Pds1p Is Required for Faithful Execution of Anaphase in the Yeast, *Saccharomyces cerevisiae*

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**Abstract.** To identify mutations that cause defects in mitosis, a collection of mutants in *Saccharomyces cerevisiae* was screened by a rapid visual assay for abnormal chromosome segregation. From this screen we identified one mutation, *pds1-1* that was independently identified in an alternative screen for mutants that exhibit inviability after transient exposure to nocodazole and precocious disassociation of sister chromatids (Guacci, V., A. Yamamoto, A. Strunnikov, J. Kingsbury, E. Hogan, P. Meluh, and D. Koshland. 1993. CSH Symp. Quant. Biol. 58:677-685; Yamamoto, T.J., G. Li, B. Schaar, I. Szilak, and D.W. Cleveland. 1992. Nature (Lond.). 359:536-539). At 23°C *pds1-1* mutants exhibit frequent cell death and a 300-fold increase in chromosome loss compared to wild type. At 37°C *pds1-1* cells fail to elongate their spindles during anaphase. This spindle defect of *pds1* mutants results from a temperature-sensitive step that occurs around the G1/S boundary about the time of spindle assembly. In the absence of spindle elongation *pds1* mutants undergo cytokinesis, leading to the missegregation of both chromosomes and spindle pole bodies. After abnormal cell division *pds1-1* mutants also initiate new rounds of DNA replication, spindle pole body duplication, and bud formation. Thus, in the *pds1-1* mutant at 37°C, cell cycle progression is uncoupled from the completion of anaphase. A *pds1* deletion allele has similar phenotypes to the original allele. Taken together these results suggest that Pds1 protein plays an important role in chromosome segregation at 23°C and an essential role for this process at 37°C. The *PDS1* gene encodes a novel 42-kD nuclear protein that has both basic and acidic domains. The level of *PDS1* mRNA varies with the cell cycle with maximal accumulation around the G1/S boundary. The stability of Pds1 protein also appears to change during the cell cycle as overproduced Pds1p is stable in S and M but degraded in early G1. Therefore, expression of Pds1p is regulated apparently both transcriptionally and posttranslationally during the cell cycle. The phenotypes of *pds1* mutants and expression pattern of Pds1p are discussed in the context of other spindle-defective mutants and the knowledge that Pds1 protein is an inhibitor of anaphase (Yamamoto, T.J., G. Li, B. Schaar, I. Szilak, and D.W. Cleveland. 1992. Nature (Lond.). 359:536-539).

The mitotic spindle is a complex macromolecular machine that mediates chromosome segregation during cell division. The cytology of the spindle has fascinated cell biologists over the last 100 years. More recently, attention has focused on identifying the spindle components, elucidating the mechanism of force generation and understanding the regulation of spindle assembly and function during the cell cycle events.

The spindle has been studied intensively in the budding yeast, *Saccharomyces cerevisiae* (for reviews see Byers, 1981; Rose et al., 1993; Winey and Byers, 1993). In this yeast the microtubule-organizing center is called the spindle pole body (SPB)1 which remains embedded in the nuclear envelope during the entire cell cycle. Microtubules are nucleated from the intra- and extranuclear surfaces of the SPB giving rise to both nuclear microtubules and cytoplasmic microtubules, respectively. In early G1, the SPB duplicates and the new SPB is embedded into the nuclear envelope concomitant with its assembly. The new and old SPB separate but remain connected by interdigitating nuclear microtubules, resulting in the formation of a short (~1-2 µm) bipolar spindle that traverses the nucleus. In budding yeast, spindle formation can occur as early as S phase. The short spindle (and associated nucleus) becomes positioned at the neck between the mother cell and the newly emerging daughter cell (the bud). At the onset of

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1. Abbreviations used in this paper: EMS, methansulfonic acid ethyl ester; ORF, open reading frame; SPB, spindle pole body.
chromosome segregation (anaphase), the spindle and nuclear envelope elongate causing one SPB and one set of chromosomes to move into the bud while the other pole and set of chromosomes stay in the mother cell moving away from the bud neck. At the end of mitosis the spindle breaks down, the nucleus divides, and cytokinesis ensues. Before its breakdown, the final length of the elongated spindle is 6–8 μm, approximately five times the length of the initial short spindle.

The assembly and function of the spindle are clearly complex and involve many structural and regulatory components. Recent biochemical and genetic studies of spindles in budding yeast have led to the identification of structural components of the SPB (Byers, 1981; Kilmartin et al., 1993; Osborne et al., 1994; Rose et al., 1993; Rout and Kilmartin, 1990; Spang et al., 1993; Winey and Byers, 1993) and microtubule-dependent motors that provide the force necessary to form the short-anaphase spindle and to elongate it at anaphase (Roof et al., 1992; Hoyt et al., 1992; Saunders and Hoyt, 1992; Saunders et al., 1995). The regulation of spindle assembly and function during the cell cycle is mediated directly or indirectly by the master kinase, p34 (Fitch et al., 1992; Lew and Reed, 1992; Grandin and Reed, 1993), and a surveillance system (checkpoint) that monitors some aspects of improper spindle assembly (Winey et al., 1991).

Clearly, the current list of SPB proteins and microtubule-based motors in budding yeast is only a subset of total components required for proper spindle assembly and elongation. In addition, many factors that coordinate spindle morphogenesis with the DNA replication, cytokinesis, budding, or other events of the cell cycle have yet to be identified. Here, we describe a simple method for identifying potential factors necessary for spindle assembly or function by visually screening a collection of temperature-sensitive conditional-lethal mutants of budding yeast for mutants that undergo gross missegregation of chromosomes at the nonpermissive temperature. This screen has the virtue that mutants defective for chromosome segregation should be identified whether or not they have associated cell cycle arrest. From this screen we identified the pds1-1 mutant that we also identified in a screen for mutants exhibiting precocious dissociation of sister chromatids in the presence of the microtubule inhibitor, nocodazole (see Yamamoto et al., 1996). We present results characterizing the temperature-sensitive defect of pds1 mutants that leads to the chromosome missegregation. The characterization of PDS1 gene and gene product is also presented.

Materials and Methods

Strains and Media

Yeast strains and genotypes are shown in Table I. All media for yeast cultures prepared as previously described (Rose et al., 1990). Solid media contained 2% agar and was otherwise identical to liquid media. TB medium contained 1.2% bacto-peptone, 2.4% yeast extract, 0.4% (wt/vol) glycerol and 9 mM potassium phosphate buffer (pH 7.0). p derivative of strain 661-1-3 (pds1) were generated at 23°C using ethidium bromide as previously described (Rose et al., 1990).

Reagents

4′,6′-diamidino-2-phenylindole (DAPI), methansulfonylic acid ethyl ester (EMS), α-factor, hydroxyurea (HU), PMSF, pepstatin A, chymostatin, p-aminobenzamidine, e-aminocaproic acid, aprotinin, leupeptin, and Diethyl pyrocarbonate (DEP) were purchased from Sigma Chem. Co. (St. Louis, MO). Nocodazole (NZ) was purchased from Aldrich Chem. Co. (Milwaukee, WI). Oxalyticase was purchased from Enzogenetics (Corvalis, OR). Pronase and Mowiol 4-88 was obtained from Calbiochem Corp. (San Diego, CA). Isopropilthio-β-D-galactoside (IPTG) was purchased from ICN Biomedical, Inc. (Aurora, OH).

Isolation of Mutants

Wild-type strain 801-6-1 was mutagenized with EMS to 50% viability. Mutagenized cells were spread on solid YEPD medium and tested for growth at 23 and 37°C. 751 temperature-sensitive (Ts) lethal mutants were isolated from a total of 60,000 mutagenized cells. To allow rapid screening of a large number of mutants, fresh patches of cells (1.5 cm²) were made on solid media at the permissive temperature, and then they were replica plated to solid media at the nonpermissive temperature for the appropriate time. Cells were removed from the plates by toothpicks, and then resuspended in 50 μl of 3.7% formaldehyde/50 mM Tris-CI (pH 7.5) in wells of a 96-well microtiter plate. After 1 h at room temperature, cells in each well were washed twice with water, and then placed in 50 μl of 50 mM Tris-CI (pH 7.5) containing 100 ng/ml DAPI. The wells were covered with the lid to the microtiter dish and then sealed with parafilm. Since yeast stick to the bottom of the wells, these changes in solution could be achieved easily by simply inverting the microtiter dish. The cells in each well were examined directly using an objective with long focal distance. Chromosome distribution was assessed using a Zeiss Axiophot microscope equipped with epifluorescent and Nomarski optics. Mutants exhibiting gross chromosomal missegregation (unequal chromosome distribution) were back crossed five times to wild-type strains congenic to 801-6-1.

Recombination and Chromosome Loss Assay

Recombination and loss of chromosome III were detected as described by Spencer et al. (1988). The rate of chromosome loss and recombination was determined using the method of the median (Lea and Coulson, 1949).

Indirect Immunofluorescence and Electron Microscopy

For immunofluorescence, cells were washed once with 0.1 M potassium phosphate buffer (pH 6.5) and fixed in the same buffer containing 3.7% formaldehyde. For microtubule staining, the cells were fixed at 23°C for 2 h. For SPB and Pds1p staining, cells were fixed for 30 min and 1 h, respectively. The fixed cells were processed for indirect immunofluorescence as described by Kilmartin and Adams (1984) except 10 μg/ml oxalyticase was used instead of zymolyase for spermatocyte formation. Microtubules were stained using rat monoclonal antibody, YOL1/34, diluted 1:200 (Cappel/
Organon Teknika, Durham, NC). For Pds1p staining, affinity-purified rabbit polyclonal anti-Pds1p antibodies were diluted 1:50, and FITC-conjugated goat anti-rabbit antibodies were diluted 1:1,000 (Cappel/Organon Teknika). All antibody reactions were carried out at 23°C for 1 h except for reactions with anti-90-kD SPB which were incubated overnight at 14°C. Samples were mounted in antifade solution (9% Mrowi 4-88, 23% glycerol in 0.1 M Tris-Cl, pH 8.5). For electron microscopy, the cells were fixed and processed as described previously (Guthrie and Fink, 1991).

**Flow Cytometric Analysis of DNA Content**

The cells were fixed with 70% ethanol and stained with propidium iodide as described by Hutter and Eipel (1978). The DNA content of 25,000 cells was determined with a Coulter EPICS 752 flow cytometer.

**Cloning and Sequencing of PDS1**

The PDS1 gene was cloned by complementing the recessive Ts- lethality associated with the pds1-1 allele. Strain 643-21 (pds1-1) was transformed with a yeast genomic library constructed in the centromere-based plasmid, p366. Approximately 10,000 transformants were replica plated onto YEPD plates and tested for growth at 37°C. Eight Ts- transformants were obtained and found to contain plasmids carrying an identical ~3.6-kb HindIII-EcoRI fragment of yeast DNA. A common ClaI-NsiI fragment containing the insert was cloned between the ClaI and PstI sites of the plasmid pRS314 (Sikorski and Hieter, 1989) to form the plasmid pAY12. Plasmid pAY30 was constructed by cloning the 1.7-kb Ndel-ScaI fragment from plasmid pAY12 between the Nhel-Scal sites of pRS314 vector. Both pAY12 and pAY39 complemented the Ts- lethality of pds1-1.

To verify that the common insert was derived from the PDS1 locus, we first cloned the 4.5-kb ClaI-NsiI fragment from plasmid pAY12 into plasmid pRS304 (Sikorski and Hieter, 1989), which bears ARS1 and TRPI but not a yeast centromere. This plasmid (pAY30) was cut within the ClaI-NsiI fragment by digestion with BglII and transformed into wild type that is trp1 (4506-2-2). Four independent Trp+ transformants shown by Southern blot to contain pAY30 integrated into the genome (data not shown) were crossed to a strain of pds1-1 (strain 619-1-2). Two of these transformants were mated to a strain 914D-2A which contains the PDS1 gene under control of the GAL1 promoter (pGAL-PDS1) and Ts- growth phenotypes to identify the PDS1 gene flanked by sequences in or adjacent to the PDS1 gene was transformed into diploid wild-type strain 663 that was homozygous leu2/leu2. LEU+ transformants were sporulated and spores were tested for LEU+ and Ts- growth phenotypes to identify spore colonies deleted for the PDS1 gene. The deletion was confirmed by Southern blot hybridization.

**Preparation of Affinity-purified Anti-Pds1 Protein Antibodies**

Bacterial Pds1 protein was expressed from plasmid pAY51 which was constructed by inserting the Nael-NcoI fragment of pAY39 between EcoRI and NdeI sites of pElectE4 bacteria expression vector (Invitrogen, San Diego, CA). Plasmid pAY51 encodes a PDS1 polypeptide which lacks 28 NH2-terminal and 18 COOH-terminal amino acids, respectively, and contains a DNA sequence encoding six histidine tandem residues in frame at the NH2 terminus. E. coli strain BL21 (Novagen, Inc., Madison, WI), carrying pAY51 was grown in 450 ml of TB medium at 37°C to an OD600 of 1.0. IPTG was added to 1 mM into the medium and the cells were incubated for 4 h at 30°C to induce expression of the fused Pds1p protein. The cells were collected and frozen at -20°C. Cells were then resuspended in binding buffer (0.5 M NaCl, and 20 mM Tris-Cl [pH 8.0]) containing 5 mM imidazole and protease inhibitors (0.5 mM PMSF, 5 μg/mL pepstatin, 1 μg/mL chymostatin, 1 mM p-aminobenzamidine, 1 mM α-aminocaproic acid, 5 μg/mL aprotinin, and 1 μg/mL leupeptin). The cell suspension was sonicated extensively and cell debris was removed by centrifugation at 100 g for 30 min. The supernatant was passed through a column containing ProBond metal-binding resin (Invitrogen, Co.). After extensively washing the column with binding buffer containing 5 mM imidazole, nonspecific-binding proteins were eluted with binding buffer containing 30 mM imidazole. The fused Pds1p was eluted with binding buffer containing 500 mM imidazole, and then precipitated by adding 1:10 vol of 100% (wt/vol) trichloroacetic acid into the eluate and incubating overnight on ice. The precipitate was pelleted by centrifugation at 185 g for 15 min at 4°C, washed twice with 80% acetic acid, solubilized by boiling in SDS-PAGE sample buffer for 5 min and run on a 10% SDS polyacrylamide gel as described by Laemmli (1970). After staining with 4 μg/mL acetic acid as described by Harlow and Lane (1988), the bands corresponding to the fused Pds1p was cut out and used directly for injection into rabbits.

IgG fraction of rabbit anti-Pds1p serum was prepared using protein A-Sepharose CL-4B (Pharmacia LKB Biotechnology, Piscataway, NY) as described by Harlow and Lane (1988). Affinity-purified anti-Pds1p antibodies were obtained as follows: the eluate of ProBond resin containing the fused Pds1p was prepared from 500 ml bacterial culture as described above and dialyzed against coupling buffer (0.5 M NaCl in 0.1 M NaHCO3 [pH 8.3]). Proteins in the dialyzed eluate were cross-linked with activated CNBr-Sepharose 4B resin (Pharmacia). The cross-linked resin was further blocked for unreacted active sites to remove uncross-linked molecules. A column containing the Pds1p protein cross-linked resin (2 ml bed volume) was washed with a solution consisting of 6 M guanidine-HCl and 0.1 M Tris-Cl (pH 7.5) and then eluted with a solution containing 4.5 M MglCl, 0.1% BSA in 0.1 M Tris-Cl (pH 7.5) successively. The column was equilibrated with 0.1 M Tris-Cl (pH 7.5), and then the IgG fraction of rabbit anti-Pds1p serum was washed through the column. After extensive washing with 0.1 M Tris-Cl (pH 8.0) and 1 M guanidine-HCl, anti-Pds1p antibodies were eluted with solution E. The eluate containing the anti-Pds1p antibodies was dialyzed against PBS (150 mM NaCl in 10 mM phosphate buffer [pH 7.4]) and Na2SO4 was added to a final concentration of 0.1% for storage of the antibodies.

**Western Blot Analysis of Overproduced Pds1p**

Strain 914D-2A which contains the PDS1 gene under control of the GAL1 promoter (pGAL-PDS1) was grown at 23°C to logarithmic phase in 20 ml of YEP+Raffinose. The culture was split into two aliquots, and galactose was added (2% final concentration) to one. After 3 more hours, cells from both aliquots were washed once with 10 mM Tris-Cl (pH 7.5) and resuspended in 100 μl of lysis buffer (1 mM EDTA, 50 mM Tris-Cl [pH 7.5]) containing protease inhibitors (see above). After adding 1 vol of acid-washed glass beads, the cell suspension was vortexed vigorously for 10 min 4°C. The cell lysate was collected and clarified by centrifugation at 18.5 g for 15 min at 4°C. After determining protein concentration by Protein Assay (Bio-Rad Labs, Hercules, CA), the clarified cell lysate was mixed with 1 vol of 2× SDS-PAGE sample buffer and boiled for 5
min. For each sample, 70 μg of protein was loaded onto 10% SDS polyacrylamide gel and electrophoresed (Laemmli, 1970). After electrophoresis, proteins were transferred to Immobilon-P membrane (Millipore Co., Bedford, MA) (Harlow and Lane, 1988). The membrane was then incubated with affinity-purified rabbit anti-Pds1p antibodies (see above) followed by horseradish peroxidase-conjugated goat anti-rabbit antibodies (Bio-Rad, No. 172-1019) diluted to 1:3,000. The reacting bands were detected using Western Biot Chemiluminescence Reagent (DuPont New England Nuclear Research Products, Boston, MA).

Quantitation of PDS1 mRNA in Cycling and Arrested Cells

To quantitate PDS1 mRNA level in synchronized cells, haploid strain 4536-151 (cdcl5-1), was grown at 23°C to logarithmic phase in 1.5 l of YEPD medium. The culture was shifted to 37°C for 3 h to arrest cells in late M. The culture was then shifted back to 23°C to release cells from arrest. Just before and every 20 min after release from arrest, 10-ml aliquots were collected and cells obtained by centrifugation were frozen immediately in liquid nitrogen for subsequent mRNA analysis. In addition, 0.5-ml aliquots were fixed and the cells processed for DNA staining with DAPI as described above. Aliquots were kept at -80°C until all samples were frozen. mRNA was prepared from the frozen cells using hot phenol as described by Körter and Domdey (1991). mRNA prepared from 10-ml aliquots was suspended in 20 μl of DEP treated water and 2 μl of each sample was electrophoresed on 1% agarose gel and processed for Northern blot analysis as described (Rose et al., 1990). The Northern blot was probed with the NheI-NcoI fragment from pAY12 to detected using Western Biot Chemiluminescence Reagent (DuPont New England Nuclear Research Products, Boston, MA).

Results

Isolation of pds1 Mutants

We sought to identify temperature-conditional mutants with defects in chromosome segregation. For this purpose, we isolated 751 mutants that were temperature sensitive for growth. Each mutant was grown at the nonpermissive temperature and then screened for abnormal chromosome segregation by examining mutant cells stained with DAPI, a DNA-specific dye, in the microscope. In the process of screening these mutants we developed a rapid method where cells were fixed, stained, and analyzed microscopically in a microtiter dish (see Materials and Methods). With this improvement several hundred mutants were screened easily in a day. In our temperature-sensitive collection, 64 mutants exhibited abnormal chromosome distribution at the nonpermissive temperature. Of these six mutants generated diploid cells that lacked most if not all chromosomes DNA. The Ts+ growth of all six mutants was recessive. These mutants were tested for complementation with mutants which have been reported to form diploid cells. Two of our mutants failed to complement exp1 and mpsl mutants, respectively. The remaining four mutants fell into three complementation groups that were previously uncharacterized.

The same Ts+ collection was also screened to identify mutants that both failed to grow after transient exposure to microtubule inhibitors and exhibited precocious dissociation of sister chromatids (pds) in the presence of these inhibitors. These mutants potentially identified regulatory or structural components of sister chromatid cohesion (for methodology and rationale of this screen see Guacci et al., 1993). We discovered that one mutant from this screen, pds1-1, was exactly the same as one of the mutants of the exp1 screen. All of the phenotypes of the pds1-1 as identified by the initial screens (Ts+ growth, aploid cell formation, death after transient exposure to microtubule inhibitors and precocious dissociation of sister chromatids) cosegregated 2:2 from the wild-type phenotypes, indicating that they were caused by a single mutation.

PDS1 Function Is Essential for Chromosome Segregation at 37°C and Important at 23°C

Normally by the time budding yeast generates a large bud anaphase is completed, and the segregated chromosomes appear as two distinct similar sized DNA masses, one in the mother and the other in the bud. This class was observed in pds1-1 cultures grown at 23°C and in wild-type cultures (Fig. 1 A, large arrows). However, it was absent in pds1-1 cultures grown at 37°C for 4 h. Instead, over 75% of these cells accumulated as either large-budded cells with only a single chromosomal DNA mass (unsegregated chromosomes) or unbudded cells with little or no chromosomal DNA mass (grossly aneuploid) (Fig. 1 A, large arrowheads; Table II). We also generated a p^ derivative of pds1-1 cells that lacked mitochondrial DNA. In these cells it was possible to follow the segregation of very small amounts of chromosomal DNA that would otherwise be obscured by mitochondrial DNA. When this strain was incubated at 37°C, we frequently observed cells with less...
Table II. Distribution of Cell Types in *pdsl*−1

| Genotype | Temp | Time | %   |
|----------|------|------|-----|
|          | (°C) | (h)  |     |
| PDS1*    | 23   | 34   | 43  |
|          | 37   | 4    | 25  |
|          |      | 19   | 3   |
|          |      | 3    | 0   |
|          |      | 2    | 7   |
|          |      | 0    | 0   |
| *pdsl*−1** | 23   | 34   | 22  |
|          | 37   | 2    | 18  |
|          |      | 5    | 2   |
|          |      | 26   | 11  |
|          |      | 13   |     |
|          |      | 3    | 18  |
|          |      | 33   | 22  |
|          |      | 11   | 13  |
|          |      | 3    | 18  |
|          |      | 33   | 22  |
|          |      | 46   | 30  |

Cells were grown to logarithmic phase in YEPD medium at 23°C and shifted to 37°C. Aliquots were fixed and stained with DAPI at designated times. Cell types of 100 cells were examined and shown in percentage.

*Strain 801-6.

**Strain 661-1-3.

(1) Unbudded cells containing little or no chromosomal DNA staining,

(2) Large budded cells with nuclei off the bud neck.

Figure 1. Distribution of chromosomal DNA and microtubules in *pdsl* mutants. (A) The *pdsl*−1 strain, 661-1-3 (Table I), was grown to logarithmic phase at 23°C, and then shifted to 37°C for 4 h. *pdsl*−1 cells were fixed before or after the temperature shift and processed for microtubule staining by anti-tubulin antibody and DNA staining using DAPI (see Materials and Methods). Typical telophase cells observed at 23°C are indicated by large arrows. An aploid cell observed at 37°C is marked by the large arrowhead. (B) The *pdsl*−1 Δ*ρ* strain, 661-1-3P1 (Table I) was grown to logarithmic phase at 23°C, and then shifted to 37°C for 8 h. Cells were fixed and processed for DAPI staining. Bars, 5 μm.

than a haploid content of chromosomal DNA as well as aploid cells (Fig. 1 B). A haploid strain with a deletion of most of the *PDS1* gene (*pdsl*−Δ2) (see below) had similar cytological phenotypes to *pdsl*−1 (data not shown). Therefore, we conclude that the Pds1p function is essential for chromosome segregation at 37°C.

The *pdsl*−Δ2 mutant was able to grow at 23°C indicating that Pds1p function was not essential for growth at 23°C. However, several observations indicated that at 23°C both *pdsl*−1 and *pdsl*−Δ2 mutants had a growth defect that probably resulted from faulty chromosome segregation. First, these mutants formed heterogenous-sized colonies, many of which were very small. Second, in cultures grown at 23°C only 60% of *pdsl*−1 and 40% of *pdsl*−Δ2 cells were able to form colonies. When *pdsl*−Δ2 cultures were grown at 19°C, the percent of cells able to form colonies increased to 67%. Cells that were unable to form colonies gave rise to microcolonies consisting of 3–50 cells. Apparently, *pdsl* cells frequently undergo lethal events that cause cell division to cease after several generations. Third, at 23°C the loss rate of chromosome III was 6.5 × 10⁻⁴ in *pdsl*−1 homozygous diploid cells and 2.2 × 10⁻⁶ in wild-type diploid cells. The recombination rate of chromosome III was 3.9 × 10⁻⁵ in *pdsl*−1 homozygous diploid cells and 3.8 × 10⁻⁶ in wild-type diploid cells. This 300-fold induction of chromosome loss in the *pdsl* mutant with only a 10-fold increase in recombination is diagnostic of a chromosome segregation defect (Hartwell and Smith, 1985). The high rate of chromosome loss in *pdsl* mutants is sufficient to explain our observations of colony growth and morphology since a particular chromosomal aneuploidy can either lead to cell death (microcolonies) or variable slow growth (colonies of heterogeneous size). We conclude that the Pds1p function is important for growth and chromosome segregation even at 23°C.

Chromosome Segregation Defect of the *pdsl* Mutants at 37°C Is Caused by a Defect in Anaphase

To assess whether the chromosome segregation defect of the *pdsl* mutants was caused by spindle malfunction, the spindle morphology in wild-type and *pdsl* cells was examined by indirect immunofluorescence and by electron microscopy. Elongated spindles typical of telophase cells were frequently observed in wild-type cells at 23 or 37°C (data not shown) or in the *pdsl* mutants at 23°C (Fig. 1 A, large arrows). In contrast, elongated spindles were absent in the *pdsl* mutant after 4 h of growth at 37°C (Fig. 1 A). The lack of elongated spindles under these conditions is consistent with the absence of segregated chromosomes in large budded cells (see above). Many large budded cells of *pdsl* mutant contained short spindles. Some of these spindles were shorter than the pre-anaphase spindles in the wild-type cells and appeared to cause invaginations of the nuclear envelope (Fig. 2 A). However, the morphology of individual microtubules and spindle pole bodies (*n* = 44) appeared normal at the level of the electron microscopy (Fig. 2 A). These results suggested that the chromosome segregation defect in the *pdsl* mutants at 37°C reflects a
Figure 3. Flow cytometry of the DNA content of pds1 and wild-type cells. Haploid wild-type strain, 5317-2-2, (A) and pds1-1 strain, 661-1-3 (B and C), were grown to logarithmic phase at 23°C and shifted to 37°C for 4 h. Cells were fixed before and after temperature shift and prepared for flow cytometry. The profile of cells wild-type cells at 23°C was similar to 37°C (data not shown). Positions of cells with 1C, 2C, and 4C DNA are shown by arrows.

Figure 2. Indirect immunofluorescence and electron microscopic analysis of spindles and spindle pole bodies (SPBs) in pds1 mutant. (A) The pds1-1 strain, 661-1-3, was grown to logarithmic phase at 23°C, and then shifted to 37°C for 4 h. Cells were fixed and processed for electron microscopy (see Materials and Methods). Three serial sections of a single cell containing multiple SPBs are shown. Three SPBs in the same nucleus are numbered from 1–3. An arrow indicates the invaginated nuclear membrane. (B) pds1-1 strain, 661-1-3, were grown as described in A. Cells were fixed before or after the temperature shift and processed for DNA staining with DAPI and SPB staining using an antibody directed against the 90-kD SPB antigen (see Materials and Methods). Arrowheads indicate cells accumulating more than two SPBs. Bars: (A) 1 μm; (B) 5 μm.

The pdsl Mutant Does Not Exhibit Cell Cycle Arrest at 37°C

When pdsl cultures were grown at 37°C, cells were observed in which cytokinesis appeared to be cleaving the undivided nucleus asymmetrically (Fig. 1 B, small arrowheads). Furthermore, pdsl cells also formed aploid cells at 37°C (Fig. 1 B, large arrowheads). We conclude that cytokinesis is not inhibited in pdsl mutants.

To examine whether DNA replication was affected in the pdsl mutants, the DNA content of wild-type and pdsl haploid cells was analyzed by flow cytometry. pds1 cells at 23°C have a similar profile to wild-type cells at 23°C and 37°C, exhibiting only 1C (G1) and 2C (G2) peaks (Fig. 3 and data not shown). In the pds1 culture grown at 37°C, the peaks of 1C and 2C cells decreased while peaks of cells containing less than 1C and more than 2C DNA appeared after 4 h temperature shift (Fig. 3). The presence of cells with less than 1C DNA content corroborated the unequal chromosome segregation observed cytologically. In addition, the presence of cells with more than 2C DNA content indicates that many pdsl cells are able to enter a second round of DNA replication at 37°C.

To assess whether the pdsl mutants affect SPB duplication, we visualized SPBs by indirect immunofluorescence with anti-tubulin and anti-SPB antibodies as well as by electron microscopy (Fig. 2). Yeast cells normally contain a single SPB in G1 and two SPBs for the remainder of the cell cycle. In contrast, when pdsl cells were grown at 37°C, they accumulated excess SPBs. The maximum number of SPB observed in a single nucleus was 4. Since the maximal DNA content also centered around 4C, these results are consistent with the hypothesis that the SPB duplication is still coupled to DNA replication and that the accumulation of SPBs occurs by their missegregation. In summary, at 37°C the pdsl mutants are defective in anaphase but still continue into the next cycle as evidenced by the completion of cytokinesis, the initiation of a new round of DNA replication and the duplication of SPBs.

The PDS1 Gene Encodes a Novel Acidic Protein of 42 kD

The PDS1 gene was cloned by transforming pdsl-1 cells with a yeast genomic DNA library and selecting for Ts+ transformants. Subcloning revealed that the minimal complementing region was contained within a 1.5-kb NheI-ScaI insert (Fig. 4 A). Introduction of a plasmid bearing the 1.5-kb insert (pAY39) complemented the Ts− and all other phenotypes of the pds1-1 mutant (Materials and Methods). The TRP1 gene was integrated adjacent to the site of the putative PDS1 gene and tetrad analysis was used to demonstrate that the 1.5-kb NheI-ScaI insert was derived from the PDS1 locus (see Materials and Methods).

The DNA sequence of the minimal-complementing insert revealed a 1.1-kb open reading frame (ORF) encoding...
a potential protein of 373 amino acids with a predicted size of 41.8 kD (Fig. 4B). Preceding this ORF was an MluI site commonly observed in genes whose transcription is cell cycle regulated (Fig. 4B). Almost all of the ORF was deleted by inserting a DNA fragment with the LEU2 gene between NheI and HindIII sites (Fig. 4A). The introduction of this deletion into a wild-type strain (see Materials and Methods) generated a mutant (pdsl-A2) that had phenotypes similar to the original pdsl-1 mutant (see below). The similarity was explained by the sequence of the pdsl-1 allele, a premature stop codon that terminates the PDS1 open reading frame at approximately two thirds its normal length (Cohen-Fix, O., and D. Koshland, unpublished results). These results confirmed that this ORF encoded the PDS1 protein (Pdslp).

The amino acid sequence deduced from the PDS1 ORF revealed that the Pdslp was overall highly acidic with an estimated pI of 4.7. However, a more detailed analysis revealed that Pdslp appears bipartite with a pI of 11.1 for residues 1-175 and 3.9 for residues 176-343. The hydropathy profile (Kyte and Doolittle, 1982) revealed no significant hydrophobic stretches. The amino acid sequence of the Pdslp was compared with those generated by translation of the GenBank nucleic acid data base using TBLASTN (Altschul et al., 1990). This comparison revealed that part of PDS1 was sequenced previously as an open reading frame fused with lacZ (GenBank accession number, T17621) (Bums et al., 1994). PDS1 is also identical to the ESP2 gene which was identified from a collection of mutants that accumulate extra SPBs and higher ploidy (Byers, B., personal communication). No other overall significant similarities were detected.

Pdslp has a region (residues 85-93) that has similarity to the cyclin degradation box (Fig. 4C). This degradation box is a 9-amino acid motif that provides a cis signal for cell cycle-dependent degradation of cyclins (Glotzer et al., 1991). The sequence in Pdslp, RLPLAAKDN, is the proper length and has three matches to the most conserved residues, the R at position 1, the L at position 4, and the N at position 9. Among the other residues in the Pdslp box, the L at position 2, the A at position 5, and the D at position 8 are found at the analogous position in other putative cyclin degradation boxes.

Figure 4. Restriction map and sequence of the PDS1 gene. (A) Shaded arrow shows the position of the open reading frame of the PDS1 gene. Bars show subclones tested for complementation of Ts- phenotype of pdsl mutants. DNA fragments which complement or fail to complement are shown as (+) or (−), respectively. The structure of the pdsl-A2 deletion allele marked by a LEU2 fragment is also shown. (B) DNA sequence of the PDS1 gene and conceptual amino acid sequence of the Pdsl protein. The MluI site upstream of the PDS1 ORF is underlined. (C) Comparison of cyclin-degradation boxes with potential degradation box of Pdslp. List of cyclin degradation boxes were taken from previous publications (Glotzer et al., 1991; Hunt, 1991; Fitch et al., 1992).
of Pds1p is lysine rich as has been observed in other proteins containing cyclin degradation boxes (Hunt, 1991).

The Pds1p Appears to be a Rare Nuclear Protein

We raised rabbit polyclonal antibodies against Pds1p expressed in E. coli and further affinity purified it (see Materials and Methods). Unfortunately, using these antibodies we were unable to detect the Pds1p in the wild-type cells by either Western blot or indirect immunofluorescence. These results suggest that Pds1p is a relatively rare protein when PDS1 transcription is driven by its own promoter. To increase the level of Pds1p, the PDS1 gene was placed under the control of the GAL1 promoter (pGAL-PDS1). When extracts of these cells were subjected to Western blot analysis, a band of 55 kD was evident (Fig. 5 A). The identity of this band as Pds1p was confirmed because it was detectable only in the extract prepared from cells grown in galactose. The difference between Pds1p mobility in gels (55 kD) and its predicted molecular mass (42 kD) may be due to its unusual charge or unidentified protein modifications. When cells expressing Pds1p from the pGAL-PDS1 construct were subjected to indirect immunofluorescence using anti-Pds1p antibody, nuclear staining was observed (Fig. 5 B). This staining was specific to the Pds1p as it was not observed in cells grown in the absence of galactose (Fig. 5 B) nor in control cells lacking the pGAL-PDS1 construct (data not shown). When myc-tagged Pds1p was expressed under its own promoter on a high copy plasmid, nuclear staining was also observed using antibodies against the myc epitope (data not shown). Furthermore, nuclear localization of the Pds1p was in agreement with the nuclear localization reported previously for the PDS1-lacZ hybrid protein (Burns et al., 1994).

PDS1 mRNA Expression Is Regulated in a Cell Cycle-dependent Manner with Maximum Expression at G1/S

Given the important role of Pds1p for anaphase spindle elongation, it was possible that expression of the PDS1 was regulated in a cell cycle-dependent manner. To test this possibility, an asynchronous population of cdc15 cells was arrested uniformly in telophase by incubation at the nonpermissive temperature (37°C), and then allowed to reenter the cell cycle synchronously by returning them to the permissive temperature (23°C). When the PDS1 mRNA levels were determined by quantitative Northern hybridization at various times after the shift to the permissive temperature, it was evident that the level of the PDS1 mRNA oscillated in a cell cycle-dependent manner (Fig. 6). The difference was sixfold between the lowest and highest levels of expression during the cell cycle. The periodicity for the highest levels of expression was 120 min which was similar to the generation time of cdc15 cells at the permissive temperature. The peak of mRNA expression correlated with the presence of unbudded cells (Fig. 6 B, a lower graph), which was shown to be a window of the cell cycle from G1 to early S phase (Strathern et al., 1981). Furthermore, cells arrested in S phase by hydroxyurea had approximately four times as much PDS1 mRNA as either those arrested in G1 by α factor or those arrested in M phase by nocodazole (data not shown). Therefore, the
Figure 6. Oscillation of PDS1 mRNA level in cycling cells. The cdc15-1 strain, 4536-151 (Table 1) was grown to logarithmic phase at 23°C, and then arrested in telophase by incubating at 37°C for 3 h. Cells were released synchronously from the telophase block by shifting the temperature to 23°C and incubating for 4 h. After the temperature shift, portions of the culture were collected at regular intervals and mRNA was isolated. mRNA from each sample was blotted on a membrane and probed with 32p-labeled fragments of PDS1 and ACT1 genes (A). Signals were quantitated using a phosphorimager, and the amounts of PDS1 mRNA normalized to the ACT1 mRNA are shown (B, upper graph). The distribution of cell types at regular time intervals was also determined by scoring 100 cells in each sample (B, lower graph). Open squares, large budded cells with two divided nuclei (telophase cells); filled diamonds, unbudded cells; open circles, small budded cells; and filled triangles, large budded cells with single nucleus.

mRNA level of the PDS1 is regulated in a cell cycle–dependent manner, and maximum mRNA accumulation occurs in a window of the cell cycle between late G1 and S phase.

Pds1p Degradation in G1

We took advantage of our ability to detect overproduced Pds1p driven by the GAL1 promoter to monitor changes in the level of Pds1p at different stages of the cell cycle. Cells with the pGAL-PDS1 construct were arrested in G1, S, or M phase with α-factor, hydroxyurea or nocodazole, respectively. Arrested cells were induced for PDS1 transcription by adding galactose in the media. The relative amount of the Pds1p was determined by Western blot, and the relative amount of PDS1 mRNA levels was determined by Northern (Materials and Methods). The levels of Pds1p in HU-arrested and nocodazole-arrested cells were similar and were at least 20 times higher than in α-factor–arrested cells (data not shown). However, the level of galactose-induced PDS1 mRNA in the α-factor–arrested and HU-arrested cells was very similar. These results suggested that the level of Pds1p was repressed in G1 either
by blocking translation of the PDS1 mRNA or by degradation of Pds1p.

To further address the potential cell cycle regulation of Pds1p levels, we followed the stability of the overproduced Pds1p (Fig. 7). The Pds1p was accumulated by driving transcription under the GAL1 promoter in nocodazole-arrested cells, and then its transcription was shut off by adding glucose into the media. After the shut off, half of the cells were maintained in the M phase by leaving nocodazole in the media. The other half were placed into fresh media that lacked nocodazole but contained α-factor. In this media, cells were released from M phase arrest and progressed to G1 of the next cell cycle. The Pds1p was stable in the cells kept in nocodazole while mRNA disappeared rapidly after shut off of its transcription (Fig. 7 A, lanes 1, 2, and 4). In contrast, in the cells released from nocodazole, Pds1p levels remained constant for 1 h but then dropped significantly by 2 h (Fig. 7 A, lanes 1, 3, and 5). As the PDS1 mRNA is already gone by 1 h, we conclude that the loss of the Pds1p between 1 and 2 h is caused by protein degradation. The degradation was not α-factor dependent since the Pds1p was degraded even in the absence of α-factor (data not shown).

The timing of the Pds1p degradation was evident by indirect immunofluorescence as well as from Western blot analysis. The nuclear staining of the Pds1p was observed in 90% of early M (large budded with a single nuclei) and late M cells (large budded with segregated nuclei) but never in G1 cells (unbudded with a single nuclei) (Fig. 7 B). Furthermore, the disappearance of Pds1p between the 1–2-h intervals correlated with the appearance of G1 cells. Therefore, we conclude that the overproduced Pds1p is degraded in G1 and suggest that the level of Pds1p is regulated in the cell cycle. The degradation of Pds1p in early G1 explains our earlier finding that the induction of PDS1 mRNA in early G1 using the pGAL-PDS1 construct does not cause accumulation of the Pds1p. Finally, these results suggest that even though PDS1 mRNA peaks at the G1/S boundary, a significant amount of Pds1p can be stable until late M.

The Temperature Sensitivity of Anaphase in pds1 Mutants Results from a Temperature-sensitive Step between G1 and S

Given that Pds1p appeared to be stable from the G1/S boundary to late M, we wanted to begin to address where in the cell cycle Pds1p function is needed. For this purpose, we asked whether the temperature sensitivity of anaphase in pds1 mutants resulted from a temperature-sensitive step at a particular stage of the cell cycle. Wild-type and pds1 cells were synchronized in G1 with α-factor, in early S with hydroxyurea or in M with nocodazole (Materials and Methods). Synchronized cells were shifted to 37°C and then allowed to progress through the cell cycle by removal of the arresting reagent (Materials and Methods). Cells were fixed at various times after release and assayed for the completion of anaphase as evidenced by the appearance of telophase cells (segregated chromosomes and elongated spindles).

A significant fraction of wild-type cells released from all three arrest points eventually progressed through mitosis to produce telophase cells (Fig. 8, A, C, and E). Similarly, after release from either hydroxyurea or nocodazole arrest, most pds1-1 cells also eventually progressed to telophase at 37°C (Fig. 8, D and F). The broad peaks in these graphs apparently reflected asynchronous recovery of cells from the arresting reagent. In contrast, during the same time course almost no telophase cells were observed for pds1-1 mutants after released from G1 arrest at 37°C (Fig. 8 B). Hence, these cells were unable to complete anaphase, and additional experiments showed that they exhibited all of the phenotypes of asynchronous cultures of pds1 mutants grown at 37°C (data not shown). This indicated that the Pds1p function is necessary after the α-factor arrest point (G1) to complete subsequent anaphase at 37°C. Finally, cells exposed to 37°C only between the α-factor and HU steps were unable to complete the ensuing anaphase (data not shown). Similar results were observed in the PDS1 deletion strain (pds1-42) (data not shown). These results suggest that the temperature sensitivity of anaphase
in cells lacking Pds1p results from a temperature-sensitive step between G1 and S. Therefore, in cells at 37°C, Pds1p function is required early in the cell cycle for subsequent spindle elongation at anaphase. Once cells progress beyond G1/S, the function of Pds1p is no longer required for the ensuing spindle elongation either because the Ts step has been completed or other proteins perform the function of Pds1p at these later stages of the cell cycle. Note these experiments do not eliminate other functions for Pds1p at later stages of the cell cycle and in fact Pds1p does have a role as an anaphase inhibitor (see Yamamoto et al., 1996).

Discussion

In this study we report the isolation of the pds1-1 mutant of S. cerevisiae by a visual screen designed to detect gross missegregation of chromosomes. Further analysis of pds1-1 and a deletion allele (pds1-Δ2) demonstrates that these pds1 mutants have defects in chromosome segregation. At 23°C pds1 cells exhibit a 300-fold increase in chromosome loss. Such a high rate of chromosome loss may be sufficient to explain the inviability of over 40% of pds1 cells grown at this temperature. At 37°C pds1 cells are inviable. They form spindles but the spindles fail to elongate leading to complete failure in proper chromosome segregation. Interestingly, the defect in anaphase at 37°C apparently results from a temperature-sensitive step at the G1-S boundary, around the time of spindle assembly. We also provide evidence that Pds1p localizes to the nucleus where spindle elongation and chromosome separation occur. Taken together these results suggest that Pds1p is required for the faithful execution of anaphase in budding yeast.

Comparison of pds1 Mutants and Other Spindle-Defective Mutants

pds1 mutants have three phenotypes that are similar to those reported for a subset of previously identified spindle-defective mutants. First, like esp1 mutants, pds1 mutants form bipolar spindles in which both SPBs appear functional. This contrasts to other spindle-defective mutants that block SPB duplication (cdc31, kar1, or mps1), fail to separate SPBs (cin8 kip1 double mutant) or form a defective new SPB (mps2 or ndc1) (Byers and Goetsch, 1974; Rose and Fink, 1987; Baum et al., 1986; Winey et al., 1991; Hoyt et al., 1992; Roof et al., 1992; Winey et al., 1993). Temporal studies of spindles in esp1 mutants indicate the spindles are normal until just before anaphase at which time the spindles begin to fray (McGrew et al., 1992). Similar results have been obtained for esp2/pds1 (Byers, B., personal communication). These similarities between pds1 and esp1 phenotypes suggest that the Pds1 and Esp1 proteins function directly or indirectly to facilitate spindle elongation. However, Pds1p is not essential for vegetative growth while Esp1p is (this study; McGrew et al., 1992), and pds1 mutants are nocodazole sensitive while esp1 mutants are not (Yamamoto et al., 1996; Yamamoto, A., and D. Koshland, unpublished data). These phenotypic differences indicate that Pds1 and Esp1 proteins may also have additional functions that differ from one another.

Second, pds1 mutants are like mps1 and esp1 mutants because at 37°C these spindle-defective mutants fail to couple cytokinesis or DNA replication with the prior completion of anaphase (this study; McGrew et al., 1992; Winey et al., 1991). This contrasts with the cell cycle arrest observed in the mps2, kar1, or cdc31 spindle-defective mutants (Pringle and Hartwell, 1981; Rose and Fink, 1987; Winey et al., 1991). One possible explanation for this difference is that cells have checkpoint(s) that respond to only a subset of spindle defects, and no checkpoint responds to the particular type of spindle defect in pds1, esp1, or mps1 mutants. A priori, it would seem extremely unlikely for a cell to have checkpoints that monitor every aspect of a complex process like mitosis. In fact, mutants in topoisomerase II or in Scc proteins have defects in mitotic chromosome structure, yet these mutants fail to arrest (Holm et al., 1985; Strunnikov et al., 1993, 1995). However, it is also possible that pds1, esp1, or mps1 mutants fail to arrest cell division because they have defects in the checkpoint mechanism itself. In support of this model, we have shown that Pds1p also functions as a regulator of anaphase and participates in a DNA-damage and possibly a spindle-damage checkpoint (Yamamoto et al., 1996). In addition, Mps1p has been shown to be a kinase that is required for a spindle-damage checkpoint (Lauzé, et al., 1995; Winey, M., personal communication). Elucidating the reason pds1 mutants fail to arrest will require a better understanding of the molecular function of Pds1p.

Finally, pds1 mutants share one phenotype with mutants defective in the kinesin-related Cin8p. At 23°C deletion mutants of either PDS1 or CIN8 show segregation defects but are viable while at 37°C both deletion mutants have dramatic spindle defects and are dead (this study; Hoyt et al., 1992; Roof et al., 1992). Thus both Pds1p and Cin8p appears to be important for chromosome segregation at 23°C but essential at 37°C. It is known that cin8 deletion mutants survive at 23°C because of a second kinesin-related protein, Kip1p (Hoyt et al., 1992; Roof et al., 1991). In this light, the survival of pds1 deletion mutants at 23°C may also reflect the existence of another protein that is functional redundant with Pds1p.

Cell Cycle Regulation of PDS1 mRNA and Pds1p

The level of PDS1 mRNA changes at least sixfold over the cell cycle with maximal expression in G1/S. Upstream from the coding region of PDS1 is a sequence that matches the consensus for the Mlu cell cycle box (Johnston et al., 1991; McIntosh et al., 1991). This sequence may well be responsible for the regulated PDS1 mRNA expression since it is present in upstream regions of genes encoding DNA synthesis proteins and mediates their mRNA induction at the G1/S boundary. While many of these genes have more than one Mlu-box in their upstream region, a single box in front of reporter gene is sufficient to confer cell cycle regulation (McIntosh et al., 1991). Interestingly, a Mlu-box has also been found upstream of the coding region of the Nuf1 spindle component and the NUF1 mRNA is also maximally expressed in G1-S (Kilmartin et al., 1993).

We also provide evidence that the level of Pds1p is regulated by cell cycle-dependent degradation. We show that overproduced Pds1p is stable from early S phase until late...
telophase but is rapidly degraded in G1. It is unlikely that Pds1p degradation in G1 is an artifact of its overproduction, however it is possible that Pds1p degradation begins earlier than the late M-G1 transition, and overproduction masks our ability to detect it. A particularly interesting possibility is that a small active pool of Pds1p may be degraded by localized degradation (Yamamoto et al., 1996).

Interestingly, both mitotic structural and regulatory proteins such as kinesins, cyclins, and INCENPs have been shown to be degraded near the late M-G1 transition (Cooke et al., 1987; Yen et al., 1992; Amon et al., 1994). Pds1p has a 9-amino acid region with similarity to the cyclin-degradation box, a cis sequence critical for cell cycle-dependent degradation of cyclins (Glotzer et al., 1991). Clearly, one attractive idea is that the Pds1p is degraded by a mechanism dependent upon its putative cyclin-degradation box. In support of this idea, the pattern of Pds1p degradation during the cell cycle is similar to the yeast mitotic cyclins (this study; Amon et al., 1994). However, the potential cyclin-degradation box in Pds1p is less similar to the consensus motif than other cyclin-degradation boxes so the relevance of the box in Pds1p for its degradation needs to be tested.

The Complex Function of Pds1p for Anaphase and the Cell Cycle

Additional studies from our laboratory show that Pds1p is an inhibitor of anaphase that can act in the G2-M window of the cell cycle (Yamamoto et al., 1996). Our results reported here extend the function of Pds1p in two ways. First, we show that Pds1p is required for faithful execution of anaphase in addition to inhibiting anaphase. Second, we show that the anaphase defect of pds1 mutants grown at 37°C apparently results from a temperature-sensitive step at the G1/S boundary. The simplest interpretation of this result is that Pds1p acts at the time of the temperature-sensitive step. If this is the case, then Pds1p acts at the G1/S boundary as well as in M, in other words, Pds1p is used during the cell cycle either at multiple discrete intervals or continuously over an extended window.

To explain these additional properties of Pds1p, we entertain three possibilities. First, Pds1p has a single activity and function which is to inhibit anaphase continuously from the G1/S boundary to the onset of anaphase. In the absence of Pds1p some anaphase functions are activated precociously crippling subsequent spindle elongation at anaphase. Second, Pds1p regulates different aspects of mitosis at different times in the cell cycle. In fact, Cdc2 kinase and other cell cycle regulators have been shown to act at multiple stages of the cell cycle presumably by modifying different targets at each stage. One particular kinase of interest is the PLK1 kinase which has recently been shown to associate with the mitotic-organizing center (higher eukaryotic equivalent of the SPB) in interphase and then to move to the midzone of the spindle around the onset of anaphase (Golsteyn et al., 1995). This pattern of localization is consistent with PLK1 kinase modifying different targets to regulate events that occur both early in spindle assembly and at anaphase, as may be the case for Pds1p. Finally, it is possible that Pds1p has two distinct activities perhaps confined to different domains. For example, one domain serves as a structural component necessary for anaphase and another domain is a regulator of anaphase. The fact that the NH2-terminal half of Pds1p is very basic while the COOH-terminal half is very acidic is consistent with the idea that Pds1p has two distinct structural domains capable of distinct functions. Furthermore, the existence of a bifunctional protein has precedent in DNA polymerase e of budding yeast (Navas et al., 1995). This protein has a catalytic domain required for its polymerase activity and a regulatory domain that functions in DNA-damage checkpoint. While discriminating between these possibilities for Pds1p will require additional experimentation, it is already clear that Pds1p is a central player in mitosis of budding yeast.

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