Dominant Alleles of *Saccharomyces cerevisiae CDC20* Reveal Its Role in Promoting Anaphase

Eric J. Schott and M. Andrew Hoyt

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Manuscript received August 13, 1997
Accepted for publication October 10, 1997

ABSTRACT

We identified an allele of *Saccharomyces cerevisiae CDC20* that exhibits a spindle-assembly checkpoint defect. Previous studies indicated that loss of *CDC20* function caused cell cycle arrest prior to the onset of anaphase. In contrast, *CDC20-50* caused inappropriate cell cycle progression through M phase in the absence of mitotic spindle function. This effect of *CDC20-50* was dominant over wild type and was eliminated by a second mutation causing loss of function, suggesting that it encodes an overactive form of Cdc20p. Overexpression of *CDC20* was found to cause a similar checkpoint defect, causing bypass of the pre-anaphase arrest produced by either microtubule-depolymerizing compounds or *MPS1* overexpression. *CDC20* overexpression was also able to overcome the anaphase delay caused by high levels of the anaphase inhibitor Pds1p, but not a mutant form immune to anaphase-promoting complex- (APC-)mediated proteolysis. *CDC20* overexpression was unable to promote anaphase in cells deficient in APC function. These findings suggest that Cdc20p is a limiting factor that promotes anaphase entry by antagonizing Pds1p. Cdc20p may promote the APC-dependent proteolytic degradation of Pds1p and other factors that act to inhibit cell cycle progression through mitosis.

ACCURATE chromosome segregation during eukaryotic cell division requires the precise assembly and function of the mitotic spindle. To ensure that replicated chromosomes are properly attached to a functioning spindle, the cell utilizes a surveillance-feedback mechanism, referred to as the spindle-assembly checkpoint (reviewed in Elledge 1996; Rudner and Murray 1996; Wells 1996). In the presence of spindle-assembly defects, this mechanism acts to inhibit progression into anaphase, the chromosome segregation stage of mitosis. It has been observed that the spindle-assembly checkpoint can respond to defects in the functions of microtubules, kinetochores, spindle pole structures, and spindle microtubule-based motor proteins.

Studies of the budding yeast *Saccharomyces cerevisiae* have revealed seven genes (*BUB1, 2,* and *3*, *MAD1, 2,* and *3*, and *MPS1*) whose functions are required to properly arrest cell cycle progression following spindle damage. Homologs of Mad2p and the Bub1p protein kinase have been localized to the kinetochores of vertebrate cells (Chen *et al.* 1996; Li and Benezra 1996; Taylor and McKeon 1997). In addition, the physical associations of *S. cerevisiae* Mad1p with Mad2p (cited in Rudner and Murray 1996) and Bub1p with Bub3p (Roberts *et al.* 1994) have been demonstrated. These findings are consistent with the hypothesis that the signal indicating improperly attached chromosomes originates from kinetochores (Nicklas 1997). Although the site of action of the Mps1p protein kinase has not been determined, it is believed to act at an early step in the generation of the spindle-damage signal. Overexpression of *MPS1* is able to block cell cycle progression prior to anaphase in a manner dependent upon the functions of the six *MAD* and *BUB* genes (Hardwick *et al.* 1996).

The mechanism by which the transduced spindle-damage signal blocks entry into anaphase is currently not known. Likely targets for this regulation are identified factors that control anaphase onset. Both entry into anaphase and subsequent exit from mitosis require the actions of the anaphase-promoting complex (APC, also known as the cyclosome), a multisubunit ubiquitin ligase (King *et al.* 1996). Ubiquitination mediated by the APC targets protein substrates to the degradative actions of the 26S proteasome. *S. cerevisiae* Pds1p, a target of APC-mediated degradation, acts as an anaphase inhibitor (Cohen-Fix *et al.* 1996; Yamamoto *et al.* 1996a,b). Cells deficient for Pds1p enter anaphase precociously or under conditions in which anaphase onset should be inhibited. Pds1p is normally degraded in anaphase in an APC-dependent manner, and nondegradable mutant forms inhibit anaphase onset. Therefore, it seems likely that a key regulatory event controlling anaphase initiation in *S. cerevisiae* is the degradation of Pds1p by the APC.

Entry into anaphase is coupled to proper assembly of the mitotic spindle. Kinesin-related Cin8p is the ma-

Corresponding author: M. Andrew Hoyt, Department of Biology, Mudd Hall, The Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218. E-mail: hoyt@jhu.edu

jor mitotic motor protein responsible for spindle assembly and elongation in *S. cerevisiae*, but is not essential for viability because of the overlapping activities of other motors (Roof *et al.* 1992; Saunders and Hoyt 1992; Saunders *et al.* 1995). In a recent study, we identified a large number of mitosis-specific genes whose functions become essential in the absence of Cin8p (Geiser *et al.* 1997). A subset of these included the spindle-assembly checkpoint genes *BUB1, 2,* and *3, MAD1* and *2,* and *MPS1*. In addition, *mad3*Δ caused a deleterious but nonlethal effect in *cin8* cells. A reasonable explanation for this finding is that Cin8p-deficient cells require the spindle-assembly checkpoint to delay anaphase entry because their spindles are assembling inefficiently.

In addition to mutants deficient in previously characterized spindle-assembly checkpoint genes, a novel checkpoint mutant, *PAC5-1*, was identified in the screen for mutants that perish in the absence of *CIN8* (Geiser *et al.* 1997). A property unique to this mutant allele is dominance for its lethal-with-*cin8*Δ phenotype, suggesting that it does not simply cause loss of function. In studies reported here, we demonstrate that *PAC5-1* is a mutant allele of *CDC20*, a gene required for anaphase entry (Sethi *et al.* 1991; O'Toole *et al.* 1997). (*PAC5-1* is redesignated here as *CDC20-50*.) We demonstrate that *CDC20-50* is dominant for the spindle-assembly checkpoint defect as well and that this effect could be mimicked by overexpression of *CDC20.* Overexpression of *CDC20* could bypass the cell cycle arrest caused by microtubule-depolymerizing compounds or *MPS1* overexpression. In addition, *CDC20* overexpression was found to bypass the arrest caused by elevated levels of the anaphase inhibitor Pds1p, but not the arrest caused by a Pds1p mutant immune to APC-mediated destruction or the arrest caused by an APC mutant defect. These findings reveal a role for Cdc20p in promoting anaphase as an antagonist of Pds1p and are consistent with the recent observation that APC-mediated Pds1p degradation requires Cdc20p function (Visintin *et al.* 1997).

MATERIALS AND METHODS

Yeast strains and media: The *S. cerevisiae* strains used in these experiments are listed in Table 1. Strains MAY2830 and MAY2831 are *cin8*Δ cyh2 and carry a plasmid (pMA1208) with *CIN8* and *CYH2* (Geiser *et al.* 1997). The loss of pMA1208 al-

TABLE 1

Yeast strains and plasmids

Strains and plasmids	Genotypes
MAY1715	*MAT***a** *his3 leu2 ura3 ade2 can1 cdc20-1*
MAY2096	*MAT*α *his3 leu2 ura3 ade2 cdc16-1*
MAY2422	*MAT***a** *his3 leu2 ura3 lys2 cyh2*
MAY2777	*MAT*α *his3 leu2 ura3 ade2 trp1 CDC20-50 cyh2 cin8::HIS3* (pMA1208)
MAY2830	*MAT***a** *his3 leu2 ura3 ade2 trp1 cyh2 cin8::HIS3* (pMA1208)
MAY2831	*MAT*α *his3 leu2 ura3 lys2 trp1 cyh2 cin8::HIS3* (pMA1208)
MAY3150	*MAT***a** *his3 leu2 ura3 ade2 trp1 bub2-71 cyh2 cin8::HIS3* (pMA1208)
MAY4366	*MAT***a** *his3 leu2 ura3 ade2 lys2 mad1::HIS3 cyh2 cin8::LEU2* (pMA1125)
MAY4403	*MAT*α *his3 leu2 ura3 lys2 cdc20-50 cyh2 cin8::LEU2* (pMA1125)
MAY4438	*MAT***a** *his3 leu2 ura3 ade2 trp1 can1 ura3:*$P_{GAL} \rightarrow$ *MPS1-myc*
MAY5197	*MAT***a** *his3 ura3 lys2 cyh2 LEU2:*$P_{GAL} \rightarrow$ *PDS1*
MAY5198	*MAT***a** *his3 ura3 lys2 cyh2 LEU2:*$P_{GAL} \rightarrow$ *PDS1-mdb*
MAY5331	*MAT***a** *his3 leu2 ura3 ade2 trp1 CDC20-50 cyh2 cin8::HIS3* (pMA1208)
p415-MET25	P_{MET} *LEU2 CEN*
p416-MET25	P_{MET} *URA3 CEN*
pES25	*CDC20-50 URA3 CEN*
pES26	*CDC20 URA3 CEN*
pES33	$P_{MET} \rightarrow$ *CDC20-50 URA3 CEN*
pES34	$P_{MET} \rightarrow$ *CDC20 URA3 CEN*
pES36	$P_{MET} \rightarrow$ *CDC20 LEU2 CEN*
pES37	*cdc20-1 URA3 CEN*
pES39	$P_{MET} \rightarrow$ *cdc20-1, 50 URA3 CEN*
pES40	$P_{MET} \rightarrow$ *cdc20-1 URA3 CEN*
pMA1125	*CIN8 URA3 CEN*
pMA1208	*CIN8 CYH2 LEU2 CEN*
pOC69	*LEU2:*$P_{GAL} \rightarrow$ *PDS1* integrating vector
pOC70	*LEU2:*$P_{GAL} \rightarrow$ *pds1-mdb* integrating vector
pRS316	*URA3 CEN*

lows cells to grow on media containing cycloheximide because of loss of the dominant *CYH2* allele that causes sensitivity. The *CDC20-50* allele, previously known as *PAC5-1*, was identified because it prevents the segregation of cycloheximide-resistant cells in the MAY2830/2831 background (Geiser *et al.* 1997). MAY4438 (a gift from M. Winey) carries a chromosomally integrated, galactose-inducible, and functional *MPS1* tagged with *myc* (Hardwick *et al.* 1996). Strains expressing *PDS1* or the destruction box mutant form *pds1-mdb* (Cohen-Fix *et al.* 1996) were created by transforming MAY2422 with pOC69 or pOC70 cut with *Eco*RI to direct integration into the *LEU2* locus. Yeast transformations were carried out using the lithium acetate procedure (Gietz *et al.* 1992).

Rich (YPD), minimal (SD), and sporulation media were as described (Sherman *et al.* 1983). Where noted, methionine was added to 3 mm or omitted to induce expression from the *MET25* promoter. For induction of the galactose promoter, cells were grown to log phase in minimal media containing 2% raffinose. Galactose was then added to 2%. To arrest cells in G_1, α-factor (Bachem, Torrence, CA) was added to 6 μg/ml to log phase cells growing in rich media at pH 4.0. To arrest cells in S phase, hydroxyurea (Sigma, St. Louis) was added to 100 mm to log phase cells growing in minimal media at pH 5.8. The benzimidazole microtubule inhibitors benomyl (DuPont, Wilmington, DE) and noco-dazole (Sigma) were added to media at 70 μg/ml and 12 μg/ml. Cycloheximide was used at 5 μg/ml.

For the analysis of nuclear morphology by microscopy, cells were fixed in 70% ethanol and stained with 4,6-diamidino-2-phenylindole (DAPI) at 0.3 μg/ml.

Linkage analysis: The diploid strain created by mating MAY4366 and MAY4403 is heterozygous for *CDC20-50* (*PAC5-1*) as well as the chromosome *VII* loci *MAD1* and *CYH2*. Tetrad analysis of this diploid revealed the linkage of all three loci, with *PAC5-1* approximately 8.5 cM from *CYH2* (39 parental ditypes, eight tetratypes, and no nonparental ditypes). Less linkage was noted for *PAC5-1* and *MAD1* (32 parental ditypes, 15 tetratypes, and no nonparental ditypes). This indicated that *PAC5-1* mapped 8.5 cM from *CYH2*, distal to *MAD1*, a position very close to *CDC20*.

DNA manipulations: Standard DNA manipulations techniques were utilized (Sambrook *et al.* 1989). Polymerase chain reaction (PCR) was performed with Vent polymerase according to the manufacturer's directions (New England Biolabs, Beverly, MA). *CDC20* was amplified from strains MAY2830, ESY52, and MAY1715, using primers beginning at −181 and +30 relative to the *CDC20* open reading frame in the Saccharomyces Genome Database. The 5′ primer was 5′-gctctagaCAGACTAAACCAGAGATC-3′ and the 3′ primer was 5′-cagccggcCATTATATGCCTTGACATG-3′ (lower case letters indicate nonyeast sequence). PCR-generated products were cloned into the *Sma*I site of pRS316 (Sikorski and Hieter 1989) to create pES26, pES25, and pES37, which carry *CDC20*, *CDC20-50*, and *cdc20-1*, respectively. All clones had the *CDC20* open reading frame in the opposite orientation relative to the nucleotide numbering system of Sikorski and Hieter (1989). To put *CDC20* alleles downstream from the *MET25* promoter, *Hin*dIII fragments from the pRS316 *CDC20* plasmids (3′ *Hin*dIII site from the pRS316 polylinker) were inserted into the *Hin*dIII site of p415-MET25 or p416-MET (Mumberg *et al.* 1994). pES40 (carrying *cdc20-1*) was made by replacing the *Msc*I-*Bcl*I fragment of pES34 with the corresponding fragment from pES37. pES39 (carrying *cdc20-1, 50*) was made by replacing the *Msc*I-*Bcl*I fragment of pES33 with the corresponding fragment from pES37. All cloning junctions and mutant alleles were verified by sequencing.

Sequencing was conducted by the dideoxy chain termination method using the Sequenase kit (United States Biochemical, Cleveland) and analyzed on 6% polyacrylamide gels or by automated analysis by the Johns Hopkins Genetics Core Facility. Sequencing primers for *CDC20* were designed based on the sequence found in the Saccharomyces Genome Data Base. *CDC20* homologs were found using the BLAST computer program (Altschul *et al.* 1990). Sequence alignment for the *CDC20* homologs was accomplished using the CLUSTAL W program (Thompson *et al.* 1994).

Checkpoint assays: For assessment of budding during microtubule disruption, cells were released from α-factor arrest onto solid rich media containing 70 μg/ml benomyl. After 6 hr, cells were examined by light microscopy and the number of cell bodies per microcolony was determined for 200 microcolonies.

For assessment of DNA content during microtubule disruption, log phase cells in liquid media were treated with 12 μg/ml nocodazole. At 0 and 4 hr, samples were fixed in ethanol and stained with propidium iodide (Hutter and Eipel 1978). The DNA content of 10,000 cells was analyzed for each sample using an EPICS 753 flow cytometer.

Sensitivity to DNA damage was assessed by spotting a dilution series of cells suspended in water onto rich agar and then by exposing the plates to increasing doses of UV light (0–300 J/m^2) using a Stratalinker (Stratagene, La Jolla, CA). Exposed plates were kept in the dark and incubated for 2 days at 26° and then evaluated for growth. The same dilution series of cells was also spotted onto rich media agar containing 0.01–0.16% methyl methanesulfonate (MMS; from Sigma) and incubated for 3 days prior to evaluation of growth. For cells expressing *CDC20* or *CDC20-50* from the *MET25* promoter, log phase cultures were transferred into minimal media lacking methionine for 1.5 hr prior to the DNA-damaging treatment described above.

RESULTS

***CDC20-50* confers a dominant checkpoint defect:** Cells deleted for *CIN8* grow at wild-type rates at 26°, but undergo prolonged M phase compared to wild-type cells, presumably because of reduced spindle function (Hoyt *et al.* 1992; Saunders *et al.* 1995). The *CDC20-50* mutation (previously referred to as *PAC5-1*) was identified in a screen for mutants that make *CIN8* essential at 26° (Geiser *et al.* 1997). A *cin8*Δ *cyh2* strain, carrying *CIN8* on a plasmid with the counter-selectable marker *CYH2*, grew well on cycloheximide plates, indicating that it was able to survive loss of the *CYH2-CIN8* plasmid (Figure 1A). An isogenic strain that also carries the *CDC20-50* mutation was unable to segregate cycloheximide-resistant cells, indicating that they were inviable without the *CIN8* plasmid and that the combination of *cin8*Δ with *CDC20-50* is lethal. In *CIN8* cells (*i.e.*, in cells with a *CIN8* plasmid), *CDC20-50* did not cause a defect in growth rate. A similar behavior was exhibited by the *bub2-71* spindle-assembly checkpoint mutant identified in the same screen as *CDC20-50* (Geiser *et al.* 1997). A *cin8*Δ/*cin8*Δ *cyh2*/*cyh2* diploid, carrying the *CIN8-CYH2* plasmid, was able to grow on cycloheximide. Isogenic strains that were either homozygous or heterozygous for *CDC20-50* were unable to segregate cycloheximide-resistant cells, indicating that they were inviable without the *CIN8* plasmid. The inviability of *CDC20*/*CDC20-*

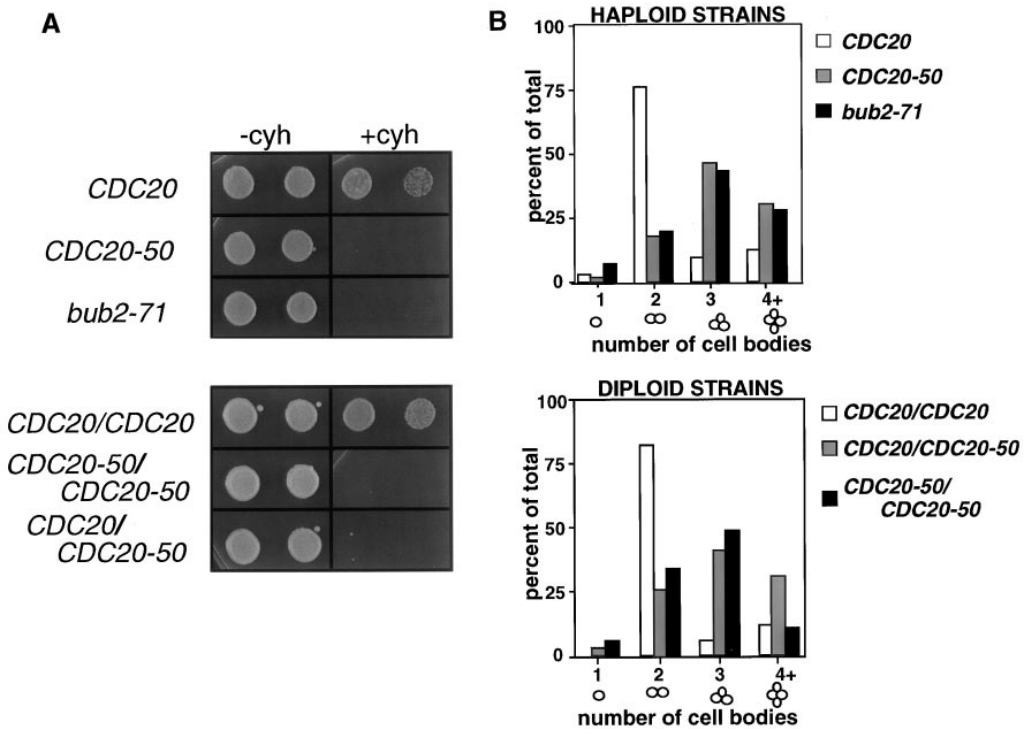

Figure 1.—Dominant phenotypes of *CDC20-50*. (A) Lethality of the *CDC20-50 cin8*Δ combination. Haploid (top) and diploid (bottom) strains with the genotype indicated on the left were spotted onto rich media with or without 5 μg/ml cycloheximide (cyh) and incubated 2 days at 26°. These strains are also *cin8*Δ *cyh2* (diploids are homozygous) and carry pMA1208 (*CIN8 CYH2*). The inability to grow on cycloheximide media indicates that the cells cannot survive loss of the *CIN8* plasmid. The spot on the right for each sample is a tenfold dilution of the spot on the left. (B) *CDC20-50* cells produce extra buds on benomyl-containing media. Haploid (top) and diploid (bottom) cells of the indicated genotype were released from α-factor synchronization onto rich solid media containing 70 μg/ml benomyl. After 6 hr, the numbers of cell bodies per microcolony was determined by microscopy; 4+ indicates four or more cell bodies per microcolony.

50 heterozygotes on cycloheximide media indicated that the lethality conferred by *CDC20-50* in the absence of *CIN8* is a dominant phenotype. The dominance of *CDC20-50* was unique among the *pac* (perish in the absence of *CIN8*) mutants previously identified (Geiser *et al.* 1997).

Exposing cells to microtubule inhibitors such as benomyl disrupts their mitotic spindles and causes them to arrest in the cell cycle with a preanaphase morphology (large-budded, mononucleate, and with a G_2 DNA content). A hallmark phenotype of spindle-assembly checkpoint mutants is continued bud emergence in the presence of microtubule inhibitors. Although we had preliminary evidence that *CDC20-50* confers a spindle-assembly checkpoint defect, it was of interest to determine whether this effect was also dominant. Spindle-assembly checkpoint function was assessed in haploid and diploid *CDC20-50* mutants by plating cells onto solid media containing a high concentration of benomyl (Figure 1B). Prior to plating, the cells were synchronized in G_1 by the α-factor mating pheromone; transfer to the benomyl-containing media released the cells from the α-factor block. After 6 hr, the plates were examined microscopically and the bud morphology of cells was determined. A majority of cells of wild-type haploid and diploid strains arrested with the characteristic large-budded morphology (≥80%). Haploid *CDC20-50* cells bypassed the large-budded arrest; over 50% of cells formed one or more extra buds. The extent to which the *CDC20-50* strain bypassed the arrest was similar to the *bub2-71* spindle-assembly checkpoint mutant strain. In addition, both *CDC20-50*/*CDC20-50* and *CDC20*/*CDC20-50* diploid strains formed extra buds in the presence of high concentrations of benomyl, indicating that *CDC20-50* is dominant for the multibudding phenotype as well. The extent to which *CDC20-50* diploids bypassed the large-budded arrest was similar to that seen with *CDC20-50* haploids. Note that the checkpoint defect is not dependent on the *cin8*Δ mutation.

Another characteristic of spindle-assembly checkpoint mutants is aberrant initiation of DNA synthesis in the presence of spindle damage, resulting in a greater than G_2 DNA content. To examine this, we added the microtubule inhibitor nocodazole to liquid exponential cultures of *CDC20-50*, *bub2-71*, and wild-type cells. At 0 and 4 hr after nocodazole addition, samples were removed and processed for flow cytometric analysis of DNA content (Figure 2). The results indicated that, like *bub2-71*, many *CDC20-50* mutant cells performed additional DNA replication steps in the presence of nocodazole-induced microtubule damage, while wild-type cells arrested with a G_2 DNA content.

Cloning of the *CDC20-50* allele: Initial evidence that *PAC5-1* is an allele of *CDC20* was obtained from crosses to test its linkage to known spindle-assembly checkpoint genes. Analysis of 47 tetrads from a diploid in which *MAD1*, *CYH2*, and *PAC5-1* (all on chromosome *VII*) were heterozygous indicated that *PAC5-1* was 8.5 cM centromere distal to *CYH2*, a position corresponding

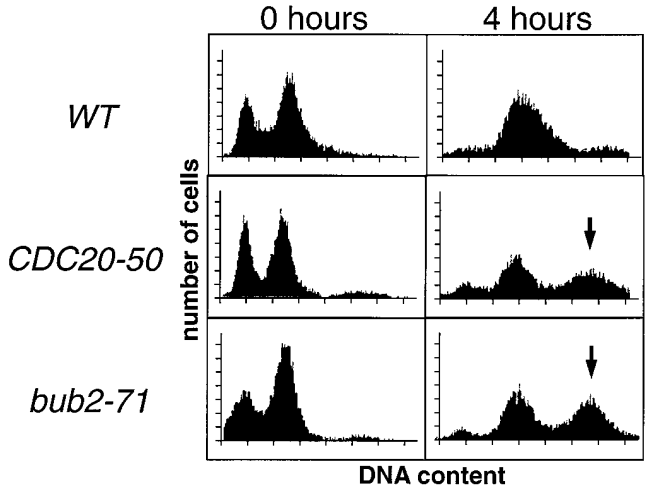

Figure 2.—Flow cytometric DNA content analysis. Nocodazole was added (12 μg/ml) to exponentially growing cultures of the indicated genotypes. At 0 and 4 hr, samples were prepared for propidium iodide DNA staining and analysis by flow cytometry. The arrows point to cells with a greater than G_2 DNA content.

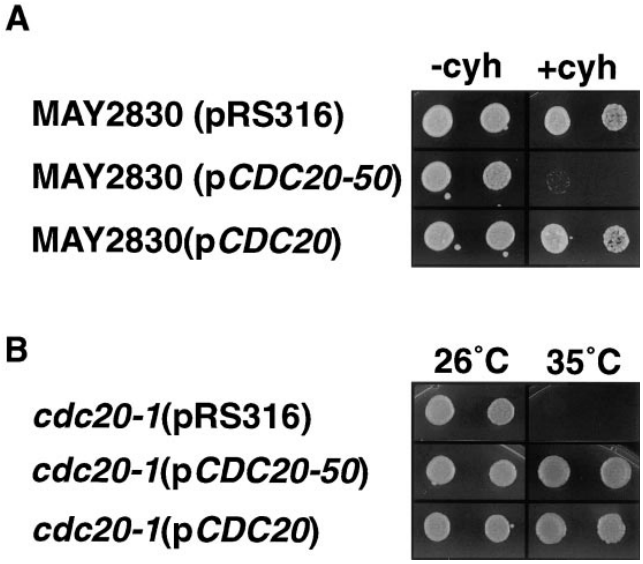

Figure 3.—Phenotypic analysis of cloned *CDC20* alleles from wild-type and *PAC5-1* strains. (A) Dominant lethality caused by the *CDC20* allele derived from the *PAC5-1* strain combined with *cin8*Δ. Strain MAY2830 [*cin8*Δ *cyh2* pMA1208 (*CIN8 CYH2*)] was transformed with a vector control (pRS316) or from a PCR fragment clone derived from a *CDC20* strain (pCDC20) or a *PAC5-1* strain (pPAC5). Transformants were spotted onto rich media with or without 5 μg/ml cycloheximide (cyh) and incubated 2 days at 26°. (B) Clones derived from *CDC20* and *PAC5-1* strains complement the temperature-sensitive *cdc20-1* allele. The three plasmids described in (A) were transformed into the *cdc20-1* strain MAY1715. Transformants were spotted onto rich media and incubated for 2 days at 26° or 35°.

to that of the *CDC20* gene (see materials and methods).

To establish that *PAC5-1* is an allele of *CDC20*, we PCR amplified and cloned the *CDC20* gene from both wild-type and *PAC5-1* strains (see materials and methods). A 2043 basepair fragment was amplified that included the *CDC20* open reading frame plus 161 base pairs 5′ and 49 base pairs 3′. Because the *CDC20-50* mutation is dominant, we reasoned that a centromere plasmid carrying *CDC20-50* should also confer lethality to *cin8*Δ cells. When a *CDC20* clone derived from the *PAC5-1* strain (labeled p*CDC20-50* in Figure 3) was transformed into a strain that carried *CIN8* on a *CYH2* plasmid, it caused a cycloheximide-sensitive phenotype similar to that caused by the original *PAC5-1* mutation (Figure 3A). Neither vector alone nor a PCR clone of *CDC20* derived from a wild-type strain caused a cycloheximide-sensitive phenotype. Therefore, *PAC5-1* (*CDC20-50*) is a mutant allele of *CDC20* (also see next section).

As a test of *CDC20* function, the PCR-derived clones of both *CDC20* and *CDC20-50* were assayed for their ability to complement the recessive temperature sensitivity of the *cdc20-1* loss-of-function mutant (Figure 3B). As expected, *CDC20* complemented the growth of the *cdc20-1* mutant at nonpermissive temperatures. The *CDC20-50* clone was equal to the *CDC20* clone in its ability to complement *cdc20-1*, even at temperatures as high as 37°. This was not unexpected, since strains carrying a genomic *CDC20-50* mutation were able to grow at 37° as well (data not shown).

***CDC20-50* carries a mutation in a residue conserved among *CDC20* homologs:** To determine the nature of the *CDC20-50* mutation, the inserts of plasmids carrying *CDC20* and *CDC20-50* were sequenced, and the sequences were compared to that of *CDC20* (YGL116W) in the Saccharomyces Genome Data Base. The 2043 base pair insert of the plasmid carrying *CDC20* was identical to the Saccharomyces Genome Data Base sequence for the *CDC20* open reading frame and surrounding sequence. The insert of the plasmid carrying *CDC20-50* differed from the *CDC20* plasmid by a single nucleotide substitution within the *CDC20* open reading frame. In *CDC20-50*, a G → A transition at base pair 1506 changes a glycine codon (GGA) to an arginine codon (AGA), corresponding to amino acid 446 of Cdc20p.

In its carboxyl-terminal end, Cdc20p contains seven copies of a motif known as the WD repeat (Sethi *et al.* 1991). Originally identified as a repeated motif in the β-subunit of trimeric guanine nucleotide-binding proteins, the approximately 40 amino acid WD motif is also found in many other proteins from a wide variety of organisms (Neer *et al.* 1994). In Cdc20p, Gly_{446} falls within the fifth WD repeat. When Cdc20p is aligned with homologs from other organisms (including human), it is seen that Gly_{446} corresponds to a residue conserved in all identified relatives (Figure 4). Most of the other WD repeats of Cdc20p and its homologs have

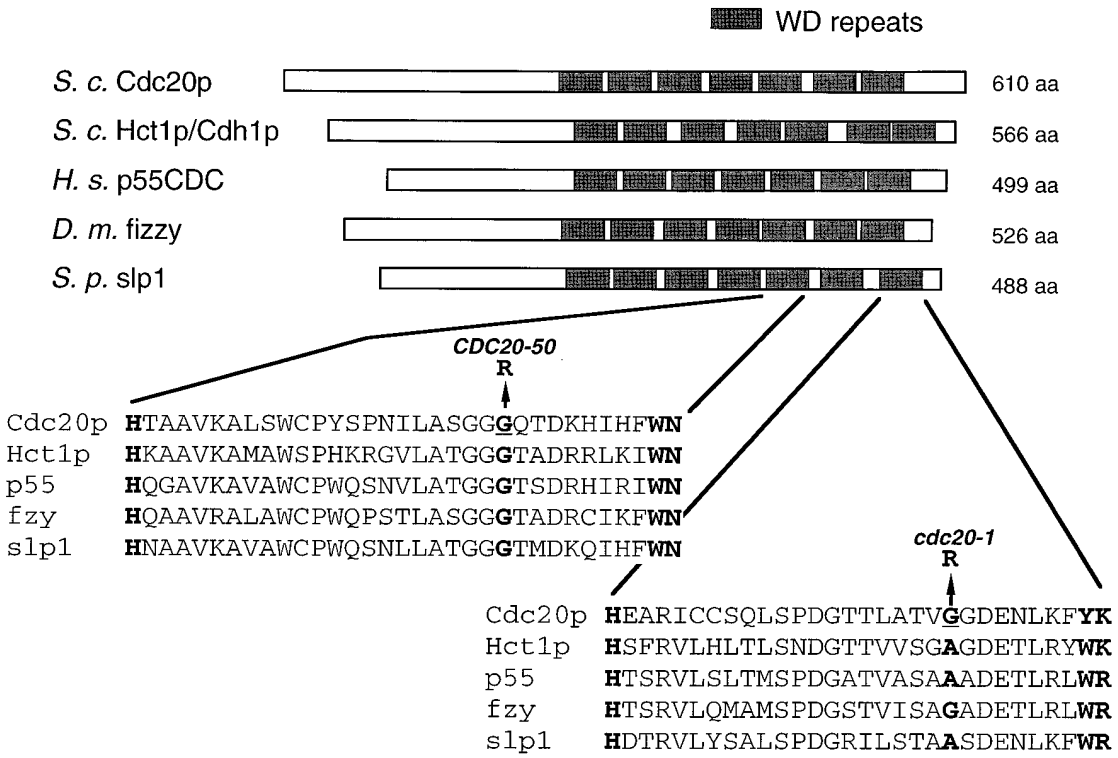

Figure 4.—*CDC20* mutant allele sequence changes. (Top) Linear representation of the positions of the WD repeats in *S. cerevisiae* Cdc20p and homologs from *S. cerevisiae* (Hct1p/Cdh1p), human (p55CDC), Drosophila (fizzy) and *Schizosaccharomyces pombe* (slp1). (Bottom) Amino acid sequence line-ups of the fifth and seventh WD repeats of Cdc20p and homologs showing the sequence changes caused by the *CDC20-50* and *cd20-1* mutations.

a glycine or a small uncharged amino acid at the corresponding position.

The dominant *CDC20-50* effect is eliminated by a loss-of-function mutation: The finding that *CDC20-50* was dominant and had full *CDC20* function suggested that *CDC20* activity was necessary for the checkpoint defect of *CDC20-50*. We investigated whether a gene that carries both *CDC20-50* (dominant gain-of-function) and *cdc20-1* (recessive temperature-sensitive loss-of-function) mutations would display the *CDC20-50* checkpoint defect.

To construct the desired double mutant, it was first necessary to determine the sequence change of the *cdc20-1* mutation (see materials and methods). The sequence of *cdc20-1* differed from the wild type by one nucleotide: a single G → A transition at position 1810, which changes codon 544 from Gly to Arg, within the seventh WD repeat motif (Figure 4). Note that this Gly to Arg change is in a position within the seventh WD repeat adjacent to the position of the *CDC20-50* Gly to Arg change within the fifth WD repeat.

We combined the *cdc20-1* mutation with the *CDC20-50* allele by replacement of a restriction fragment carrying the 1810 G → A mutation of *cdc20-1* for the same region of a *CDC20-50* clone. The double change mutant allele is referred to as *cdc20-1, 50*. This allele was placed downstream of the *MET25* promoter to create P_{MET} → *cdc20-1, 50*. For comparison, P_{MET} → *CDC20-50* and P_{MET} → *cdc20-1* were similarly constructed. The *MET25* promoter is induced approximately ninefold by growth in the absence of methionine and retains some basal activity in the presence of methionine (Mumberg *et al.* 1994). In addition, all constructs possess 76 base pairs 5′ from the translation initiation codon, an amount sufficient to allow *CDC20* complementation of the *cdc20-1* mutant (Sethi *et al.* 1991). Therefore, although these constructs are inducible, they are not strongly repressed in noninducing conditions (in the presence of methionine).

The P_{MET} → *CDC20* constructs were tested for their ability to confer *CDC20*-complementing activity to a *cdc20-1* strain and for their ability to dominantly kill *cin8*Δ cells (Figure 5). When transformed into a temperature-sensitive *cdc20-1* mutant, only the P_{MET} → *CDC20-50* construct was able to efficiently relieve the growth defect at 33° (Figure 5A). Partial complementation at 33° was provided by P_{MET} → *cdc20-1*, but only when methionine was omitted from the media to induce high expression of *cdc20-1*. The P_{MET} → *cdc20-1, 50* construct provided no complementing activity, indicating that this double mutant provided even less *CDC20* activity than *cdc20-1*. Since we do not possess the ability to measure Cdc20p protein levels, we could not directly test whether the *cdc20-1, 50* form was stably ex-

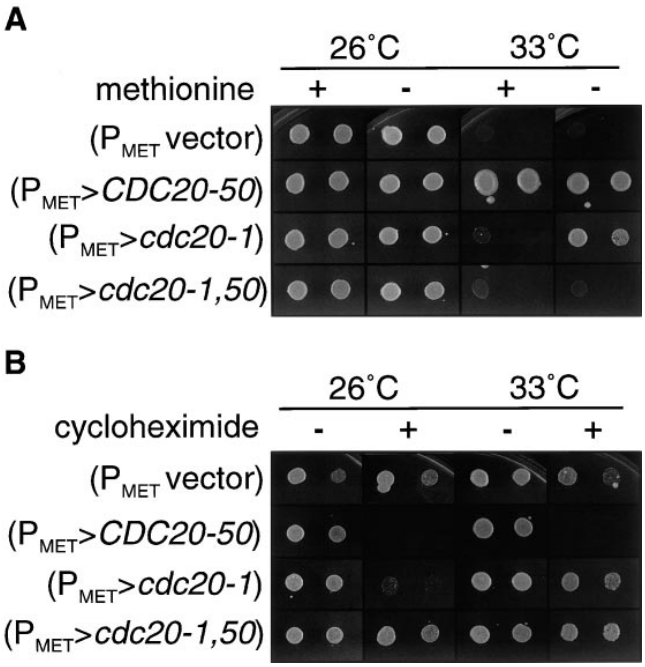

Figure 5.—The *cdc20-1* mutation eliminates the effects of the *CDC20-50* mutation. (A) Abilities of *CDC20* alleles to complement the temperature sensitivity of *cdc20-1*. The P_{MET} plasmid vector or constructs in which the indicated *CDC20* alleles were placed downstream of the promoter were transformed into the *cdc20-1* strain MAY1715. Transformants were spotted onto minimal media with or without 3 mM methionine and incubated at the indicated temperatures. (B) Abilities of *CDC20* alleles to dominantly cause lethality in the absence of *CIN8*. The same plasmids tested in (A) were transformed into MAY2830 (*cin8*Δ *cyh2* pMA1208 [*CIN8 CYH2*]). Transformants were tested for their ability to segregate cycloheximide-resistant cells by spotting onto minimal media lacking uracil (to select for the P_{MET} plasmid) and methionine (to induce expression from P_{MET}). A reduced nonpermissive temperature of 33° was used for this experiment to demonstrate the ability of overexpressed *cdc20-1* to partially complement the chromosomal *cdc20-1* allele (row 3 in [A]).

pressed. However, we did note that this allele uniquely caused a deleterious phenotype. Expression of $P_{MET} \rightarrow$ *cdc20-1, 50* induced by methionine-less media inhibited the growth of *cdc20-1* cells at 26° (data not shown). The other *CDC20* forms did not cause this effect. This indicates that the product of the *cdc20-1, 50* allele is probably stably expressed. When transformed into *cin8*Δ cells carrying a *CIN8 CYH2* plasmid, $P_{MET} \rightarrow$ *CDC20-50* prevented segregation of cycloheximide-resistant cells, but $P_{MET} \rightarrow$ *cdc20-1, 50* did not (Figure 5B). (The temperature-sensitive effects of $P_{MET} \rightarrow$ *cdc20-1* on cycloheximide resistance will be discussed below.)

In summary, the combination of both *CDC20-50* and *cdc20-1* changes in the same gene product abrogates the dominant *cin8*Δ synthetic lethality caused by the *CDC20-50* form. Therefore, the gain of function exhibited by the *CDC20-50* product requires some aspect of normal Cdc20p function.

Overexpression of *CDC20* causes a spindle-assembly checkpoint defect: The temperature-sensitive mutant *cdc20-1* is unable to enter anaphase at the restrictive temperature. The effect of the *CDC20-50* mutation is inappropriate progression through mitosis when spindles are damaged. The findings that *CDC20-50* is dominant, provides *CDC20*-complementing function, and causes a phenotype that appears to be the opposite to that of loss of function led us to examine whether overexpression of *CDC20* may cause consequences similar to that of *CDC20-50*. *CDC20*, expressed from the P_{MET} promoter induced by omitting methionine from the media, was found to cause lethality in combination with *cin8*Δ (Figure 6). This effect required full induction since the addition of methionine to the media now permitted the appearance of cycloheximide-resistant cells. $P_{MET} \rightarrow$ *CDC20-50* caused cycloheximide sensitivity independent of methionine, consistent with our conclusion that it represents an overactive form and does not require overexpression for its effect. Significantly, $P_{MET} \rightarrow$ *cdc20-1*

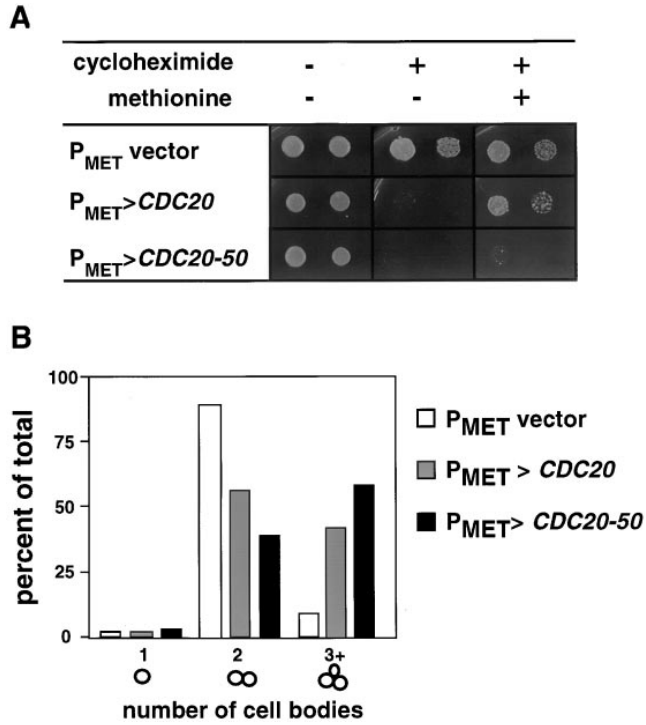

Figure 6.—Overexpression of *CDC20* causes a phenotype similar to that of *CDC20-50*. (A) Lethality of P_{MET}-expressed *CDC20* in the absence of *CIN8*. MAY2830 (*cin8*Δ *cyh2* pMA1208 [*CIN8 CYH2*]) was transformed with P_{MET} vector only or by P_{MET} driving expression of *CDC20* or *CDC20-50*. Transformants were spotted onto minimal media lacking uracil (to select for the P_{MET} plasmid) and with or without cycloheximide and methionine as indicated. (B) Extra bud formation on benomyl-containing media caused by P_{MET}-driven expression of *CDC20*. Wild-type cells transformed with the indicated plasmid genotype were released from α-factor synchronization onto solid minimal media lacking uracil (to select for the P_{MET} plasmid) and methionine (to induce expression from P_{MET}) and containing 70 μg/ml benomyl.

also caused lethality with *cin8*Δ, but only in the absence of methionine and only at 26° (Figure 5B). At 33°, this effect was eliminated, consistent with the temperature-sensitivity of the *cdc20-1* product. None of the $P_{MET} \rightarrow$ *CDC20* forms caused slow growth of wild-type cells on media lacking methionine, although slow growth caused by *CDC20* expression from a higher-level galactose-inducible promoter has been reported (Lim and Surana 1996).

Using the benomyl arrest assay, $P_{MET} \rightarrow$ *CDC20* and P_{MET}*CDC20-50* were tested for the ability to cause cells to bypass the spindle-assembly checkpoint (Figure 6B). Wild-type haploid cells carrying $P_{MET} \rightarrow$ *CDC20*, $P_{MET} \rightarrow$ *CDC20-50* or vector only were arrested in G_1 with α-factor and then released onto benomyl-containing solid media (70 μg/ml) lacking methionine. After 6 hr, the proportion of cells that had arrested with a single large bud or had produced more than one bud was determined. A high percentage of cells carrying vector only arrested with a large-budded morphology on benomyl; only 11% produced cell groupings with three or more cell bodies. In contrast, a high number of cells overexpressing *CDC20* or *CDC20-50* produced extra buds indicating bypass of the benomyl-induced cell cycle arrest; 40% of $P_{MET} \rightarrow$ *CDC20* cells produced extra buds as did 55% of $P_{MET} \rightarrow$ *CDC20-50* cells. In summary, both the perish-in-absence-of-*CIN8* phenotype and the spindle checkpoint defect of *CDC20-50* could be mimicked simply by increased expression of *CDC20*.

A recently reported study found that *CDC20* overexpressed from a high-level galactose-inducible promoter caused increased sensitivity to UV irradiation (Lim and Surana 1996). This finding suggested that the normal G_2 delay imposed by the DNA damage response checkpoint could be eliminated by high levels of Cdc20p. However, we were unable to detect any increased sensitivity to DNA damage caused by UV irradiation or MMS for cells expressing $P_{MET} \rightarrow$ *CDC20* or $P_{MET} \rightarrow$ *CDC20-50* (see materials and methods). It is possible that the difference between these findings is the result of the increased strength of the P_{GAL} promoter relative to the P_{MET} promoter (Mumberg *et al.* 1994). Cells expressing *CDC20-50* from its normal chromosomal locus and promoter were also no more sensitive to these DNA-damaging treatments than *CDC20* cells (data not shown).

Overexpression of *CDC20* bypasses the preanaphase arrest caused by overexpressed *MPS1*: Damaged spindles generate a signal that is translated into a preanaphase cell cycle arrest by the spindle-assembly checkpoint gene products. The observation that overproduced Mps1p could induce a similar preanaphase arrest, dependent upon the *BUB* and *MAD* gene products, suggested that it functions upstream in the signal transduction pathway (Hardwick *et al.* 1996). We investigated whether overexpressed *CDC20* could bypass the arrest caused by *MPS1* overexpression.

A strain carrying an *MPS1* gene under the control of a high-level, galactose-inducible P_{GAL} promoter was transformed with $P_{MET} \rightarrow$ *CDC20* or a vector plasmid for control. Cells were grown in raffinose-containing media (plus methionine, but lacking leucine to select for the plasmid) and synchronized in S phase with the DNA synthesis inhibitor hydroxyurea. For the last 30 min of hydroxyurea treatment, galactose was added to the media to induce *MPS1* expression and methionine was omitted to induce *CDC20* expression. The cells were then released from the hydroxyurea block into galactose-containing media lacking methionine. At intervals, samples were removed, fixed, and stained with DAPI and observed microscopically to determine whether nuclear division had occurred (Figure 7A). In addition, at the point of release from hydroxyurea, a sample was spread onto solid galactose media lacking methionine, allowing us to score bud emergence (Figure 7B). The cells carrying the vector plasmid were inhibited from nuclear division and new bud emergence for the course of the experiment. In contrast, the cells carrying the $P_{MET} \rightarrow$ *CDC20* plasmid efficiently entered anaphase, divided their nuclei, and created new buds. A very similar effect was exhibited by cells carrying a $P_{MET} \rightarrow$ *CDC20-50* plasmid (data not shown). Therefore, the preanaphase arrest caused by overexpression of *MPS1* can be bypassed by overexpression of *CDC20*.

Overexpression of *CDC20* overcomes the preanaphase arrest caused by overexpressed *PDS1*, but not loss of APC function: Loss of *CDC20* prevents entry into anaphase (Sethi *et al.* 1991; O'Toole *et al.* 1997), and we have demonstrated that overexpression of *CDC20* causes inappropriate entry into anaphase. Entry into anaphase normally requires the APC-mediated degradation of Pds1p (Cohen-Fix *et al.* 1996). It is possible, therefore, that Cdc20p exerts its effects through either the APC or Pds1p. To examine this possibility, we tested the ability of overexpressed *CDC20* to bypass the preanaphase arrest caused by either loss of APC function or overexpression of *PDS1*.

A *cdc16-1* strain, temperature sensitive for the function of the APC (Zachariae and Nasmyth 1996; Zachariae *et al.* 1996), and a *CDC16* strain were transformed with $P_{MET} \rightarrow$ *CDC20* or a vector plasmid for control. Cells were synchronized with hydroxyurea in methionine-containing media (minus uracil, to hold the plasmid) at 26°. Fifteen minutes prior to release from the hydroxyurea block, the cells were transferred to media lacking methionine (to induce *CDC20* expression) and the incubation temperature was raised to 37°. The cells were released from the hydroxyurea into the same media at 37°. Samples were removed at time intervals, stained with DAPI, and observed to determine whether they had performed nuclear division (Figure 8). The *CDC16* cells efficiently performed nuclear division within 1.5 hr, while the

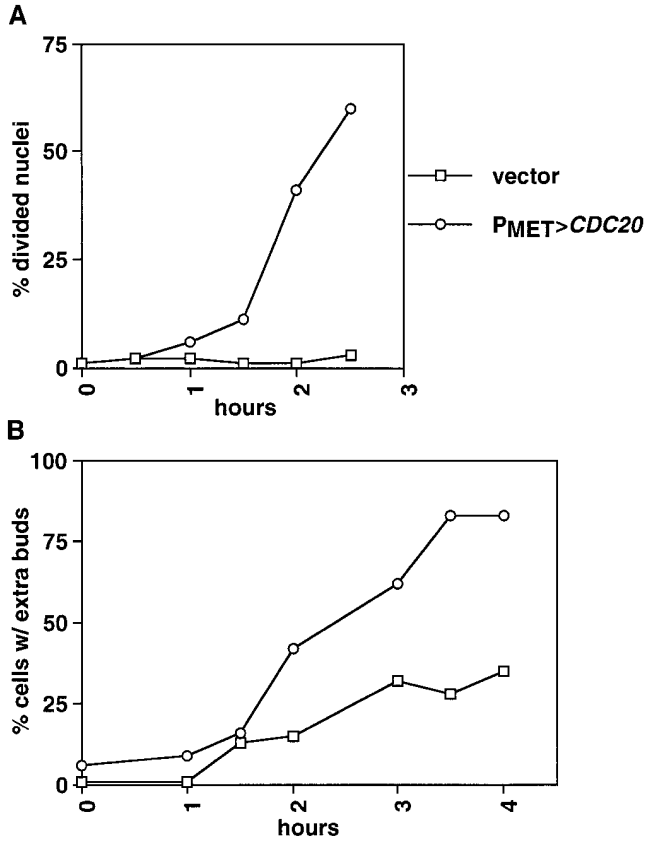

Figure 7.—Overexpressed *CDC20* overcomes the preanaphase arrest caused by *MPS1* overexpression. MAY4438, carrying a chromosomally integrated $P_{GAL} \rightarrow$ *MPS1*, was transformed with P_{MET} vector only (□) or a $P_{MET} \rightarrow$ *CDC20* plasmid (○). Transformants were grown in minimal raffinose media and synchronized with hydroxyurea. For the last 30 min of hydroxyurea treatment, galactose was added to the media to induce *MPS1* expression and methionine was omitted to induce *CDC20* expression. The cells were then released from the hydroxyurea block into liquid and solid galactose-containing media lacking methionine at 26°. (A) The liquid media cells were fixed at timepoints, stained with DAPI, and observed for nuclear division. Percent divided nuclei indicates the percentage of total cells with two DAPI-staining chromosomal masses. (B) The solid media cells were examined by microscopy for bud emergence. The percent cells with extra buds is the percent microcolonies with three cell bodies.

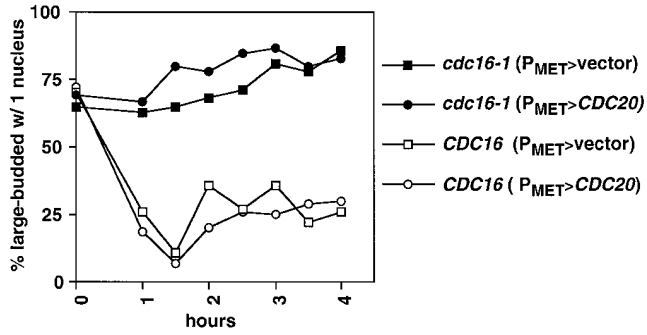

Figure 8.—*CDC20* overexpression did not overcome the preanaphase arrest caused by *cdc16-1*. *CDC16* strain MAY2422 and *cdc16-1* strain MAY2096 were transformed with P_{MET} vector and $P_{MET} \rightarrow$ *CDC20* plasmids and synchronized with hydroxyurea. Fifteen minutes prior to release from the hydroxyurea block, the cells were transferred to liquid media lacking methionine (to induce *CDC20* expression) and the incubation temperature was raised to 37°. The cells were released from the hydroxyurea into the same media at 37°. Samples were removed at time intervals, fixed, stained with DAPI, and observed. The percent of total cells that are large-budded (bud 50% diameter of mother or greater) and have one DAPI-staining chromosomal mass is displayed. Symbols: ■, *cdc16-1* (P_{MET} vector); ●, *cdc16-1* ($P_{MET} \rightarrow$ *CDC20* plasmid); □, *CDC16* (P_{MET} vector); ○,*CDC16* ($P_{MET} \rightarrow$ *CDC20* plasmid).

cdc16-1 cells were inhibited for the course of the experiment. The presence of the $P_{MET} \rightarrow$ *CDC20* plasmid did not allow the *cdc16-1* cells to enter anaphase at 37°. Therefore, *CDC20*, overexpressed under our experimental conditions, was unable to bypass loss of APC function. The expression of *CDC20-50* also did not cause *cdc16-1* or *cdc23-1* (encodes another component of the APC) cells to overcome their preanaphase arrest (data not shown).

Transient overexpression of *PDS1* has been observed to cause a preanaphase delay (Cohen-Fix *et al.* 1996). This study also reported that transient overexpression of the *pds1-mdb* mutant form, altered within the "destruction box" sequence required for ubiquitination and subsequent degradation, caused a more pronounced delay in anaphase onset. To examine whether overexpression of *CDC20* could alleviate this delay, strains expressing the *PDS1* forms from P_{GAL} were transformed with $P_{MET} \rightarrow$ *CDC20* or a vector plasmid. Following hydroxyurea synchronization in raffinose plus methionine media, cells were transferred for 2 hr to media containing hydroxyurea and galactose (to induce expression of *PDS1*) and lacking methionine (to induce expression of *CDC20*). Cells were released from the hydroxyurea block by transfer to liquid or solid glucose media (to repress further *PDS1* expression) lacking methionine. Liquid culture samples were removed and examined for nuclear division (Figure 9A). In addition, the cells spread onto solid media were observed for bud emergence (Figure 9B). A delay in nuclear division and bud emergence caused by overexpressed *PDS1* could be detected under these conditions (compare $P_{GAL} \rightarrow$ *PDS1* [P_{MET} vector] with WT [P_{MET} vector] in Figure 9, A and B). The extent of the *PDS1*-induced delay was reduced in cells that overexpressed *CDC20*; $P_{GAL} \rightarrow$ *PDS1* ($P_{MET} \rightarrow$ *CDC20*) cells entered anaphase and budded approximately 30 min earlier than $P_{GAL} \rightarrow$ *PDS1* (P_{MET} vector) cells. Therefore, the preanaphase delay caused by Pds1p could be relieved by overexpression of Cdc20p. In contrast, expression of *pds1-mdb* caused a strong block to anaphase onset that was not relieved by the overexpression of *CDC20*. Interestingly, overexpression of *CDC20* produced a slight but reproducible increase in the rate of nuclear division and bud emergence in the wild-type cells as well.

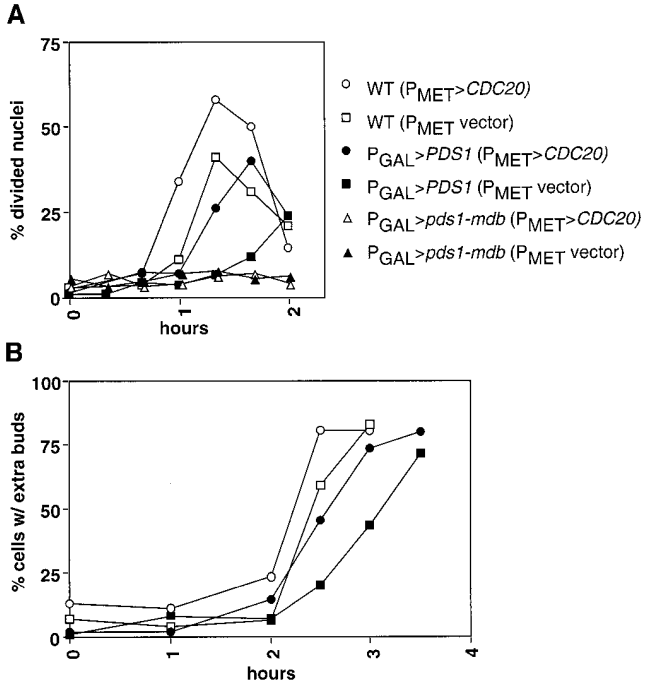

Figure 9.—Overexpressed *CDC20* overcomes the pre-anaphase arrest caused by *PDS1* overexpression. MAY5197 and MAY5198 carry galactose-inducible chromosomal *PDS1* and *pds1-mdb* (mutant destruction box) alleles, respectively. MAY2422 is a wild-type strain that expresses *PDS1* from its normal promoter. These strains were transformed with P_{MET} vector or $P_{MET} \rightarrow$ *CDC20* plasmids. Transformants were synchronized with hydroxyurea in raffinose media and were transferred for 2 hr to media containing hydroxyurea and galactose (to induce expression of *PDS1*) and lacking methionine (to induce expression of *CDC20*). Cells were released from the hydroxyurea block by transfer to liquid or solid glucose media (to repress further *PDS1* expression) lacking methionine at 26°. Liquid and solid media cells were observed for nuclear division (A) and extra bud formation (B), respectively, as described in the legend for Figure 7. In addition, the plating efficiencies of these cells were examined prior to and following the 2 hr galactose addition. For all six genotypes, the plating efficiency of the treated cells remained high, indicating that neither this protocol nor bypass of the Pds1p delay induced lethality. The data presented are for a representative experiment. The relative order of anaphase entry and bud emergence depicted here was reproduced in three additional repeats of this protocol. Symbols: ○, wild-type strain ($P_{MET} \rightarrow$ *CDC20* plasmid); □, wild-type strain (P_{MET} vector); ●, $P_{GAL} \rightarrow$ *PDS1* strain ($P_{MET} \rightarrow$ *CDC20* plasmid); ■, $P_{GAL} \rightarrow$ *PDS1* strain (P_{MET} vector); △, $P_{GAL} \rightarrow$ *pds1-mdb* strain ($P_{MET} \rightarrow$ *CDC20* plasmid); ▲, $P_{GAL} \rightarrow$ *pds1-mdb* strain (P_{MET} vector).

DISCUSSION

The actions of the spindle-assembly checkpoint prevent cell cycle progression from metaphase into anaphase in response to mitotic spindle defects. We have identified and characterized the *CDC20-50* mutant allele that caused *S. cerevisiae* cells to bypass this cell cycle arrest in a dominant fashion. In contrast, loss of *CDC20* function (*e.g.*, caused by the recessive temperature-sensitive *cdc20-1* allele) caused cells to arrest prior to anaphase with duplicated chromosomes and an assembled bipolar spindle (Sethi *et al.* 1991; O'Toole *et al.* 1997). A similar defect in cell cycle progression has been noted for loss of function of Drosophila *fizzy*, a *CDC20* homolog (Dawson *et al.* 1995; Sigrist *et al.* 1995). The dominance and inappropriate cell cycle progression exhibited by *CDC20-50* suggested that it may represent a gain-of-function allele. Indeed, we were able to mimic the phenotypes of *CDC20-50* by overexpressing *CDC20*. It seems likely, therefore, that the Cdc20-50p form is overactive, producing aberrantly high levels of what is a normal Cdc20p function. The Cdc20-50p change, affecting a conserved amino acid within the fifth of seven WD repeats, may create an intrinsically overactive protein or may destroy an interaction with a function that negatively regulates Cdc20p activity.

The ability of overexpressed *CDC20* to promote entry into anaphase suggests that it acts as a limiting activator of anaphase. Our findings indicate that a likely target of Cdc20p regulation is the anaphase inhibitor Pds1p. Entry into anaphase requires the APC-mediated proteolytic degradation of Pds1p (Cohen-Fix *et al.* 1996). We found that the delay to anaphase entry caused by transient high levels of Pds1p could be relieved by overexpression of *CDC20*. This suggests that Cdc20p acts as an antagonist of Pds1p. The observed antagonism required that Pds1p be susceptible to APC-mediated destruction. The block to anaphase progression caused by a destruction box mutant form of Pds1p, immune to the actions of the APC, could not be relieved by overexpression of *CDC20*. It seems most likely, therefore, that Cdc20p can promote the destruction of Pds1p in an APC-dependent fashion. Consistent with this hypothesis is our finding that the metaphase arrest caused by loss of APC function could not be bypassed by *CDC20* overexpression. Pds1p is stabilized in APC-mutant cells (Cohen-Fix *et al.* 1996). If a role of Cdc20p is to direct the APC to Pds1p, high levels of Cdc20p would not be expected to compensate for an APC defect. In agreement with our findings is the recently reported observation that Pds1p is stabilized in *cdc20-1* cells and destabilized in cells overexpressing *CDC20* (Visintin *et al.* 1997).

PDS1 function is also required to prevent DNA-damaged cells from entering anaphase prematurely (Yamamoto *et al.* 1996b). We also note the recent report demonstrating that overexpression of *CDC20* allows cells with damaged DNA, caused by a temperature-sensitive *cdc13* mutation, to enter anaphase (Lim and Surana 1996). It is possible that *CDC20*-induced degradation of Pds1p in the *cdc13* cells promoted aberrant anaphase onset.

Cells deficient for *PDS1* enter anaphase inappropriately but do not exit mitosis into the G_1 phase. In nocodazole-treated *pds1* cells, sister chromatids can disjoin, but new bud emergence or DNA replication

rounds remain inhibited (Yamamoto *et al.* 1996b). However, we observed new bud emergence and DNA replication in nocodazole-treated cells expressing *CDC20-50* or overexpressing *CDC20*, demonstrating exit from M phase and progression through G_1. This indicates that there must exist additional targets of activated Cdc20p beyond Pds1p. Exit from M phase requires the APC-mediated destruction of the mitotic cyclin proteins (Surana *et al.* 1993), and normal spindle disassembly requires the APC-mediated destruction of the spindle protein Ase1p (Juang *et al.* 1997). Recent studies demonstrated that destruction of Ase1p and the Clb2p mitotic cyclin occur under the control of *HCT1/CDH1*, an *S. cerevisiae CDC20* homolog (Schwab *et al.* 1997; Visintin *et al.* 1997). It is possible that additional targets must be degraded as well to complete exit from mitosis. While the degradation of these targets may not normally be under *CDC20* control, nocodazole-treated cells overexpressing *CDC20* were able to exit M phase and therefore were able to overcome all regulatory obstacles to progression. Perhaps under these conditions, Cdc20p can direct the APC to targets beyond its normal scope. Indeed, it was reported that although the mito-tic cyclin Clb2p is not stabilized in *cdc20-1* cells, Clb2p is destabilized in cells overexpressing *CDC20* (R. Visintin, S. Prinz and A. Amon, unpublished results). Functional overlap between *CDC20* and *HCT1/CDH1* was suggested by the observation that overexpression of *HCT1/CDH1* could suppress the temperature sensitivity of *cdc20-1* (Schwab *et al.* 1997). Perhaps overexpressed *CDC20* can lead to the destruction of protein targets normally under the control of *HCT1/CDH1*.

We have demonstrated that overexpression of *CDC20* can bypass the cell cycle arrest caused by the spindle-assembly checkpoint. The mutant phenotypes of the seven spindle-assembly checkpoint genes characterized to date (*BUB1, 2, 3, MAD1, 2, 3,* and *MPS1*) suggest that they act as negative regulators of anaphase onset and cell cycle progression. In contrast, the phenotypes of *CDC20* loss- and gain-of-function mutants indicate that it must be an activator of anaphase. Our findings suggest the interesting possibility that Cdc20p is actually the target of regulation for the spindle-assembly checkpoint. In this formulation, the checkpoint gene products would act upstream of Cdc20p to transduce the spindle-damage signal and to inhibit the Cdc20p anaphase-promoting function. *CDC20-50* or overexpression of *CDC20* may create forms that are insensitive to the cycle-arresting signals produced by the checkpoint mechanism. However, our data do not rule out the possibility that Cdc20p function is unrelated to spindle-assembly checkpoint function. High levels of Cdc20p may simply be able to drive anaphase entry in a manner such that the arrest signal originating from the checkpoint mechanism is bypassed. The *MPS1*-encoded protein kinase is believed to act at an early step in the spindle-damage signaling pathway, and its overexpression appears to mimic normal pathway activation (Hardwick *et al.* 1996). We found that *CDC20* overexpression could overcome the metaphase arrest caused by overexpression of *MPS1*. This could indicate that Cdc20p antagonizes Mps1p function, perhaps in a manner similar to its antagonism of Pds1p. It seems more likely, however, that the *MPS1*-induced arrest resembles that caused by spindle disruption and that both produce a similar signal that is ignored or bypassed by the downstream-acting overexpressed Cdc20p.

In summary, we have found that the *CDC20* product behaves like a limiting factor regulating the entry into anaphase. *CDC20* homologs exist throughout the eukaryotes, including Drosophila *fizzy*, which also appears to be required for anaphase entry (Dawson *et al.* 1995; Sigrist *et al.* 1995). The metaphase-anaphase transition is a critical regulatory point in the eukaryotic cell cycle. At this stage, numerous regulatory influences reporting the readiness of the cell to segregate chromosomes must be integrated and acted upon. It seems likely that Cdc20p homologs perform a central role in this important regulatory mechanism.

The authors thank Penny Tavormina, Dan Burke, Mark Winey, and Orna Cohen-Fix for the gifts of strains and DNAs; Angelika Amon and Wolfgang Seufert for the communication of unpublished findings; and Orna Cohen-Fix, Cindy Dougherty, Katie Farr, John Geiser, and Penny Tavormina for helpful discussions and comments on the manuscript. This work was support by National Institutes of Health grant GM-49363 awarded to M.A.H.

LITERATURE CITED

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

Chen, R., J. C. Waters, E. D. Salmon and A. W. Murray, 1996 Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. Science **274:** 242–246.

Cohen-Fix, O., J.-M. Peters, M. W. Kirschner and D. Koshland, 1996 Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev. **10:** 3081–3093.

Dawson, I. A., S. Roth and S. Artavanis-Tsakonas, 1995 The *Drosophila* cell cycle gene *fizzy* is required for normal degradation of cyclin A and B during mitosis and has homology to the *CDC20* gene of *Saccharomyces cerevisiae*. Development **129:** 725–737.

Elledge, S. J., 1996 Cell cycle checkpoints: preventing an identity crisis. Science **274:** 1664–1672.

Geiser, J. R., E. J. Schott, T. J. Kingsbury, N. B. Cole, L. J. Totis *et al.*, 1997 *S. cerevisiae* genes required in the absence of the *CIN8*-encoded spindle motor act in functionally diverse mitotic pathways. Mol. Biol. Cell **8:** 1035–1050.

Gietz, D., A. St. Jean, R. A. Woods and R. H. Schiestl, 1992 Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:** 1425–1426.

Hardwick, K. G., E. Weiss, F. C. Luca, M. Winey and A. W. Murray, 1996 Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption. Science **273:** 953–956.

Hoyt, M. A., L. He, K. K. Loo and W. S. Saunders, 1992 Two *Saccharomyces cerevisiae* kinesin-related gene-products required for mitotic spindle assembly. J. Cell Biol. **118:** 109–120.

Hutter, K. J., and H. E. Eipel, 1978 Flow cytometric determinations of cellular substances in algae, bacteria, molds and yeast.

Antonie Van Leeuwenhoek J. Microbiol. Ser. **44:** 269–282.
Juang, Y., J. Huang, J. Peters, M. E. McLaughlin, C. Tai *et al.*, 1997 APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle. Science **275:** 1311–1314.
King, R. W., R. J. Deshaies, J. Peters and M. W. Kirschner, 1996 How proteolysis drives the cell cycle. Science **274:** 1652–1659.
Li, Y., and R. Benezra, 1996 Identification of a human mitotic checkpoint gene: *hsMAD2*. Science **274:** 246–248.
Lim, H. H., and U. Surana, 1996 Cdc20, a β-transducin homologue, links *RAD9*-mediated G2/M checkpoint control to mitosis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **253:** 138–148.
Mumberg, D., R. Müller and M. Funk, 1994 Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res. **22:** 5767–5768.
Neer, E. J., C. J. Schmidt, R. Nambudripad and T. F. Smith, 1994 The ancient regulatory-protein family of WD-repeat proteins. Nature **371:** 297–300.
Nicklas, R. B., 1997 How cells get the right chromosomes. Science **275:** 623–637.
O'Toole, E. T., D. N. Mastronarde, T. H. Giddings Jr., M. Winey, D. J. Burke *et al.*, 1997 Three-dimensional analysis and ultrastructural design of mitotic spindles from the *cdc20* mutant of *Saccharomyces cerevisiae*. Mol. Biol. Cell **8:** 1–11.
Roberts, B. T., K. A. Farr and M. A. Hoyt, 1994 The *Saccharomyces cerevisiae* checkpoint gene *BUB1* encodes a novel protein kinase. Mol. Cell. Biol. **14:** 8282–8291.
Roof, D. M., P. B. Meluh and M. D. Rose, 1992 Kinesin-related proteins required for assembly of the mitotic spindle. J. Cell Biol. **118:** 95–108.
Rudner, A. D., and A. W. Murray, 1996 The spindle assembly checkpoint. Curr. Opin. Cell Biol. **8:** 773–780.
Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Saunders, W. S., and M. A. Hoyt, 1992 Kinesin-related proteins required for structural integrity of the mitotic spindle. Cell **70:** 451–458.
Saunders, W. S., D. Koshland, D. Eshel, I. R. Gibbons and M. A. Hoyt, 1995 *Saccharomyces cerevisiae* kinesin- and dynein-related proteins required for anaphase chromosome segregation. J. Cell Biol. **128:** 617–624.
Schwab, M., A. S. Lutum and W. Seufert, 1997 Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. Cell **90:** 683–693.
Sethi, N., M. C. Monteagudo, D. Koshland, E. Hogan and D. J. Burke, 1991 The *CDC20* gene product of *Saccharomyces cerevisiae*, a β-transducin homolog, is required for a subset of microtubule-dependent cellular processes. Mol. Cell. Biol. **11:** 5592–5602.
Sherman, F., G. R. Fink and J. B. Hicks, 1983 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sigrist, S., H. Jakobs, R. Stratmann and C. F. Lehner, 1995 Exit from mitosis is regulated by *Drosophila fizzy* and the sequential destruction of cyclins A, B and B3. EMBO J. **14:** 4827–4838.
Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.
Surana, U., A. Amon, C. Dowzer, J. McGrew, B. Byers *et al.*, 1993 Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. EMBO J. **12:** 1969–1978.
Taylor, S. S., and F. McKeon, 1997 Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. Cell **89:** 725–735.
Thompson, J. D., D. G. Higgins and T. J. Gibson, 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673–4680.
Visintin, R., S. Prinz and A. Amon, 1997 CDC20 and CDH1, a family of substrate-specific activators of APC-dependent proteolysis. Science **278:** 460–463.
Wells, W. A. E., 1996 The spindle-assembly checkpoint: aiming for a perfect mitosis, every time. Trends Cell Biol. **6:** 228–234.
Yamamoto, A., V. Guacci and D. Koshland, 1996a Pds1p is required for faithful execution of anaphase in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. **133:** 85–97.
Yamamoto, A., V. Guacci and D. Koshland, 1996b Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). J. Cell Biol. **133:** 99–110.
Zachariae, W., and K. Nasmyth, 1996 TPR proteins required for anaphase progression mediate ubiquitination of mitotic B-type cyclins in yeast. Mol. Biol. Cell **7:** 791–801.
Zachariae, W., T. H. Shin, M. Galova, B. Obermaier and K. Nasmyth, 1996 Identification of subunits of the anaphase-promoting complex of *Saccharomyces cerevisiae*. Science **274:** 1201–1204.

Communicating editor: M. D. Rose