Copyright © 1991 by the Genetics Society of America

Genetic and Molecular Analysis of *cdr1/nim1* in *Schizosaccharomyces pombe*

H. Feilotter,*,†,[1] P. Nurse† and P. G. Young*

**Department of Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6, and* †*ICRF Cell Cycle Group, Department of Biochemistry, University of Oxford, Oxford, England, OX1 3QU*

Manuscript received June 5, 1990
Accepted for publication October 19, 1990

ABSTRACT

The *cdr1* gene in *Schizosaccharomyces pombe* was identified as a mutation affecting the nutritional responsiveness of the mitotic size control. *cdr1* alleles have been further analyzed for genetic interactions with elements of the mitotic control pathway and cloned by plasmid rescue of a conditional lethal *cdr1-76 cdc25-22* double mutant. These analyses show that the *cdr1* gene is allelic to *nim1*, a gene identified as a high copy number plasmid suppressor of the mitotic control gene, *cdc25*. The gene structure for *cdr1* differs from the described *nim1* gene in the carboxyl-terminal portion of the gene. The published *nim1* sequence encoded a product of predicted M_r 45,000, and included 356 amino acids from the amino-terminal region of the gene and 14 amino acids from a noncontiguous carboxyl-terminal fragment. The *cdr1* sequence includes an additional 237 amino acids of the contiguous fragment and encodes a product of predicted M_r 67,000. The sequence shows a high level of identity with protein kinases over the amino-terminal catalytic domain, and limited identity with yeast protein kinases *SNF1*, *KIN2* and *KIN1* over part of the carboxyl-terminal domain. The effect of overexpression of the full length gene has been examined in various genetic backgrounds. These data show that the full length gene product is required to give a normal cell cycle response to nitrogen starvation. A detailed examination of the genetic interaction of *cdr1* mutants with various mutants of mitotic control genes (*cdc2, cdc25, wee1, cdc13*) demonstrated strong interactions with *cdc25*, some *cdc2* alleles, and with *cdc13-117*. Overall, the results are interpretable within the framework of the existing model of *cdr1/nim1* action in mitotic control, *i.e.*, *cdr1* functions upstream of *wee1* to relieve mitotic inhibition.

IN *Schizosaccharomyces pombe*, cell division is modulated by a size control acting at mitosis (for review see Lee and Nurse 1988; Fantes 1989). The mitotic control is sensitive to the nutritional status of the cell as is shown by the transient increase or decrease in cell division rate and cell size following shifts to nutritionally poorer or richer medium (Fantes and Nurse 1977). Nutritional cues or growth status activate or delay a network of control gene products, the best studied of which is the protein kinase, p34, encoded by the *cdc2* gene (Nurse, Thuriaux and Nasmyth 1976). *wee1*, a protein kinase acts as a mitotic inhibitor (Nurse 1975; Russell and Nurse 1987a). *cdc25* and probably *stf1* (Nurse, Thuriaux and Nasmyth 1976; Fantes 1979; Russell and Nurse 1986; Hudson, Feilotter and Young 1990) operate on an induction pathway in opposition to *wee1* to regulate *cdc2* activity. The product of the *cdc13* gene, a mitotic cyclin, associates with p34 and is involved in controlling the timing of mitosis (Booher and Beach 1988; Draetta and Beach 1988; Hagan, Hayles and Nurse 1988; Goebl and Byers 1988; Solomon *et al.* 1988). These genes have been shown to have highly conserved homologs in all higher eukaryotic cells examined (Draetta *et al.* 1987; Lee and Nurse 1987; Labbe *et al.* 1988).

In the absence of *wee1* function, cells are advanced into mitosis prematurely and thus undergo division at a small cell size (Nurse 1975; Nurse and Thuriaux 1977, 1980; Thuriaux, Nurse and Carter 1978). Such cells are not responsive to changes in nutritional status (Fantes and Nurse 1977), suggesting that this gene is normally required for correct response to nutritional signals.

Candidates for genes functioning in transducing nutritional cues to the mitotic control have been identified as nonconditional mutants with altered responses to nutritional deprivation (Young and Fantes 1984; 1987). These mutants (*cdr1*, *cdr2*) display altered cell division kinetics in response to starvation for various nutrients, resulting in a long cell phenotype even after extended starvation periods. The mutants display strong negative interactions with *cdc25* mutants; $cdr1^-$ *cdc25-22* or $cdr2^-$ *cdc25-22* double mutants are very elongated relative to wild-type cells and grow slowly with a reduced restrictive temperature of 27°. Mutations in *wee1* are epistatic to *cdr* mutations with respect to cell size (Young and Fantes 1987) suggesting that the *cdr* genes might play a role in nutritional modulation of the mitotic size control, functioning upstream of *wee1*.

[1] Present address: Cold Spring Harbor Laboratories, Cold Spring Harbor, New York 11724.

Other mutants have been identified which have nutritionally modulated phenotypes and thus may play a role in nutritional sensing or response. The *mcs4-13* mutant displays sensitivity to nutritional status in certain genetic backgrounds. An *mcs4-13 wee1-50 cdc25-22* triple mutant is viable at 35° in rich medium but displays a cell cycle arrest terminal phenotype at this temperature on minimal medium (MOLZ *et al.* 1989). The *mcs4-13* mutation alone causes delay of cell division on minimal medium, although this effect is abolished on medium containing yeast extract (MOLZ *et al.* 1989). In addition, the *mcs4-13 cdr1-76* double mutant displays a greatly elongated phenotype during starvation on medium lacking a nitrogen source (P. YOUNG, L. MOLZ and D. BEACH, unpublished observations) suggesting a role for both of these gene products in the nutritional response. Another gene whose expression is affected by the nutritional environment is the *win1* gene. In a *cdc25-22 wee1-50* background the presence of the *win1-1* mutation reverses the *wee1* suppression of *cdc25* on minimal medium, although not on complex medium (OGDEN and FANTES 1986).

We have exploited the strong interactions between *cdr1* and *cdc25* mutants to clone the *cdr1* gene. This has revealed allelism between *cdr1* and the *nim1* protein kinase (RUSSELL and NURSE 1987b). It has clarified the structure of the gene as the original *nim1* sequence included a noncontiguous genomic fragment. We provide the complete sequence of the *cdr1* gene and have also undertaken an extensive genetic analysis of interactions between *cdr1* mutants and various alleles of genes in the mitotic control in order to further examine the function of the *cdr1* gene.

MATERIALS AND METHODS

Strains, media and growth: The origin of yeast strains is shown in Table 1. Cells were grown on YEA (MITCHISON 1970) or minimal medium (MITCHISON 1970 as modified by NURSE 1975) as indicated. Cells were tested for temperature sensitivity after growth on YEA agar with phloxin B (GUTZ *et al.* 1974). The *cdr*⁻ phenotype was scored on minimal agar minus NH_4Cl after growth for several days at 20° (YOUNG and FANTES 1987). Matings were done on malt extract agar at 20°. Cell length measurements were made on cells displaying a septum during logarithmic growth in liquid medium.

Cloning and plasmids: The *cdr1* gene was cloned from both *Hin*dIII and *Sau*3a partial digest genomic libraries in the vector pWH5 (WRIGHT *et al.* 1986; P. YOUNG and D. BEACH, unpublished results). Yeast transformations and plasmid isolation were as previously described (BEACH and NURSE 1981). Integration of the pcdr1 plasmid was done by removal of the 2-μm region of pWH5 by *Sal*I digestion followed by retransformation and selection for stable $LEU2^+$ transformants.

DNA sequencing: DNA sequencing was performed using the dideoxynucleotide method (SANGER, NICKLEN and COULSON 1977) on subclones in the vectors pUC118 and pUC119. Data base searches were carried out using the FASTA programs at GenBank (PEARSON and LIPMAN 1988).

RESULTS

Molecular cloning of *cdr1*: Mutations in *cdr1* cause cells to divide at an elongated size under all growth conditions (YOUNG and FANTES 1987). This size phenotype was shown to be recessive by constructing a diploid *cdr1-76/+ ade6-210/ade6-216* h^-/h^+ (Q388) and assessing cell length during growth. *cdr1-76/+* cells were the same length as an *ade6-210/ade6-216* diploid alone and thus the *cdr1* elongated cell phenotype was recessive.

The *cdr1* gene was cloned by rescue of the *cdr1-76 cdc25-22* double mutant at 29°, a temperature at which the *cdc25-22* lesion alone is not lethal. The strain *cdr1-76 cdc25-22 leu1-32* (Q334) was transformed with two different gene banks and cells allowed to recover for a day at 20° and then selected at 29°.

Eight plasmids were recovered and shown to retransform the original mutant strain. Restriction map analysis showed that one of these plasmids was the *cdc25* gene. Four versions of a second sequence were recovered. This plasmid was shown to be *cdr1* by integration into a *cdr1-76 leu1-32* strain and outcrossing to wild-type cells. Progeny were scored for the appearance of the *cdr1*⁻ phenotype following nitrogen starvation. Only one of the 408 colonies scored in this manner displayed a *cdr1*⁻ phenotype showing that the plasmid was integrated close to the *cdr1* locus.

***cdr1* gene structure:** Comparison of restriction map data from pcdr1 and pnim1 (RUSSELL and NURSE 1987b) shows that they contain a common 6.1-kb *Hin*dIII fragment (Figure 1). By Southern blotting the *cdr1* 6.1-kb *Hin*dIII fragment gave a positive hybridization signal with the equivalent region from *nim1* (data not shown). The *nim1* open reading frame extends beyond the 6.1-kb fragment into a downstream 3-kb *Hin*dIII fragment (RUSSELL and NURSE 1987b) which is not the same as the downstream 4.1-kb *Hin*dIII fragment in pcdr1 (Figure 1). The 4.1-kb *Hin*dIII *cdr1* fragment was likely to be the correct contiguous fragment since the *cdr1* gene was cloned independently from two libraries, giving identical clones (Figure 1). To confirm this, genomic Southern blots hybridized with either a part of the 6.1-kb or the 4.1-kb *Hin*dIII fragments shows that both probes hybridize to the same *Eco*R1 and *Bam*H1 fragments spanning the region (Figure 2). The 3-kb fragment shown in the *nim1* map (RUSSELL and NURSE 1987b) thus belongs elsewhere in the genome.

To confirm the allelism of *cdr1* and *nim1* genetically, crosses of *cdr1-76* and a *nim1* disruption [*nim1::ura4*⁺ (Q649) RUSSELL and NURSE 1987b] were undertaken. No wild-type recombinants were seen in

TABLE 1

Strain list

Strain	Genotype	Origin
Q64	*cdr1-34 h⁻*	YOUNG and FANTES (1987)
Q65	*cdr1-34 h⁺*	YOUNG and FANTES (1987)
Q160	*cdc2-M72 h⁻*	
Q161	*cdc2-L7 h⁻*	
Q164	*cdc2-M76 h⁺*	
Q165	*cdc2-M26 h⁺*	
Q247	*975 h⁺*	
Q250	*972 h⁻*	
Q258	*cdr1-76 h⁺*	YOUNG and FANTES (1987)
Q265	*cdc2-33 cdr1-76 h⁻*	This work
Q267	*cdr1-76 h⁺*	YOUNG and FANTES (1987)
Q271	*cdc13-117 h⁻*	
Q308	*cdc2-33 h⁺*	
Q310	*cdc2-130 h⁻*	
Q314	*cdc13-117 cdr1-76 h⁻*	This work
Q321	*cdc25-M51 h⁺*	
Q334	*cdr1-76 cdc25-22 leu1-32 h⁻*	This work
Q356	*cdc25-22 leu1-32 h⁺*	D. BEACH
Q361	*cdc2-56 h⁻*	
Q377	*cdc2-L7 cdr1-76 h⁺*	This work
Q381	*cdc25-43 h⁻*	
Q388	*cdr1-76/+ ade6-210/ade6-216 h⁺/h⁻*	This work
Q397	*leu1-32 h⁻*	
Q403	*cdr1-76 leu1-32 h⁻*	This work
Q409	*cdc2-56 cdr1-76 h⁻*	This work
Q415	*cdc2-M72 cdr1-76 h⁻*	This work
Q416	*cdc2-M72 cdr1-34 h⁻*	This work
Q419	*cdc13-117 cdr1-34 h⁻*	This work
Q422	*cdc2-M26 cdr1-76 h⁻*	This work
Q423	*cdc2-L7 cdr1-34 h⁻*	This work
Q429	*cdc2-M35R20 h⁺*	
Q430	*cdc2-M35 h⁻*	
Q438	*cdc2-M26 cdr1-34 h⁻*	This work
Q445	*cdc2-130 cdr1-76 h⁻*	This work
Q447	*cdc2-130 cdr1-34 h⁻*	This work
Q452	*cdc2-33 cdr1-34 h⁻*	This work
Q454	*cdc2-M76 cdr1-76 h⁻*	This work
Q455	*cdc2-M76 cdr1-34 h⁻*	This work
Q484	*cdc2-56 cdr1-34 h⁻*	This work
Q489	*cdc13-117 leu1-32 h⁻*	This work
Q546	*cdr1-76 ade6-216 h⁻*	This work
Q649	*nim1::ura4⁺ ade6-210 leu1-32 ura4-D18 h⁻*	RUSSELL and NURSE (1987b)
Q652	*cdc2-M35R20 cdr1-34 h⁻*	This work
Q653	*cdc2-48 cdr1-34 h⁻*	This work
Q668	*cdr1-76/nim1::ura4⁺ ade6-210/ade6-216 h⁺/h⁻*	This work
Q679	*cdc25::ura4⁺ cdc2-3w cdr1-34 h⁻*	This work
Q708	*cdc25::ura4⁺ cdc2-3w h⁻*	HUDSON, FEILOTTER and YOUNG (1990)
Q756	*cdc2-48 h⁻*	P. NURSE
Q757	*cdc2-48 cdr1-76 h⁻*	This work

Strains not specifically designated are from the Edinburgh strain collection, University of Edinburgh, courtesy of P. FANTES.

35 complete tetrads. It is known that the *cdc16* locus maps at a distance of 9.7 cM from the *cdr1* locus (YOUNG and FANTES 1987). To confirm the mapping of the *nim1* disruption at or near the *cdr1* locus, *nim1::ura4⁺* was crossed to *cdc16* and the map distance between *nim1* and *cdc16* was calculated following random spore analysis. For a total of 145 spores scored the data indicated a map distance of 8.3 cM. Finally, *cdr1-76 ade6-210 h⁻* was crossed to *nim1::ura4⁺ ade6-216 h⁺* to generate a diploid (Q668). The resulting diploid had an elongated *cdr⁻* phenotype (28.9 μm in length at septation) relative to a wild-type diploid (20.5 μm). The two strains thus did not complement.

The 6.1-kb fragment of pcdr1 alone is known to rescue *cdc25-22* although it is lacking an undefined length of the carboxyl terminus of the gene (RUSSELL and NURSE 1987b). To estimate the distance which the transcript extends into the downstream fragment,

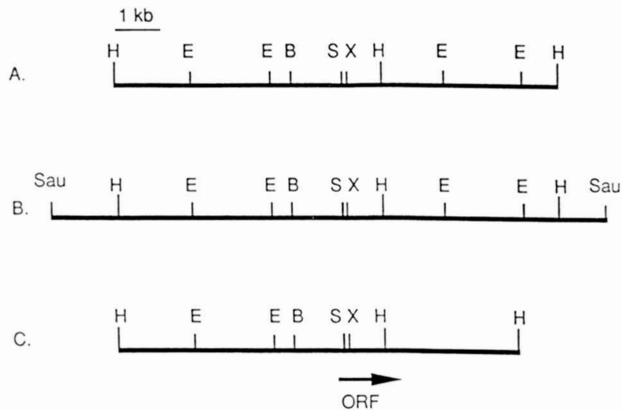

FIGURE 1.—Restriction maps of independent *cdr1* clones and of *nim1*. A, pcdr1 from the *Hin*dIII partial genomic library; B, pcdr1 from the *Sau*3a partial genomic library; C, pnim1 with open reading frame (ORF) indicated from RUSSELL and NURSE (1987b). B = *Bam*HI; E = *Eco*RI; H = *Hin*dIII; S = *Sph*I; X = *Xho*I.

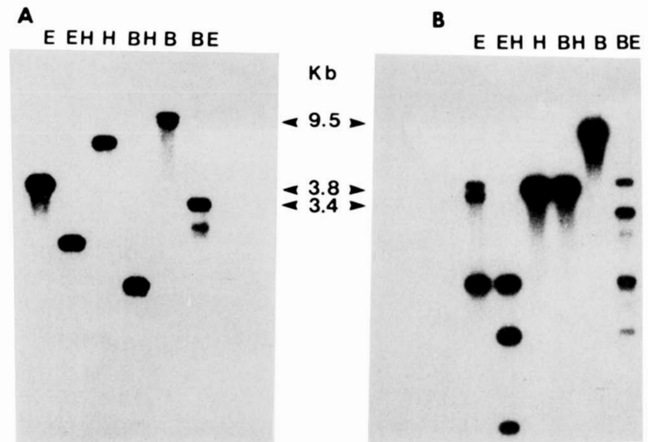

FIGURE 2.—Southern blots of *S. pombe* genomic DNA were probed with the 0.125-kb *Sph*I/*Xho*I fragment (A) or the 4.1-kb *Hin*dIII fragment of *cdr1* (B). Diagnostic fragments spanning the common *Hin*dIII site are the 3.8-kb *Eco*RI fragment, the 9.5-kb *Bam*HI fragment and the 3.4-kb *Eco*RI/*Bam*HI fragment. The top bands in the *Eco*RI and *Bam*HI/*Eco*RI lanes in blot B are the result of partial digestion. E = *Eco*RI; H = *Hin*dIII; B = *Bam*HI.

a Northern blot of total cellular RNA was hybridized with a single-stranded DNA probe made from the *Xho*I/*Hin*dIII region of the 6.1-kb pcdr1 *Hin*dIII fragment. A major transcript at 2.4 kb and two minor transcripts at 1.0 kb and 0.5 kb were found. High stringency washes did not remove the signals for any of the transcripts (data not shown). The previously sequenced coding region (RUSSELL and NURSE 1987b) is 1.1 kb long, suggesting substantial transcript overlap into the downstream 4.1-kb *Hin*dIII fragment.

Structure of the *cdr1* gene: The published *nim1* sequence included 759 bases of upstream untranslated sequence and a 370-amino acid open reading frame (RUSSELL and NURSE 1987b). The first 260 amino acids of this sequence encodes a putative protein kinase domain (RUSSELL and NURSE 1987b). The internal *Hin*dIII site marks the beginning of a non-contiguous fragment at residue 356 of the *nim1* amino acid sequence. In the full length genomic clone, starting at the *Hin*dIII site, we have sequenced downstream a further 1200 bp into the appropriate contiguous fragment. This has revealed an additional 237 amino acids before termination of the open reading frame (Figure 3). The continuous open reading frame is 593 amino acids in length encoding a polypeptide of predicted M_r 67,010. Sequencing of 500 bases downstream of the termination codon revealed no further substantial open reading frame. Three potential polyadenylation signals (AATAAA) are indicated (PROUDFOOT and BROWNLEE 1976).

Based on the sequence, no striking secondary structural features were noted (GARNIER, OSGUTHORPE and ROBSON 1978). Involvement of the gene product in nutritional signal transduction suggests a possible plasma membrane location. Two potential glycosylation sites were found at residues 156 and 367 (MARSHALL 1972); however no hydrophobic membrane spanning domain is present (RAO and ARGOS 1986).

By analysis of concensus sequences, the $p67^{cdr1}$ kinase has three potential cAMP/GMP-dependent protein kinase phosphorylation sites, all in the carboxyl-terminal half of the protein (residues 298, 376 and 387) (FREMISCO, GLASS and KREBS 1980; GLASS and SMITH 1983; GLASS, EL-MAGHRABI and PILKIS 1986). Numerous potential protein kinase C and casein kinase II phosphorylation sites were found both in the kinase domain and the carboxyl-terminal portions of the gene.

Sequence comparisons: The complete *cdr1* amino acid sequence was compared with available databases using the FASTA program (PEARSON and LIPMAN 1988). The search revealed significant similarities to protein kinases, as noted previously for the region from residues 15–260 of the $p67^{cdr1}$ sequence (RUSSELL and NURSE 1987b; HANKS, QUINN and HUNTER 1988). The highest levels of identity were with *S. cerevisiae* protein kinases *SNF1*, *KIN2* and *KIN1* and with the family of calcium calmodulin-dependent protein kinases. With the exception of *SNF1*, *KIN2*, *KIN1*, and the catalytic subunit of phosphorylase *b* kinase, the region of identity between all of these kinases and $p67^{cdr1}$ extended only over the kinase domain. Considering only amino acid identity in the kinase domain, $p67^{cdr1}$ has 37.1% identity with *KIN1*, 38.8% with *KIN2*, 41.6% with *SNF1*, 39.4% with cAMPK from rat, 39.4% with cAMPK from mouse and 31.4% with phosphorylase *b* kinase.

The protein kinases which show some similarity to $p67^{cdr1}$ outside of the catalytic domain are not similar to each other in this region. They represent several classes of kinases, apparently regulated in different ways. The *KIN1* and *KIN2* protein kinases are closely related serine-threonine kinases of unknown regulation and function (LEVIN *et al.* 1987). The *SNF1*

```
-779  ggatcctgtttgtattaatattgtaggatcgatatttgaaacatctgtaggccttctatata
-716  gtgggttgttctacatattatttatttattcattcatctttctttgttttacttactgttctc
-653  catattttatctgattatacagggtttgaattaaaatccgttagtttattttattctacctt
-590  tttcaactatttgaccgccccaatatttccatttttactttctccttttcttgtttcttctctc
-527  ttttaatttatgaagttcaatccttattcgaatatcattaacttaacctttattggatttata
-464  catcacgaatcttatacactttacatactttctttatttacgctccccttcgtttcttttttc
-401  tttgtctttaccttttttttattgctgttactcaaaacgtcttgtaacttacatttgttatttg
-338  tgttcttgtgctacttcccatttttttttcaaggtccgtctatttccgttccacgtttcattt
-275  ttctgttctacaatacactcagaaaagtgctttaggttatttatgaggcactcttattttatt
-212  aattcgtttccagtatctattttgtgatcacaccctatttgtatcgtggtgtcattcacttt
-149  ttcctttacttattccaaatttcatgctacttttcgacatttattctacttttcatcataag
-86   tatttcagattttctgaagttgctattattcagtctgcaattttacttttggaaagcctttc
-23   ccttaataaaaggttagatttga ATG GTG AAG CGA CAC AAA AAT ACT ATT GGC
                              Met Val Lys Arg His Lys Asn Thr Ile Gly
31    GTA TGG CGT CTT GGG AAG ACT TTA GGG ACT GGT TCC ACT AGC TGT GTG
      Val Trp Arg Leu Gly Lys Thr Leu Gly Thr Gly Ser Thr Ser Cys Val
                          SphI
79    CGT CTT GCC AAG CAT GCT AAA ACT GGT GAT TTG GCT GCC ATC AAA ATT
      Arg Leu Ala Lys His Ala Lys Thr Gly Asp Leu Ala Ala Ile Lys Ile
127   ATC CCT ATT CGA TAT GCC TCC ATA GGA ATG GAA ATC TTA ATG ATG CGT
      Ile Pro Ile Arg Tyr Ala Ser Ile Gly Met Glu Ile Leu Met Met Arg
175   TTG TTG CGC CAT CCT AAT ATT CTT CGA CTA TAC GAT GTT TGG ACC GAT
      Leu Leu Arg His Pro Asn Ile Leu Arg Leu Tyr Asp Val Trp Thr Asp
              NdeI                XhoI
223   CAT CAG CAT ATG TAC TTA GCA CTC GAG TAC GTC CCT GAT GGT GAG CTT
      His Gln His Met Tyr Leu Ala Leu Glu Tyr Val Pro Asp Gly Glu Leu
271   TTT CAC TAT ATA CGC AAA CAT GGA CCT TTG AGC GAA AGA GAG GCT GCC
      Phe His Tyr Ile Arg Lys His Gly Pro Leu Ser Glu Arg Glu Ala Ala
319   CAT TAT CTT TCC CAA ATT CTT GAT GCT GTA GCG CAT TGT CAT CGC TTT
      His Tyr Leu Ser Gln Ile Leu Asp Ala Val Ala His Cys His Arg Phe
367   CGC TTT CGC CAT CGG GAT TTA AAA CTT GAG AAT ATT CTC ATC AAG GTA
      Arg Phe Arg His Arg Asp Leu Lys Leu Glu Asn Ile Leu Ile Lys Val
415   AAT GAG CAA CAA ATT AAA ATA GCT GAT TTT GCC ATG GCC ACT GTT GAA
      Asn Glu Gln Gln Ile Lys Ile Ala Asp Phe Gly Met Ala Thr Val Glu
463   CCA AAT GAC TCT TGT CTT GAG AAT TAT TGC GGC TCT TTA CAT TAT TTG
      Pro Asn Asp Ser Cys Leu Glu Asn Tyr Cys Gly Ser Leu His Tyr Leu
511   GCT CCC GAA ATT GTA TCT CAC AAA CCA TAT CGT GGA GCT CCT GCT GAT
      Ala Pro Glu Ile Val Ser His Lys Pro Tyr Arg Gly Ala Pro Ala Asp
559   GTT TGG TCT TGT GGT GTC ATC TTG TAT TCA TTG TTG TCG AAT AAG CTC
      Val Trp Ser Cys Gly Val Ile Leu Tyr Ser Leu Leu Ser Asn Lys Leu
607   CCC TTT GGT GGT CAA AAC ACA GAT GTT ATT TAT AAT AAG ATT CGC CAT
      Pro Phe Gly Gly Gln Asn Thr Asp Val Ile Tyr Asn Lys Ile Arg His
          NdeI
655   GGC GCA TAT GAT CTT CCT TCT TCA ATT TCT TCG GCT GCG CAA GAT TTG
      Gly Ala Tyr Asp Leu Pro Ser Ser Ile Ser Ser Ala Ala Gln Asp Leu
703   TTA CAC AGA ATG CTA GAT GTA AAT CCA AGC ACG CGT ATA ACA ATT CCG
      Leu His Arg Met Leu Asp Val Asn Pro Ser Thr Arg Ile Thr Ile Pro
751   GAA TTT TTT TCT CAT CCA TTT CTA ATG GGC TGT ACA TCA TTG AGC TCT
      Glu Phe Phe Ser His Pro Phe Leu Met Gly Cys Thr Ser Leu Ser Ser
799   ATG GAT AGC ACT ACC CCT CCT ACC CCT TCA CTG TCA ATT GAC GAA ATT
      Met Asp Ser Thr Thr Pro Pro Thr Pro Ser Leu Ser Ile Asp Glu Ile
847   GAT CCA TTA GTA GTT GAT TGC ATG TGT GTG CTT TGG AAA AAA TCA TCC
      Asp Pro Leu Val Val Asp Cys Met Cys Val Leu Trp Lys Lys Ser Ser
895   TCT AAA AAA GTC GTT CGT CGT CTT CAA CAG CGA GAT GAT AAC GAC GAA
      Ser Lys Lys Val Val Arg Arg Leu Gln Gln Arg Asp Asp Asn Asp Glu
943   AAA TAT GTG TAT AAA GTT TTA TCC GAG ATT TTG CGC GAT GAT ATG TTG
      Lys Tyr Val Tyr Lys Val Leu Ser Glu Ile Leu Arg Asp Asp Met Leu
991   AAA AAA CAA CGT TTT GAT GAA AAT AAA TAT CTC AGT CTT TAC GAT TTA
      Lys Lys Gln Arg Phe Asp Glu Asn Lys Tyr Leu Ser Leu Tyr Asp Leu
                                  HindIII
1039  ATT CAC GAC AAT AAT CTT TTC ACC AAA GCT TCT ATT TCA ACA ACA TCG
      Ile His Asp Asn Asn Leu Phe Thr Lys Ala Ser Ile Ser Thr Thr Ser
1087  TTA GTA AAA TCC AAT GTT TCG ACA AAC TCT CGC AAA AGT AGT AAT TTT
      Leu Val Lys Ser Asn Val Ser Thr Asn Ser Arg Lys Ser Ser Asn Phe
1135  GAA GAT GAA CTT GCT AGA AGA GTT TCT AGT CCG CTT TCA GCC TTA AAT
      Glu Asp Glu Leu Ala Arg Arg Val Ser Ser Pro Leu Ser Ala Leu Asn
1183  CAA ATG TCT CAA TCT CCA ATA CCG ATC AGG GTG TCT TCT GAC AAG GAT
      Gln Met Ser Gln Ser Pro Ile Pro Ile Arg Val Ser Ser Asp Lys Asp
1231  TAT GAC AGC TAT GCC TGT CAT GAA GTA GTT TCA AAC CCA TCG ACT TTG
      Tyr Asp Ser Tyr Ala Cys His Glu Val Val Ser Asn Pro Ser Thr Leu
1279  GAT GAT GAC TAT AAT TAT ATG TTT GTT TGT CCA CCA GAA GAG TAT ACT
      Asp Asp Asp Tyr Asn Tyr Met Phe Val Cys Pro Pro Glu Glu Tyr Thr
1327  TAT TCA ACT GAT AAT GTT AGA ACT GAT TCT CTG GAC TTA CAA TCT CTC
      Tyr Ser Thr Asp Asn Val Arg Thr Asp Ser Leu Asp Leu Gln Ser Leu
1375  CCC ACT CCT ACC CTT GAG CAA TTA GAA TCC GTA CCA TTC AAT CGG TAT
      Pro Thr Pro Thr Leu Glu Gln Leu Glu Ser Val Pro Phe Asn Arg Tyr
1423  GGC TAT GTC CGC ATC TTT CCT AGC ACC ACT CTG TCT AGC ACA GCC AGT
      Gly Tyr Val Arg Ile Phe Pro Ser Thr Thr Leu Ser Ser Thr Ala Ser
1471  GGC TAC TAC ACT CCT GAT TCA CTT TCA ACT CCT GAA CCA TCA ATA GAT
      Gly Tyr Tyr Thr Pro Asp Ser Leu Ser Thr Pro Glu Pro Ser Ile Asp
1519  GGG CTA ACC AAT TTG GAC GAT GTC CAG GTT GGA GGC TTT GTT CAA GGA
      Gly Leu Thr Asn Leu Asp Asp Val Gln Val Gly Gly Phe Val Gln Gly
1567  TCA GGC AAT CAA AAT AGA CGT CCC ATA TCT TTT CCT GTA ATC AGT AAT
      Ser Gly Asn Gln Asn Arg Arg Pro Ile Ser Phe Pro Val Ile Ser Asn
1615  ATG CAG CCG AAC ATA ACT AAT GTG AGA TCT GCT TCT GCT CCT TTA TGT
      Met Gln Pro Asn Ile Thr Asn Val Arg Ser Ala Ser Ala Pro Leu Cys
1663  TCA TCT CCA GTT CCA AGT CGG AGA TAT TCT CAA TAT GCT ACT AAT ATT
      Ser Ser Pro Val Pro Ser Arg Arg Tyr Ser Gln Tyr Ala Thr Asn Ala
1711  AGA TAT ACT CCC AGA AAA GTT TCT TCC GGT TCT GTA TTA CGA AAG ATT
      Arg Tyr Thr Pro Arg Lys Val Ser Ser Gly Ser Val Leu Arg Lys Ile
1759  TCT TCA TTC TTT CGG AAG GAT TAA ataggaatttttcaaaaacacagtcttctt
      Ser Ser Phe Phe Arg Lys Asp
1813  cctcaaaatcaaccattaatgtctatactgtgggcggctattcaaatgttaagatctgtttat
1876  aatttaatgaggatgataattatttttttggatctcccgtcgtgtgtacctgtacctttcta
1939  cgaatttaatgatctttttggcaattggacctttgagtctattagcaatttaatggtttagg
2002  gtttacgttatatatcgttaatgatttgggcacactagtttatatatgtaactaatgaatatc
2065  ttctattacaatgttccagaaatcagtagtctgtataaagtaaaatttgttgtgaacattag
2128  ttttatatgaaatttgggtgttttatgtaataacgtctgttggattttgaaaattgtcgaat
2191  ataccggagtgtttagtaataaaattgaggtttaagagttatcgcaataaataaaatatgaag
2254  aaataattcctataaaccgtttacctct
```

FIGURE 3.—The complete DNA sequence of the *cdr1* gene. The upstream noncoding sequence and the first 356 amino acid residues are taken from RUSSELL and NURSE (1987b). Relevant restriction enzyme sites are indicated above the sequence. Three putative polyadenylation signal sequences are underlined in the carboxyl-terminal noncoding sequence.

protein kinase plays a role in catabolic derepression of glucose-repressed genes and is regulated by nutritional cues (CELENZA and CARLSON 1986), while rabbit phosphorylase *b* kinase is a calcium-regulated protein kinase, essential for glycogen metabolism (PICKETT-GIES and WALSH 1986; REIMANN *et al.* 1984). Downstream of the catalytic domains, the level of identity to $p67^{cdr1}$ is 17.4% with *KIN1* and 17.4% with *KIN2* over 46 amino acids. For *SNF1* the identity was 9.0% over 152 residues and for phosphorylase *b* kinase, 10.4% over 96 residues.

A separate data base search was performed for the carboxyl-terminal 245 amino acids of $p67^{cdr1}$. No conserved resgions were identified. Because of the high identity between $p67^{cdr1}$ and a number of Ca^{2+}/calmodulin-dependent protein kinases, this region was examined for potential calmodulin binding sites. An amphiphilic helix representing a potential calmodulin binding site is located at residues 330–344.

Genetic interactions: Overexpression of the 6.1-kb *Hin*dIII fragment of the *cdr1* gene results in a smaller cell size at division (RUSSELL and NURSE 1987b). Analysis of a *cdr1* disruption in combination with a *cdc25* overexpressor and with a *wee1* disruption strain suggested that *cdr1* normally functions upstream of *wee1* as a negative regulator of that gene (RUSSELL and NURSE 1987b). Earlier observations of the epistatic interaction between *wee1* and *cdr1* (YOUNG and FANTES 1987) may now be viewed in light of this model, as may the observation that reduced *cdc25*

TABLE 2

Interactions affecting cell length at division

Strain	26°	32°	36°
Part a			
cdc25::ura4 cdc2-3w	18.8 ± 0.6	ND	ND
cdc25::ura4 cdc2-3w cdr1-34	30.0 ± 1.1*	ND	ND
Part b			
972	15.0 ± 0.4	15.1 ± 0.3	15.8 ± 0.4
cdr1-76	20.4 ± 0.3*	18.7 ± 0.3*	16.8 ± 0.4*
cdr1-34	17.0 ± 0.4*	17.6 ± 0.3*	16.6 ± 0.3
cdc2-33	15.9 ± 0.3	17.8 ± 0.6	cdc⁻
cdc2-33 cdr1-76	20.3 ± 0.4*	cdc⁻	cdc⁻
cdc2-33 cdr1-34	20.5 ± 0.6*	31.7 ± 0.6*	cdc⁻
cdc2-M72	16.7 ± 0.4	16.2 ± 0.5	cdc⁻
cdc2-M72 cdr1-76	23.1 ± 0.5*	cdc⁻	cdc⁻
cdc2-M72 cdr1-34	22.6 ± 0.5*	19.8 ± 0.4*	cdc⁻
cdc2-M26	17.5 ± 0.3	18.5 ± 0.4	cdc⁻
cdc2-M26 cdr1-76	21.0 ± 0.4*	cdc⁻	cdc⁻
cdc2-M26 cdr1-34	28.7 ± 0.8*	cdc⁻	cdc⁻
cdc2-M35	28.8 ± 0.4	28.7 ± 0.6	cdc⁻
cdc2-M35 cdr1-76	cdc⁻	cdc⁻	cdc⁻
cdc2-M35 cdr1-34	cdc⁻	cdc⁻	cdc⁻
cdc2-M35R20	20.4 ± 0.5	ND	27.4 ± 0.7
cdc2-M35R20 cdr1-76	cdc⁻	ND	cdc⁻
cdc2-M35R20 cdr1-34	31.5 ± 0.5*	ND	cdc⁻
cdc2-48	26.0 ± 0.5	cdc⁻	cdc⁻
cdc2-48 cdr1-76	cdc⁻	cdc⁻	cdc⁻
cdc2-48 cdr1-34	cdc⁻	cdc⁻	cdc⁻
cdc2-130	11.9 ± 0.4	10.2 ± 0.3	13.8 ± 0.4
cdc2-130 cdr1-76	16.4 ± 0.9*	16.5 ± 0.8*	18.5 ± 0.7*
cdc2-130 cdr1-34	ND	ND	ND
cdc2-M76	15.3 ± 0.6	16.3 ± 0.4	cdc⁻
cdc2-M76 cdr1-76	16.6 ± 0.4*	19.9 ± 0.5*	cdc⁻
cdc2-M76 cdr1-34	18.4 ± 0.3*	20.0 ± 0.4*	cdc⁻
Part c			
cdc13-117	17.9 ± 0.4	20.2 ± 0.8	cdc⁻
cdc13-117 cdr-176	23.9 ± 0.4*	cdc⁻	cdc⁻
cdc13-117 cdr1-34	26.9 ± 0.6*	cdc⁻	cdc⁻

Values indicate cell length in μm. Single and double mutants were grown to mid-logarithmic phase in liquid medium at the indicated temperature. Cell lengths of cells with a complete septum were measured microscopically. Statistically significant differences ($P < 0.05$) are indicated with an asterisk.

activity is lethal in a *cdr1⁻* background (YOUNG and FANTES 1987). Further genetic analysis was therefore undertaken to examine the position of *cdr1* activity in the mitotic control pathway.

Specifically, crosses of two mutant alleles of *cdr1*, *cdr1-76* and *cdr1-34*, to alleles of *cdc25*, *cdc2* and *cdc13* were performed. Cells carrying the *cdr1-76* mutation are similar in length at division to cells carrying a gene disruption. Cells carrying the *cdr1-34* mutation are shorter at division (Table 2) and therefore this allele is unlikely to represent complete loss of function. Both alleles are conditionally lethal in a *cdc25-22* background (YOUNG and FANTES 1987).

Crosses to *cdc25*: The interaction between *cdc25-22* and *cdr1-76* has been shown to result in greatly elongated cells with a restrictive temperature of 27° (YOUNG and FANTES 1987). We investigated the effects of other *cdc25* alleles on *cdr1-76* and *cdr1-34* mutants. Both *cdc25-43* and *cdc25-M51* resulted in the same double mutant phenotype (data not shown). A *cdr1* disruption (*nim1::ura4⁺*, Q649) in a *cdc25-22* background results in a similar phenotype. Loss of the *cdc25* gene product is lethal for cells which retain wild-type *wee1* and *cdc2* genes; strains which have either lost *wee1* function or which carry a *cdc2–3w* allele no longer require the mitotic inducing function of *cdc25* (RUSSELL and NURSE 1986). If *cdr1* functions as a mitotic inducer in a pathway independent from *cdc25* activity, it might be expected that cells rescued for a *cdc25* disruption by the presence of *cdc2-3w* may be adversely affected by further loss of *cdr1* function. We tested the effect of a *cdc2-3w* mutation in a *cdr1-34* background carrying a disruption for *cdc25*. Triple mutants (*cdc25::ura4⁺ cdc2-3w cdr1-34*, Q679) were

TABLE 3

Interactions affecting starvation response

	Wild type	*cdr1-76*	*cdr1-34*
Wild type	+	++	++
cdc2-33	+	+++	+++
cdc2-L7	+	+++	+++
cdc2-M26	+	++++	++++
cdc2-M72	++	+++++	+++++
cdc2-56	+	+	+

Single and double mutants and wild-type controls were plated onto EMM-N agar and grown for 3–4 days at 20°. Cell size was scored microscopically on plates and cell length was estimated relative to wild-type length under these conditions. Cell lengths at septation could not be measured under these conditions since cells arrest elsewhere in the cell cycle. A score of + is the same as wild-type following starvation (about 6–7 μm); a score of ++ is about 9–10 μm; a score of +++ is similar in size to wild-type cells growing on Edinburgh minimal medium (about 14–15 μm at septation); a score of ++++ is almost twice the size of wild-type cells growing on EMM (about 25–26 μm), a score of +++++ is greater than 26 μm.

elongated compared to the parental strains (Table 2a).

Crosses to *cdc2* mutants: Since *cdr1-76* was known to depress the restrictive temperature of *cdc2-33* (Young and Fantes 1987), the interaction with various *cdc2* alleles was investigated. The most striking effects were found with double mutants of *cdr1-* and *cdc2-M35*, *cdc2-M35R20* or *cdc2-48*. Each of these strains is extremely elongated with a restrictive temperature of 25° or less (Table 2b). This phenotype is similar to that seen for the interaction of $cdr1^-$ with *cdc25-22*. Interestingly, the interactions with *cdc2-M35R20*, a non cdc^{ts} strain, showed allele specificity: the double mutant with *cdr1-76* was cdc^- at 26° while that with *cdr1-34* was elongated but still grew. Several alleles of *cdc2* in a $cdr1^-$ background resulted in a lowered restrictive temperature of 32° for the double mutant. In some cases, this was seen as an allele specific interaction with the *cdr1* mutants (*e.g.*, *cdr1-76 cdc2-M72*, *cdr1-76 cdc2-33*) (Table 2b). We conclude from these results that the *cdr1-34* mutation probably has residual $cdr1^+$ activity.

Double mutants with phenotypically *wee* alleles of *cdc2* (*cdc2-1w*, *cdc2-3w*) were indistinguishable in size from the wee parent (data not shown).

Negative interactions (*i.e.*, increases in cell length) following nitrogen starvation were noted with several *cdc2* alleles (Table 3). *cdc2-M26* and *cdc2-M72* showed particularly strong interactions. There was no simple relationship between starved size and the length of the double mutant in liquid culture.

Crosses to *cdc13*: *cdc13-117* is a temperature-sensitive lethal mutation which arrests at 35.5° in mid-mitosis (Nurse, Thuriaux and Nasmyth 1976; Nasmyth and Nurse 1981). Deletion of the gene causes arrest before mitotic initiation (Booher and Beach 1988; Hagan, Hayles and Nurse 1988). In the double mutants *cdr1-76 cdc13-117* and *cdr1-34 cdc13-117*, the cells display a terminal arrest phenotype very much more extreme in terms of cell length than the *cdc13-117* parent alone. These mutants resemble $cdc25^-$ mutants in that they are extremely long and lack septae, in contrast to cells carrying the *cdc13-117* mutation alone. This cdc^- phenotype is evident at a lowered restrictive temperature of 32° (Table 2c).

TABLE 4

Plasmid transformations

Cells	pWH5	pcdr1	pcdr1^{tr}	pcdc25
leu1-32	14.4 ± 0.3	10.4 ± 0.4	13.3 ± 0.3	11.2 ± 0.4
cdr1-76	18.3 ± 0.3	15.0 ± 0.6	15.9 ± 0.6	13.9 ± 0.7
cdc25-22	18.9 ± 0.7	17.1 ± 0.3	20.3 ± 0.8	13.5 ± 0.2

Transformed cells were grown in minimal medium at 25° and cells with complete septae were measured. Twelve cells were measured for each sample. Values are given as the mean cell length in microns with standard errors.

Plasmid transformations: The effect of overexpression of the full length *cdr* gene (pcdr1) and a version truncated at the *Hin*dIII restriction site (pcdr1^{tr}) as well as overexpression of pcdc25 in wild-type, *cdr1-76* and *cdc25-22* strains was examined.

The pcdr1 plasmid in wild-type cells resulted in a population of cells which divided at a size smaller than control transformed wild-type cells (Table 4). The plasmid restored *cdr1-76 leu1-32* cells to wild-type size during logarithmic growth. pcdr1 also shortened the length at septation of *cdc25-22* mutants following incubation at the permissive temperature of 25° (Table 4) and rescued this strain at the restrictive temperature (data not shown).

Strong overexpression of the truncated gene (pnim1) in wild-type cells results in premature mitosis (Russell and Nurse 1987b). We asked whether the presence or absence of the downstream segment of the gene resulted in a change in the phenotype of cells carrying plasmid-borne copies of the gene at relatively low copy number (pWH5). The full length *cdr1* gene in the vector pWH5 (Figure 1) was digested with *Hin*dIII and religated. Plasmids retaining the 6.1-kb *Hin*dIII fragment but missing the downstream 4.1-kb fragment were selected and used to transform wild-type and cdr^- cells. During growth on minimal medium the truncated gene caused a slight advancement of wild-type cells into mitosis, though this was not as pronounced as that caused by either the full length pcdr1 gene or pcdc25 (Table 4). pcdr1^{tr} was capable of restoring *cdr1-76* cells to almost wild-type length during logarithmic growth, but could not do the same for *cdc25-22* mutants at the permissive temperature (Table 4).

cdr1-76 leu1-32 strains transformed with pcdr and pcdr1^{tr} were then grown on minimal medium plates lacking a nitrogen source until growth arrested. Con-

trol cells (transformed with pWH5 alone) arrested in G_1 at a large cell size after several days on this medium. Mutants transformed with full length pcdr1 gave a typical wild-type starvation response after the same length of time with the cells dividing down to a reduced cell size. Mutants transformed with pcdr1^{tr} were not capable of the wild-type response and arrested as long cells like the *cdr1-76* control cells. Therefore, the truncated form of *cdr1*, though capable of causing small cell size at high expression levels (RUSSELL and NURSE 1987b) and of rescuing *cdc25-22*, was not capable of restoring complete wild-type function to the *cdr1-76* mutant.

Overexpression of the *cdc2* gene (pcdc2–14 in DB248X) (BOOHER and BEACH 1988) was also tested and allowed the partial rescue of the *cdr1-76* long-cell phenotype both on its own and in combination with *cdc25-22* at the restrictive temperature, although overexpression of *cdc2* could not rescue *cdc25-22* strains at 36°.

DISCUSSION

Gene structure: Restriction map, Southern blot and genetic analysis of the *cdr1* gene has shown that it is allelic to the *nim1* gene but differs from the *nim1* plasmid at the carboxyl terminus. We have provided evidence for the contiguity of the 4.1-kb *Hin*dIII fragment of the *cdr1* clone. The major transcript length (2.4 kb) suggests that the reading frame extends a substantial distance into the downstream 4.1-kb fragment. This was confirmed by sequencing of the downstream region of the gene.

The sequence of the *cdr1* gene has revealed a protein of predicted M_r 67,010. The kinase domain (by comparison to known kinases) resides in the amino-terminal region of 260 residues of this protein. Following this is a carboxyl-terminal region of 333 amino acids which is involved in mitotic activation upon nitrogen starvation. Several protein kinases have been identified which contain amino-terminal kinase domains and a substantial carboxyl-terminal domain. Within this group of kinases (*e.g.*, *KIN1*, *KIN2*, *SNF1*, CaMPK, phosphorylase *b* kinase) the carboxyl-terminal domains appear to be unique to each individual kinase. It has been suggested that these domains may be important in assembly of the holoenzyme in those cases where this is required (*i.e.*, cAMPK or phosphorylase *b* kinase) (LIN *et al.* 1987; BENNETT and KENNEDY 1987). In the case of CaMPK, the domain carboxyl to the kinase and calmodulin-binding domain is hypothesized to act as a regulatory hinge region which blocks activation of the kinase when calmodulin is not bound and to expose the active site following calmodulin binding (LIN *et al.* 1987). It is not evident at this point what the biochemical function of the carboxyl-terminal domain of the p67^{cdr1} protein kinase might be. The *KIN1* and *KIN2* genes were identified during a search for yeast protein tyrosine kinases (LEVIN *et al.* 1987). They appear to encode serine-threonine specific kinases (HANKS, QUINN and HUNTER 1988). Both have their kinase domains in the amino-terminal region of the protein and have a carboxyl terminus of undefined function (LEVIN *et al.* 1987). The identity between *KIN1*, *KIN2* and p67^{cdr1} extends past the kinase domains into the carboxyl-terminal domain for 46 amino acids before diverging completely. This may suggest the presence of subdomains within the region which play a role in the activity of the kinase. A similar situation exists with the *SNF1* kinase, but the conserved regions extends for 152 amino acids past the p67^{cdr1} kinase domain. This is of particular interest because of the role of the *SNF1* kinase in catabolic derepression (CARLSON, OSMOND and BOTSTEIN 1981; CARLSON *et al.* 1984). In *S. cerevisiae* a subset of genes are repressed in the presence of glucose; reversal of this repression requires the activity of the *SNF1* kinase which may act through the negative regulation of a transcriptional regulator of invertase (CARLSON *et al.* 1984). *SNF1* activation is sensitive to nutritional cues with activation being a response generated by the absence of glucose. The *cdr1* kinase is also sensitive to nutritional cues and this sensitivity has been shown to depend on the presence of the carboxyl-terminal fragment of the gene.

Relation to nitrogen starvation: On multiple copy plasmids, the truncated version of the gene ending at the *Hin*dIII site is capable of rescuing loss of *cdc25* gene function (RUSSELL and NURSE 1987b) as is the full-length *cdr1* gene. Transformation of the truncated and full-length genes into a number of mutant strains results in the same phenotypes in all cases tested under nonstarvation conditions. However, in a *cdr1-76* mutant during nitrogen starvation, the truncated version of the *cdr1* gene is not capable of restoring the wild-type mitotic response to starvation. This suggests that the carboxyl terminus of the gene plays a role in regulation of the activity of the kinase. It also suggests that the kinase might normally be regulated by nutritional cues.

Genetic interactions: The *nim1* gene has been described as a mitotic inducer which operates through negative regulation of *wee1* (RUSSELL and NURSE 1987b). This model predicts that cells defective for *cdr1* activity should be less capable of down-regulating *wee1* activity, so that mitosis should be at least partially inhibited at all times, resulting in a constitutively long cell. Mutations in *wee1* would be epistatic to mutations in *cdr1*. Although not explicitly tested in the initial experiments, any loss of *cdc25* function should be exacerbated by loss of *cdr1*.

All genetic interaction data presented here are consistent with this model. We have shown that loss of

cdr1 function strongly delays mitosis in combination with any deficit in *cdc25* function. The overexpressed *cdc25* plasmid is able to advance *cdr1-76* mutants into early mitosis and a *cdc25* disruption rescued by *cdc2-3w* is sensitive to reductions in *cdr1* activity. These results are consistent with *cdr1* operating on a pathway distinct from the *cdc25* activation pathway.

Strong lethal interactions were noted for *cdr1⁻* with *cdc2-M35*, *cdc2-M35R20* and *cdc2-48*. These particular *cdc2* strains also were lethal at temperatures above 27° in *cdc25⁻* backgrounds, suggesting that they may be deficient for *cdc25* interaction. Results with *stf1*, a semidominant mutation epistatic to *cdc25* (HUDSON, FEILOTTER and YOUNG 1990), show partial alleviation of the *cdc2-M35* and *cdc2-M35R20* phenotypes in a *stf1⁻* background, suggesting again that *cdc25* interaction may be particularly deficient in these *cdc2* alleles. These results are consistent with increased *wee1* inhibition in a *cdr1⁻* background which then becomes lethal with *cdc25* deficiency or *cdc2* alleles deficient in *cdc25* response.

The nitrogen limited stimulation of division is affected by loss of *cdr1*. At least two alleles of *cdc2* (*cdc2-M26*, *cdc2-M72*) display *cdc⁻*-like elongation in *cdr1⁻* backgrounds when deprived of nitrogen. This is similar to the *mcs4-13 cdr1-76* response (P. YOUNG, L. MOLZ and D. BEACH, unpublished observations) and may suggest that these two *cdc2* alleles and *mcs4* share some common interaction or function.

***cdr1* and mitotic initiation:** Overexpression of the *cdc2* gene can reverse the lethal interaction of mutations in *cdr1* and *cdc25* together. This suggests that the availability of free subunits of the *cdc2* kinase complex overrides increased *wee1* inhibition resulting from *cdr1* deficiency. Thus, the normal function of *cdr1*, and by extension, *wee1*, may be concerned with availability or the regulation of assembly of complex components.

Similarities to some of the interactions observed for *cdr1* mutants have been noted in interactions involving a number of *mcs* mutants. Rescue of the lethal interaction of *mcs4-13 cdc25-22 wee1-50* on minimal medium by p*cdc2* (MOLZ *et al.* 1989) may be similar to the rescue of the conditional lethal *cdc25-22 cdr1-76* interaction by p*cdc2* observed here. The observed nutritionally conditional negative interaction between the *mcs4-13* and *cdr1-76* (P. YOUNG, L. MOLZ and D. BEACH, unpublished observations) suggests that the *cdr1* gene product has a role in a pathway which also requires the activity of *mcs4*. The demonstrated interaction between the *mcs* genes and mitotic control genes *cdc2*, *cdc25* and *cdc13* strengthens the hypothesis that this nutritional sensing pathway converges directly on the mitotic initiation control pathway.

The *cdr1* gene product appears to have a direct interaction with elements of the mitotic size control. It is likely that the interaction is subject to regulation by nutritional cues. As a protein kinase, it is a good candidate for an early element in the signal transduction cascade connecting extracellular sensing to cell division control. The fact that it affects response to several different nutrients suggests a central role (YOUNG and FANTES 1987). The carboxyl-terminal domain of the protein is necessary to enable the triggering of the cell cycle response to starvation. As such, it could be a target for phosphorylation by other kinases or the binding site for a regulatory subunit. Because the truncated protein is not capable of sustaining this response, the interaction must be of a positive nature. It is clear, however, that it is not the only such element since *cdr1* mutants are still capable of modulating cell size at the G_2 control point, though not as effectively as a wild-type cell (YOUNG and FANTES 1987).

In the original *cdr* mutant screen two major complementation groups were found. Both *cdr1* and *cdr2* have elongated cell phenotypes and this is not additive in the double mutant suggesting that they operate on the same pathway. It is conceivable that the *cdr2* gene product plays a role in the activation or regulation of *cdr1*. This possibility is currently being examined.

We thank J. HUDSON, K. GOULD and S. SAZAR for useful discussion of this work and C. WEAVER for help with the restriction mapping. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to P.G.Y. (A9335) and an MRC (UK) and ICRF grant to P.N. H.F. was supported by a predoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada and by the Ontario Graduate Scholarship Program.

LITERATURE CITED

BEACH, D., and P. NURSE, 1981 High frequency transformation of the fission yeast *Schizosaccharomyces pombe*. Nature **290:** 140–142.

BENNETT, M., and M. KENNEDY, 1987 Deduced primary structure of the subunit of brain type II Ca^{2+}/calmodulin-dependent protein kinase determined by molecular cloning. Proc. Natl. Acad. Sci. USA **84:** 1794–1798.

BOOHER, R., and D. BEACH, 1988 Involvement of *cdc13+* in mitotic control in *Schizosaccharomyces pombe*: possible interaction of the gene product with microtubules. EMBO J. **7:** 2321–2327.

CARLSON, M., B. OSMOND and D. BOTSTEIN, 1981 Mutants of yeast defective in sucrose utilization. Genetics **98:** 25–40.

CARSLON, M., B. OSMOND, L. NEIGEBORN and D. BOTSTEIN, 1984 A suppressor of *SNF1* mutations causes constitutive high-level invertase synthesis in yeast. Genetics **107:** 19–32.

CELENZA, J., and M. CARLSON, 1986 A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science **233:** 1175–1180.

DRAETTA, G., and D. BEACH, 1988 Activation of *cdc2* protein kinase during mitosis in human cells: cell cycle dependent phosphorylation and subunit rearrangement. Cell **54:** 17–26.

DRAETTA, G., L. BRIZUELA, J. POTASHKIN and D. BEACH, 1987 Identification of p34 and p13, human homologs of the cell cycle regulators of fission yeast encoded by *cdc2+* and *suc1+*. Cell **50:** 319–325.

FANTES, P., 1979 Epistatic gene interactions in the control of division in fission yeast. Nature **279:** 428–430.

FANTES, P., 1989 Cell cycle controls, pp. 128–204 in *Molecular Biology of the Fission Yeast* edited by A. NASIM, P. YOUNG and B. JOHNSON. Academic Press, New York.

FANTES, P., and P. NURSE, 1977 Control of the timing of cell division in fission yeast. Exp. Cell Res. **115:** 317–329.

FREMISCO, J., D. GLASS and E. KREBS, 1980 Optimal spatial requirements for the location of basic residues in peptide substrates for the cyclic AMP-dependent protein kianse. J. Biol. Chem. **255:** 4240–4245.

GARNIER, J., D. OSGUTHORPE and B. ROBSON, 1978 Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120:** 97–120.

GLASS, D., M. EL-MAGHRABI and S. PILKIS, 1986 Synthetic peptides corresponding to the site phosphorylated in 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase as substrates of cyclic nucleotide-dependent protein kinase. J. Biol. Chem. **261:** 2987–2993.

GLASS, D., and S. SMITH, 1983 Phosphorylation by cyclic GMP-dependent protein kinase of a synthetic peptide corresponding to the autophosphorylation site in the enzyme. J. Biol. Chem. **258:** 14797–14803.

GOEBL, M., and B. BYERS, 1988 Cyclin in fission yeast. Cell **54:** 739–740.

GUTZ, H., H. HESLOT, U. LEUPOLD and N. LOPRIENO, 1974 *Schizosaccharomyces pombe*, pp. 395–446 in *Handbook of Genetics, Vol I: Bacteria, Bacteriophage and Fungi*, edited by R. C. KING. Plenum Press, New York.

HAGAN, I., J. HAYLES and P. NURSE, 1988 Cloning and sequencing of the cyclin-related *cdc13*$^{+}$ gene and a cytological study of its role in fission yeast mitosis. J. Cell Sci. **91:** 587–595.

HANKS, S., A. QUINN and T. HUNTER, 1988 The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science **241:** 42–52.

HUDSON, J., H. FEILOTTER and P. G. YOUNG, 1990 *stf1*: non-wee mutations epistatic to *cdc25* in the fission yeast *Schizosaccharomyces pombe*. Genetics **126:** 309–315.

LABBE, J., M. LEE, P. NURSE, A. PICARD and M. DOREE, 1988 Activation at M-phase of a protein kinase encoded by a starfish homolog of the cell cycle control gene *cdc2*. Nature **335:** 251–254.

LEE, M., and P. NURSE, 1987 Complementation used to clone a human homologue of the fission yeast cycle control gene *cdc2*. Nature **327:** 31–35.

LEE, M., and P. NURSE, 1988 Cell cycle control genes in fission yeast and mammalian cells. Trends Genet. **4:** 287–290.

LEVIN, D., C. HAMMOND, R. RALSTON and J. BISHOP, 1987 Two yeast genes that encode unusual protein kinases. Proc. Natl. Acad. Sci. USA **84:** 6035–6039.

LIN, C., M. KAPILOFF, S. DURGESIAN, K. TATEMOTO, A. RUSSO, P. HANSON, M. SCHULMAN and M. ROSENFELD, 1987 Molecular cloning of a brain-specific calcium/calmodulin-dependent protein kinase. Proc. Nat. Acad. Sci. USA **84:** 5962–5966.

MARSHALL, R., 1972 Glycoproteins. Annu. Rev. Biochem. **41:** 673–702.

MITCHISON, M., 1970 Physiological and cytological mutants of *Schizosaccharomyces pombe*. Methods Cell Physiol. **4:** 131–165.

MOLZ, L., R. BOOHER, P. YOUNG and D. BEACH, 1989 *cdc2* and the regulation of mitosis: six interacting *mcs* genes. Genetics **122:** 773–782.

NASMYTH, K., and P. NURSE, 1981 Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **182:** 119–124.

NURSE, P., 1975 Genetic control of cell size at cell division in yeast. Nature **256:** 574–551.

NURSE, P., and P. THURIAUX, 1977 Controls over the timing of DNA replication during the cell cycle of fission yeast. Exp. Cell Res. **107:** 365–375.

NURSE, P., and P. THURIAUX, 1980 Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. Genetics **96:** 627–637.

NURSE, P., P. THURIAUX and K. NASMYTH, 1976 Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **146:** 167–178.

OGDEN, J., and P. FANTES, 1986 Isolation of a novel type of mutation in the mitotic control of *Schizosaccharomyces pombe* whose phenotypic expression is dependent on the genetic background and nutritional environment. Curr. Genet. **10:** 509–514.

PEARSON, W., and D. LIPMAN, 1988 Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:** 2444–2448.

PICKETT-GIES, C., and D. WALSH, 1986 pp. 395–459 in *The Enzymes*, Vol. XVII, edited by P. BOYER and E. KREBS. Academic Press, London.

PROUDFOOT, N., and G. BROWNLEE, 1976 3′ Non-coding region sequences in eukaryotic messenger RNA. Nature **263:** 211–214.

RAO, M., and P. ARGUS, 1986 A conformational preference parameter to predict helices in integral membrane proteins. Biochim. Biophys. Acta **869:** 197–214.

REIMANN, E., K. TITANI, L. ERICSSON, R. WAKE, E. FISCHER and K. WALSH, 1984 Homology of the subunit of phosphorylase *b* kinase with cAMP-dependent protein kinase. Biochemistry **23:** 4185–4192.

RUSSELL, P., and P. NURSE, 1986 *cdc25+* functions as an inducer in the mitotic control of fission yeast. Cell **45:** 145–153.

RUSSELL, P., and P. NURSE, 1987a Negative regulation of mitosis by *wee1+*, a gene encoding a protein kinase homolog. Cell **49:** 559–567.

RUSSELL, P., and P. NURSE, 1987b The mitotic inducer *nim1+* functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. Cell **49:** 569–576.

SANGER, F., S. NICKLEN and A. COULSON, 1977 DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5469.

SOLOMON, M., R. BOOHER, M. KIRSCHNER and D. BEACH, 1988 Cyclin in fission yeast. Cell **54:** 738–740.

THURIAUX, P., P. NURSE and B. CARTER, 1978 Mutants altered in the control coordinating cell division with cell growth in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **161:** 215–220.

WRIGHT, A., K. MAUNDRELL, W. HEYER, D. BEACH and P. NURSE, 1986 Vector for the construction of gene banks and the integration of cloned genes in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Plasmid **15:** 156–158.

YOUNG, P. G., and P. FANTES, 1984 Changed division response mutants function as allosuppressors, pp. 221–228 in *Growth, Cancer and the Cell Cycle*, edited by P. SKEHAN and S. J. FRIEDMAN. Humana Press, Clifton, N.J.

YOUNG, P. G., and P. FANTES, 1987 *Schizosaccharomyces pombe* mutants affected in their division response to starvation. J. Cell Sci.**88:** 295–304.

Communicating editor: D. BOTSTEIN