little primary structure homology, they are similar in that they contain PEST sequences, suggesting functional homology. The *CLN2-1* mutation, which is a truncation of the carboxyl-terminal 30% of the *CLN2* coding region, removes the principal PEST sequence (Fig. 1*C*) but leaves the cyclin box intact. Thus, though the mutant protein presumably retains its inductive functions, the mutational removal of a destabilizing PEST sequence may eliminate the possibility of regulation based on oscillating cyclin levels or on induced cyclin destruction. The phenotypes of the *CLN2-1* mutation thus suggest that cyclins in yeast are important in the regulation of division by external signals in addition to their previously proposed role in the coordination of division with internal requirements for cell cycle progression.

Recently, mutations in a gene, known alternately as *DAF1* (16) or *WHI1* (23, 35), have been described that bear a marked resemblance to the *CLN2-1* mutation. Dominant *DAF1* and *WHI1* mutants have a small cell size, bypass the G_1 phase of the cell cycle, and show a reduced sensitivity to mating pheromone (16, 23, 35). We have observed that *DAF1-1* mutants are also somewhat limited in their ability to G_1 arrest in response to starvation (C.W. and S.I.R., unpublished). Although the *DAF1-1* mutation appears to confer greater insensitivity to mating pheromone but less pronounced insensitivity to starvation-mediated G_1 arrest than does the *CLN2-1* mutation, the same constellation of phenotypes is observed. It is noteworthy, then, that *DAF1/WHI1* encodes a cyclin-like protein structured similarly, in terms of domains, to *CLN1* and *CLN2*. *DAF1-1* and *WHI1-1* are translational termination mutations that remove PEST-sequence-containing carboxyl-terminal segments (16, 23). Interestingly, the homology between *DAF1/WHI1* and *CLN1* or *CLN2* is no greater than that with any other cyclin and is limited to the cyclin box region. Therefore, in terms of primary structure, the products of these genes are not closely related. The obvious functional relatedness, based on mutant phenotypes is, thus, enigmatic. It remains to be determined whether *DAF1/WHI1*, which is not itself essential, provides the cyclin function that allows the double mutant *cln1 cln2* cells to survive.

We thank G. Cole, F. Cross, B. Futcher, F. Ho, P. Russell, and D. Stone for helpful discussions, F. Cross for the *DAF1-1* mutation, and Cordelia Rauskolb for assistance in some of the earlier experiments. C.W. acknowledges postdoctoral support from the Research Institute of Scripps Clinic. This work was funded by Public Health Service Grant GM38328 to S.I.R. from the National Institute of General Medical Sciences. S.I.R. also acknowledges support from American Cancer Society Faculty Research Award FRA-248.

1. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D. & Hunt, T., (1983) *Cell* **33**, 389–396.
2. Rosenthal, E. T., Hunt, T. & Ruderman, J. V. (1980) *Cell* **20**, 487–494.
3. Swenson, K. I., Farrell, K. M. & Ruderman, J. V. (1986) *Cell* **47**, 861–870.
4. Reed, S. I. (1980) *Genetics* **95**, 561–577.
5. Lörincz, A. T. & Reed, S. I. (1984) *Nature (London)* **307**, 183–185.
6. Reed, S. I., Hadwiger, J. A. & Lörincz, A. T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4055–4059.
7. Mendenhall, M D., Jones, C. A. & Reed, S. I. (1987) *Cell* **50**, 927–935.
8. Wittenberg, C. & Reed, S. I. (1988) *Cell* **54**, 1061–1072.
9. Reed, S. I., Ferguson, J. & Groppe, J. C. (1982) *Mol. Cell. Biol.* **2**, 412–425.
10. Rothstein, R. J. (1983) *Methods Enzymol.* **101**, 202–211.
11. Tschumper, G. & Carbon, J. A. (1980) *Gene* **10**, 157–166.
12. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153**, 163–168.
13. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. (1983) *Methods Enzymol.* **101**, 228–245.
14. Nasmyth, K. A. & Tatchell, K. (1980) *Cell* **19**, 753–764.
15. Broach, J. R., Strathern, J. M. & Hicks, J. B. (1979) *Gene* **8**, 121–133.
16. Cross, F. (1988) *Mol. Cell. Biol.* **8**, 4675–4684.
17. Struhl, K., Stinchcomb, D., Scherer, S. & Davis, R. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1035–1039.
18. Hutter, K.-J. & Eipel, H. E. (1979) *J. Gen. Microbiol.* **113**, 369–375.
19. Chvatchko, Y., Howald, I. & Riezman, H. (1986) *Cell* **46**, 355–364.
20. Hadwiger, J. A., Wittenberg, C., Mendenhall, M. D. & Reed, S. I. (1989) *Mol. Cell. Biol.*, in press.
21. Johnston, M. & Davis, R. W. (1984) *Mol. Cell. Biol.* **4**, 1440–1448.
22. Wittenberg, C., Richarson, S. L. & Reed, S. I. (1987) *J. Cell. Biol.* **105**, 1527–1538.
23. Nash, R., Tokiwa, G., Anand, S., Erickson, K. & Futcher, A. B. (1988) *EMBO J.* **7**, 4335–4346.
24. Pines, J. & Hunt, T. (1987) *EMBO J.* **6**, 2987–2995.
25. Booher, R. & Beach, D. (1988) *EMBO J.* **7**, 2321–2327.
26. Johnston, G. C., Pringle, J. R. & Hartwell, L. H. (1977) *Exp. Cell Res.* **105**, 79–98.
27. Masui, Y. & Markert, C. L. (1971) *J. Exp. Zool.* **177**, 129–146.
28. Newport, J. W. & Kirschner, M. W. (1984) *Cell* **37**, 731–742.
29. Gerhardt, J., Wu, M. & Kirschner, M. W. (1984) *J. Cell Biol.* **98**, 1247–1255.
30. Dunphy, W. G., Brizuela, L., Beach, D. & Newport, J. (1988) *Cell* **54**, 423–431.
31. Gautier, J., Norbury, C. J., Lohka, M., Nurse, P. & Maller, J. (1988) *Cell* **54**, 433–439.
32. Labbe, J. C., Lee, M. G., Nurse, P., Picard, A. & Doree, M. (1988) *Nature (London)* **335**, 251–254.
33. Arion, D., Meijer, L., Brizuela, L. & Beach, D. (1988) *Cell* **55**, 371–378.
34. Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science* **234**, 364–368.
35. Sudbury, P. E., Goodey, A. R. & Carter, B. L. A. (1980) *Nature (London)* **288**, 401–404.