A Late Mitotic Regulatory Network Controlling Cyclin Destruction in *Saccharomyces cerevisiae*

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Exit from mitosis requires the inactivation of mitotic cyclin-dependent kinase–cyclin complexes, primarily by ubiquitin-dependent cyclin proteolysis. Cyclin destruction is regulated by a ubiquitin ligase known as the anaphase-promoting complex (APC). In the budding yeast *Saccharomyces cerevisiae*, members of a large class of late mitotic mutants, including cdc15, cdc5, cdc14, dbf2, and tem1, arrest in anaphase with a phenotype similar to that of cells expressing nondegradable forms of mitotic cyclins. We addressed the possibility that the products of these genes are components of a regulatory network that governs cyclin proteolysis. We identified a complex array of genetic interactions among these mutants and found that the growth defect in most of the mutants is suppressed by overexpression of *SPO12*, *YAK1*, and *SIC1* and is exacerbated by overproduction of the mitotic cyclin Clb2. When arrested in late mitosis, the mutants exhibit a defect in cyclin-specific APC activity that is accompanied by high Clb2 levels and low levels of the anaphase inhibitor Pds1. Mutant cells arrested in G1 contain normal APC activity. We conclude that Cdc15, Cdc5, Cdc14, Dbf2, and Tem1 cooperate in the activation of the APC in late mitosis but are not required for maintenance of that activity in G1.

INTRODUCTION

Progression through the eukaryotic cell division cycle is governed by oscillations in the activities of cyclin-dependent kinases (CDKs). Entry into mitosis is initiated by mitotic CDK–cyclin complexes, including the Cdc2–cyclin B complex in vertebrates and the Cdc28–Clb complex of *Saccharomyces cerevisiae* (King *et al.*, 1994; Nasmyth, 1996; Morgan, 1997). Exit from mitosis requires CDK inactivation, which is accomplished primarily by ubiquitin-dependent destruction of the cyclin subunit (Murray, 1995; King *et al.*, 1996; Hoyt, 1997). The importance of cyclin destruction for exit from mitosis is underscored by the observation in a wide range of eukaryotes that overexpression of nondestructible forms of mitotic cyclins causes cells to arrest in anaphase (Murray *et al.*, 1989; Gallant and Nigg, 1992; Holloway *et al.*, 1993; Surana *et al.*, 1993; Rimmington *et al.*, 1994; Sigrist *et al.*, 1995; Yamano *et al.*, 1996). Under some conditions, however, additional CDK inactivation mechanisms allow mitotic exit in the absence of complete cyclin destruction (Minshull *et al.*, 1996; Toyn *et al.*, 1996; Schwab *et al.*, 1997; Visintin *et al.*, 1997; Jin *et al.*, 1998).

Mitotic cyclin destruction requires the covalent attachment of a chain of ubiquitin molecules to a region near the amino terminus of the cyclin protein (Glotzer *et al.*, 1991). Ubiquitination of cyclin, like that of other proteins, begins with the transfer of ubiquitin from the ubiquitin-activating enzyme (E1) to a ubiquitin-conjugating enzyme (E2) (Hershko *et al.*, 1994; King *et al.*, 1995; Hochstrasser, 1996). The E2, together with a ubiquitin ligase (E3), transfers the ubiquitin onto the cyclin substrate. The E3 required for cyclin ubiquitination is a multisubunit protein complex known as the anaphase-promoting complex (APC) or cyclosome (Irniger *et al.*, 1995; King *et al.*, 1995; Sudakin *et al.*, 1996; Peters *et al.*, 1996; Zachariae *et al.*, 1996, 1998; Hwang and Murray, 1997; Kramer *et al.*, 1998; Yu *et al.*, 1998). Several lines of evidence suggest that the APC mediates the key regulatory step in cyclin destruction (Hershko *et al.*, 1994; King *et al.*, 1995; Sudakin *et al.*, 1995).

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In addition to being required for the ubiquitination of mitotic cyclins, the APC also catalyzes the ubiquitination of other mitotic regulatory proteins. APC-dependent degradation of the Pds1 protein of *S. cerevisiae* (or Cut2 of *Schizosaccharomyces pombe*) is required for progression from metaphase to anaphase (Cohen-Fix *et al.*, 1996; Funabiki *et al.*, 1996); thus, mutation or inhibition of the APC causes a metaphase arrest and not the anaphase arrest that results from overexpression of nondegradable cyclin (Holloway *et al.*, 1993; Irie *et al.*, 1995; Cohen-Fix *et al.*, 1996; Zachariae *et al.*, 1998). Other APC substrates have also been identified in *S. cerevisiae*, including the microtubule-associated protein Ase1, whose destruction is necessary for efficient disassembly of the mitotic spindle (Juang *et al.*, 1997). The APC is also required for the destruction of the WD40 repeat protein Cdc20 and the Polo-related protein kinase Cdc5 (Charles *et al.*, 1998; Prinz *et al.*, 1998; Shirayama *et al.*, 1998).

Studies of APC regulation have focused almost exclusively on its cyclin–ubiquitin ligase activity, which increases in metaphase or anaphase and remains high throughout G1 (Amon *et al.*, 1994; King *et al.*, 1995; Lahav-Baratz *et al.*, 1995; Sudakin *et al.*, 1995; Brandeis and Hunt, 1996; Zachariae and Nasmyth, 1996; Charles *et al.*, 1998). In higher eukaryotes, activation of the APC toward cyclin substrates is initiated by Cdc2–cyclin B (Felix *et al.*, 1990; Lahav-Baratz *et al.*, 1995; Sudakin *et al.*, 1995), whereas in budding yeast there is evidence that Cdc28-associated kinase activity inhibits cyclin ubiquitination by the APC (Amon, 1997). Recent studies have also implicated other protein kinases in APC regulation: Polo-related kinases (Plk1 in mammals, Plx1 in *Xenopus*, and Cdc5 in budding yeast) promote APC activation, whereas in mammals and fission yeast protein kinase A (PKA) appears to inhibit cyclin-directed APC activity (Yamashita *et al.*, 1993; Charles *et al.*, 1998; Descombes and Nigg, 1998; Kotani *et al.*, 1998; Shirayama *et al.*, 1998). Little is known about how these various regulatory influences are integrated to provide the correct timing of cyclin destruction.

To ensure the proper order of mitotic events, the APC may also be regulated at the level of substrate specificity. APC-dependent ubiquitination of proteins involved in sister chromatid cohesion (Pds1) occurs at the metaphase-to-anaphase transition, whereas mitotic cyclins (e.g., Clb2, Cdc20, and Ase1) remain stable until the end of anaphase (Pellman *et al.*, 1995; Cohen-Fix *et al.*, 1996; Zachariae *et al.*, 1996; Shirayama *et al.*, 1998). Recent work suggests that this additional level of regulation may be conferred in *S. cerevisiae* by Cdc20 and Hct1/Cdh1 (Schwab *et al.*, 1997; Visintin *et al.*, 1997; Lim *et al.*, 1998; Shirayama *et al.*, 1998). Overexpression of CDC20 results in APC-dependent destabilization of Pds1 but has little effect on the destruction of Ase1 and Clb2; cdc20 mutants arrest in metaphase with stable Pds1 (Sethi *et al.*, 1991; Visintin *et al.*, 1997; Shirayama *et al.*, 1998). Similar evidence suggests that HCT1 promotes the destruction of Clb2 and Ase1 but not that of Pds1 (Schwab *et al.*, 1997; Visintin *et al.*, 1997). The regulation of these putative specificity factors is not well understood, although recent studies suggest that Cdc20 may be regulated by multiple mechanisms: its levels increase during mitosis, and its function may be negatively regulated in response to spindle damage (Hwang *et al.*, 1998; Kim *et al.*, 1998; Prinz *et al.*, 1998; Shirayama *et al.*, 1998).

In *S. cerevisiae*, various genetic screens have led to the identification of a group of mutants that arrest in late anaphase with large buds, an elongated spindle, and separated DNA (Hartwell *et al.*, 1973; Johnston and Thomas, 1982; Johnston *et al.*, 1990; Molero *et al.*, 1993; Shirayama *et al.*, 1994a,b; Luca and Winey, 1998). This arrest phenotype is similar to that observed in yeast overexpressing a nondegradable form of Clb2, raising the possibility that the late mitotic gene products are required for the inactivation of Cdc28–Clb complexes (Surana *et al.*, 1993). Interestingly, the late mitotic mutants all encode potential regulatory proteins, including the protein kinases Cdc15 and Dbf2, the Polo-like kinase Cdc5, the protein phosphatase Cdc14, and the Ras-like GTPase Tem1 (Johnston *et al.*, 1990; Schweitzer and Philipsen, 1991; Wan *et al.*, 1992; Kitada *et al.*, 1993; Shirayama *et al.*, 1994b). Recent studies suggest that Cdc5 promotes mitotic exit by stimulating APC activity toward cyclins (Charles *et al.*, 1998; Shirayama *et al.*, 1998), and it seems likely that the other late mitotic proteins also contribute to the control of cyclin destruction.

In the present work, we address the hypothesis that the proteins encoded by the late mitotic gene family form a regulatory network governing Cdc28 inactivation in late mitosis. In support of this hypothesis, we find that several late mitotic mutants display an extensive array of genetic interactions. These mutants arrest with elevated levels of Clb2, decreased amounts of Pds1, and negligible cyclin-specific APC activity. We therefore conclude that the proteins encoded by the late mitotic genes promote mitotic exit by activating the cyclin–ubiquitin ligase activity of the APC.

**MATERIALS AND METHODS**

**Yeast Strains and Plasmids**

All strains (Table 1) were derivatives of W303 (MATa ade2-1 trp1-1 leu2-3, 112 his3-11, 15 ura3-1 can1-100). Strains were made cogenic by backcrossing at least four times to AF534 and were made barA by a subsequent cross to AF592 (a gift from A. Straight, University of California, San Francisco, CA) or were constructed in AF592 using a pop-in, pop-out strategy (Guthrie and Fink, 1991).

Multicopy plasmids carrying the genes encoded by the late mitotic mutants were cloned as follows. pSS107 (pRS426-CDC15HA) was made by cloning the hemagglutinin (HA) epitope into a PstI site generated by oligonucleotide mutagenesis at the stop codon of a
Table 1. Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
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<td>A. Straight</td>
</tr>
<tr>
<td>AFS92</td>
<td>MATa bar1</td>
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</tr>
<tr>
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<td>MATa bar1 cdc6-1</td>
<td>A. Straight</td>
</tr>
<tr>
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<td>MATa cdc6-2</td>
<td>A. Rudner</td>
</tr>
<tr>
<td>SLJ127</td>
<td>MATa bar1 cdc6-2</td>
<td>This work</td>
</tr>
<tr>
<td>JC34</td>
<td>MATa bar1 cdc5-1</td>
<td>J. Charles</td>
</tr>
<tr>
<td>SLJ250</td>
<td>MATa bar1 cdc14-1</td>
<td>This work</td>
</tr>
<tr>
<td>SLJ256</td>
<td>MATa bar1 dpf2-2</td>
<td>This work</td>
</tr>
<tr>
<td>SLJ200p</td>
<td>MATa bar1 tem1-1-3</td>
<td>This work</td>
</tr>
<tr>
<td>ADR58</td>
<td>MATa bar1 ura3::GAL-CLB2-URA3 (pDK27)</td>
<td>A. Rudner</td>
</tr>
<tr>
<td>ADR1002</td>
<td>MATa pds1::PDS1HA-URA3</td>
<td>This work</td>
</tr>
<tr>
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<td>MATa bar1 pds1::PDS1HA-URA3</td>
<td>This work</td>
</tr>
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<td>MATa bar1 cdc6-1 pds1::PDS1HA-URA3</td>
<td>This work</td>
</tr>
<tr>
<td>SLJ425</td>
<td>MATa bar1 cdc6-2 pds1::PDS1HA-URA3</td>
<td>This work</td>
</tr>
<tr>
<td>SLJ426</td>
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</tr>
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<td>SLJ272</td>
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</tr>
<tr>
<td>SLJ23</td>
<td>MATa bar1 cdc15::CDC15HA3</td>
<td>This work</td>
</tr>
</tbody>
</table>

* All strains are in a W303 background.

** Constructed in AFS92 as described (Shirayama et al., 1994b).

4-kb genomic CDC15 fragment. pJC29 (pRS426-HACDC5) was created by inserting the HA epitope into an Nco1-EcoRI site generated at the start codon of CDC5. The construct contains 300 bp of 5' sequence and 500 bp of 3' sequence in addition to the CDC5 open reading frame. pF3-2 (pRS426-CDC14HA3) contains 564 bp of promoter sequence and the open reading frame of CDC5 fused in frame to a triple HA (HA3) tag in pRS426. To construct pJC57 (pRS426-HA3DBF2), the DBF2 open reading frame and 380 bp of 3' sequence were ligated in frame into a 2μ plasmid containing the DBF2 promoter sequence and a single HA tag. Finally, pJS6 (pRS426-TEM1HA3) was generated by fusing the 3' end of the TEM1 open reading frame (accompanied by 300 bp of 5' sequence) to an HA3 tag in pRS426. All of these constructs were shown to complement the appropriate temperature-sensitive mutant in single copy and on the multicopy plasmid.

Strains containing GAL-CLB2-URA3 were obtained from crosses to ADR58 (a gift from A. Rudner, University of California, San Francisco, CA; Hwang and Murray, 1997). Wild-type and mutant strains containing PDS1HA-URA3 were obtained from crosses to ADR1002, a wild-type strain containing PDS1HA-URA3 integrated at the PDS1 locus (a gift from D. Kosholand, Carnegie Institution of Washington, Baltimore, MD; Cohen-Fix et al., 1996). To construct pSJ50 (GAL-CLB2HA) and pRTK-C1 (GAL-PDS1HA), the open reading frames of CLB2 and PDS1 were amplified from genomic DNA by PCR and cloned into a pRS304-based plasmid (Sikorski and Hieter, 1989) containing the GAL1/10 promoter and a single C-terminal HA tag. Strains containing GAL-CLB2HA or GAL-PDS1HA were made by digesting pSJ50 and pRTK-C1 with BstXI for integration at TRP1.

All CDC15 constructs were derived from a 4-kb PruU genomic fragment containing the CDC15 gene (a gift from A. Rudner; Schweitzer and Philippsen, 1991). To create SLJ23, CDC15 was tagged at the carboxyl terminus with an HA3 tag and integrated into AFS92 by a pop-in, pop-out strategy (Guthrie and Fink, 1991). pSJ103 (pRS426-CDC15HA3) was made by subcloning the CDC15HA3 genomic fragment into pRS426. A kinase-deficient mutant CDC15 (pSJ59) was generated by site-directed mutagenesis of pSJ103 using the following oligonucleotide to change lysine 54 to a leucine (K54L): 5'-GTACACGACCTCTAGAATTGCCACGAC-3'. The wild-type HA3-tagged CDC15 constructs fully complement the growth defects of cdc15-2 and cdc15Δ. The K54L mutant does not complement either strain (our unpublished data).

### Yeast Standards

Standard protocols were used for yeast transformation, genetic analysis, and cell propagation (Guthrie and Fink, 1991). To arrest temperature-sensitive strains, cells were grown at 23°C to midlog phase and arrested with 1 μg/ml α-factor or 15 μg/ml nocodazole at 23°C for 3.5 h or by shifting cells to 37°C for 3.5 h. During the last 30 min of the arrests, α-factor- and nocodazole-arrested cultures were shifted to 37°C in the continued presence of the arresting agent. To measure the turnover of Pds1 and Clb2, cells were grown in YP/2% raffinose to an OD600 of 0.3 and arrested. Expression from the GAL promoter was induced by the addition of galactose to 2% for 30 min. Transcription and translation were then repressed with 2% dextrose and 10 μg/ml cycloheximide, and cells were harvested at the indicated times. Arrest and release from α-factor were done by growing cells at 30°C to an OD600 of 0.3. α-Factor (1 μg/ml) was added for 3 h, cells were pelleted, washed three times in fresh media, and released in fresh media at 30°C.

### High-Copy Suppressor Screen

To screen for high-copy suppressors of cdc15-2, SLJ02 was transformed with a URA3-marked GAL-cDNA library (a gift from Aaron Straight, University of California; Liu et al., 1992). Transformants were selected on SC-ura/dextrose plates at 23°C. Cells were washed off the plates and resuspended in 5C-ura/galactose-nocodazole media and allowed to grow for 6 h at 23°C. The culture was then diluted and plated onto YP/galactose-raffinose plates at 37°C to select for suppressors. From ~25,000 SC-ura/dextrose transformants, 312 colonies formed on YP/galactose-raffinose at 37°C. The putative sup-

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pressors were retested for growth at 37°C. Growth at 37°C was then shown to be plasmid and galactose dependent for 189 of the suppressors.

Ninety-two suppressors were chosen for further analysis. Restriction mapping and sequence analysis of 12 cDNAs revealed that SPO12 or SIC1 were responsible for suppression. To allow rapid analysis of the remaining suppressors, whole-colony PCR was done using a primer complementary to the GAL promoter and a primer in the SPO12 gene or the SIC1 gene. In two independent PCR analyses, 71 of the suppressors were found to be SPO12, and 15 of the suppressors were SIC1. Sequencing of plasmids rescued from the six remaining suppressors revealed that there were three identical fusion genes with the kinase domain of YAK1 (594 bp downstream of the start codon), one was CDC15, and two were YGR230W, an open reading frame with homology to SPO12 on chromosome VII. All of these plasmids retested in their ability to restore growth to cdc15-2 at 37°C.

**Lysate Preparation and Immunoblotting**

Yeast lysates were prepared by resuspending cells in 3–5 pellet vol of ice cold LLB (50 mM HEPES–NaOH, pH 7.4, 75 mM KCl, 50 mM NaF, 50 mM β-glycerophosphate, 1 mM EGTA, 0.1% NP40, 1 mM dithiothreitol [DTT], 1mM phenylmethylsulfonylfluoride, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin) and lysing by mechanical disruption in a Beadbeater (Biospec Products, Bartlesville, OK). Lysates were clarified by centrifugation at 14,000 X g for 10 min at 4°C. Protein concentrations of extracts were determined with the Bio-Rad (Hercules, CA) protein assay, using BSA as a standard.

For immunoblots, equal amounts of total protein were loaded on SDS-PAGE gels, and proteins were electrophoretically transferred to nitrocellulose. Cdc2 and Cdc28 proteins were detected with affinity-purified polyclonal antibodies as described (Gerber et al., 1995; Charles et al., 1998). For detection of HA-tagged proteins, the mouse monoclonal antibody 12CA5 was used as previously described (Gerber et al., 1995). Sic1 immunoblots were performed with a 1:1000 dilution of α-Sic1 polyclonal antibodies (a gift from M. Tyers, University of Toronto, Toronto, Canada; Skowyra et al., 1997).

**Kinase Assays**

To measure Cdc15-associated kinase activity, cell lysates (250 μg) were incubated with 3 μg of 12CA5 and protein A-Sepharose (Sigma, St. Louis, MO) for 2 h at 4°C. Immune complexes were washed three times in LLB and once in kinase buffer (50 mM HEPES–NaOH, pH 7.4, 5 mM MgCl2, 2.5 mM MnCl2, 5 mM β-glycerophosphate, and 1 mM DTT) and incubated for 10 min at 23°C in a 20-μl reaction mixture containing 20 μM ATP, 2 μg of myelin basic protein (MBP), and 5 μCi of [γ-32P]ATP (3000 mCi/mmol) in kinase buffer. Reaction products were analyzed on 12% SDS-PAGE gels followed by autoradiography.

Cdc2-associated kinase activity was measured as described (Gerber et al., 1995) by immunoprecipitating Cdc2 from 100 μg of yeast lysate with 0.3 μg of affinity-purified anti-Cdc2 antibody and protein A-Sepharose for 2 h at 4°C.

**In Vitro Ubiquitination Assay**

Ubiquitin ligase activity of the APC was measured as described (Charles et al., 1998). Briefly, the APC was immunoprecipitated with 12CA5 monoclonal antibodies from 500 μg of yeast lysate (containing Cdc27HA, a gift from P. Hieter, University of British Columbia, Vancouver, Canada; Lamb et al., 1994). Immune complexes were washed three times in LLB, once in high-salt QA (20 mM Tris-HCl, pH 7.6, 250 mM KCl, 1 mM MgCl2, and 1 mM DTT), and twice in buffer QA (20 mM Tris–HCl, pH 7.6, 100 mM KCl, 1 mM MgCl2, and 1 mM DTT) and were then incubated for 15 min at 23°C in a 15-μl reaction containing 3.5 pmol of Uba1, 47 pmol of Ubc4, 1 mM ATP, 20 μg of bovine ubiquitin (Sigma), and 0.25 μl of 125I-labeled sea urchin (13–91) cyclin B1 in buffer QA. Reaction products were electrophoresed on 7.5–15% gradient gels and analyzed for ubiquitin conjugates by autoradiography with the Bio-MaxMS system (Eastman Kodak, Rochester, NY).

**RESULTS**

**Genetic Interactions among Late Mitotic Mutants**

The similar anaphase arrest phenotype of cdc15-2, cdc5-1, cdc14-1, dbf2-2, and tem1-3 mutants suggests that the proteins encoded by these genes may have overlapping functions in the control of mitotic exit. Consistent with this possibility, a variety of previous studies have revealed that overexpression of some late mitotic genes results in growth of other late mitotic mutants at the nonpermissive temperature (Kitada et

<table>
<thead>
<tr>
<th>Vector</th>
<th>CDC15HA</th>
<th>HACDC5</th>
<th>CDC14HA3</th>
<th>HA3DBF2</th>
<th>TEM1HA3</th>
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<td>WT</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

* Wild-type and mutant strains were transformed with the indicated gene on a 2μ plasmid carrying a URA3 marker (pRS426; Sikorski and Hieter, 1989) and transformants were selected on SC-ura plates at 23°C. Suppression of each mutant arrest was analyzed by streaking for single colonies on SC-ura plates at 37°C. +, Robust growth on SC-ura plates at 37°C; +, weak growth on SC-ura plates at 37°C; –, no growth on SC-ura plates at 37°C. In some cases, similar results have been obtained in previous studies, as indicated by the superscripts.

b Kitada et al. (1993).

c Result differs from that previously reported; previous work used the cdc15-1 and dbf2-1 alleles in a different strain background.

d Shirayama et al. (1996).

e Shirayama et al. (1994b).
We extended these studies by carrying out a systematic high-copy suppression analysis of the major late mitotic mutants in a common strain background. Multicopy plasmids carrying CDC15, CDC5, CDC14, DBF2, and TEM1 were each sufficient to rescue the temperature-sensitive growth defects of many of the late mitotic mutants (Table 2). The tem1-3 mutant was suppressed by all of the late mitotic genes except DBF2, whereas cdc14-1 and dbf2-2 grew only when their wild-type genes were supplied. Interestingly, CDC14 was unique in its ability to restore growth to the majority of mutants at 37°C.

Further evidence that the late mitotic mutants are functionally linked is that many double mutants are inviable (Table 3). In addition, most of the viable double mutants exhibited growth defects and reduced viability at semipermissive temperatures (Table 3). In particular, the cdc5-1 and tem1-3 mutants exhibited synthetic interactions with all other late mitotic family members examined. In contrast, the cdc14-1 mutant had no obvious synthetic interaction with cdc15-2 and dbf2-2 mutants and only minor interactions with cdc5-1 and tem1-3. These genetic interactions suggest that the proteins encoded by the late mitotic mutants work together to coordinate exit from mitosis.

### Table 3. Synthetic interactions between late mitotic mutants

<table>
<thead>
<tr>
<th></th>
<th>Maximum permissive temperature (°C)</th>
<th>Maximum permissive temperature of double mutants (°C)</th>
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<tr>
<td></td>
<td></td>
<td>cdc15-2</td>
</tr>
<tr>
<td>cdc15-2</td>
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<tr>
<td>cdc5-1</td>
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<tr>
<td>tem1-3</td>
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</table>

Heterozygous diploids were generated by crossing single haploid mutant strains. Diploids were sporulated for 4 d at 23°C. For each cross, 20–40 tetrads were dissected and analyzed. Dead indicates that no viable double-mutant spores were obtained from nonparental ditype tetrads at 23°C. The maximum permissive temperature for growth of viable double mutants was determined by comparing growth with that of the single-mutant parents at 23, 30, 33, and 37°C. The data are presented twice for clarity.

### High-Copy Suppressors of cdc15-2

To identify additional genes involved in control of exit from mitosis, we performed a screen for GAL-driven cDNAs that allowed growth of a cdc15-2 strain at 37°C.
Table 4. Suppression of late mitotic mutants by GAL-cDNAs

<table>
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<tr>
<th>Effect of GAL-cDNA on colony growth at 37°C*</th>
<th>Vector</th>
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</tr>
<tr>
<td>tem1-3</td>
<td>−</td>
<td>+/+</td>
<td>+</td>
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* Wild-type and mutant strains were transformed with the indicated GAL-cDNA plasmids, and transformants were selected on SC-ura/dextrose at 23°C. To analyze the ability of the GAL-cDNA to restore growth of the mutant at 37°C, cultures were grown overnight to OD600 = 1.0 and spotted onto YP/galactose-raffinose plates in a series of six fivefold dilutions. After 3 d at 37°C, growth on the plates was scored as follows: ++++, robust growth (at the fourth dilution and beyond); ++, growth at the third dilution; +, growth at the first and second dilution; +/−, growth of only the first dilution; −, no growth above background.

b The YAK1 clone contains only the 3′ end of the gene (see MATERIALS AND METHODS for details).

c Growth was very poor, mainly microcolonies.

Finally, growth of cdc15-2 cells was partially restored at 37°C by GAL-driven overexpression of SIC1 (Figure 1), which encodes an inhibitor of Cdc28–Clb kinases and has previously been reported to suppress cdc15 mutants when overexpressed (Mendenhall, 1993; Schwob et al., 1994; Toyn et al., 1996). Growth of cdc15-2 cells was rescued even more effectively by SIC1 on a 2μ plasmid (our unpublished data). The ability of SIC1 to suppress the growth defect in the cdc15 mutant is of particular interest, because it suggests that the primary defect in this mutant is an inability to inactivate Cdc28.

Overexpression of SIC1, SPO12, and Truncated YAK1 Allows Growth of Late Mitotic Mutants

If Cdc15 cooperates with the other late mitotic proteins to regulate exit from mitosis, then high-copy suppressors of cdc15-2 should also allow growth of the other mutants at 37°C. Indeed, overexpression of SIC1 partially restored growth to all of the late mitotic mutants at 37°C (Table 4) (Donovan et al., 1994; Toyn et al., 1996; Charles et al., 1998). SPO12 overexpression resulted in robust growth of cdc15-2, cdc5-1, dfb2-2, and tem1-3 at 37°C but did not restore growth to cdc14-1 cells (Table 4) (Parkes and Johnston, 1992; Toyn and Johnston, 1993; Shirayama et al., 1996). Similarly, overproduction of truncated Yak1 partially rescued the temperature-sensitive growth defect of all late mitotic mutants except cdc14-1 (Table 4).

Overexpression of CLB2 Is Toxic in Late Mitotic Mutants

Mutants defective in cyclin destruction should be sensitive to increased production of cyclin protein. Overproduction of Clb2 is known to be toxic in cdc5-1 and tem1-3 mutants at the permissive temperature but has no effect.
on growth of wild-type strains (Shirayama et al., 1994b; Charles et al., 1998). In the present work, we found that overexpression of CLB2 also prevents growth of cdc14-1 and dbf2-2 mutants at 23°C (Figure 2). Although a cdc15-2 strain overexpressing CLB2 was able to grow at the permissive temperature, the excess CLB2 was lethal in this mutant at a semipermissive temperature (Figure 2). These synthetic interactions are consistent with the possibility that the late mitotic proteins act as positive regulators of cyclin destruction.

Clb2 Destruction Is Reduced in Late Mitotic Mutants

The late mitotic mutants arrest in anaphase with separated chromosomes, suggesting that mutants in these genes may be defective in the destruction of cyclins but not that of Pds1 (Hartwell et al., 1973; Kitada et al., 1993; Surana et al., 1993; Shirayama et al., 1994b; Toyn and Johnston, 1994). We therefore compared Clb2 and Pds1 protein levels in the late mitotic mutants at their arrest point. As previously reported, cdc15-2 and cdc5-1 mutants arrest with high Clb2 levels, whereas only a small fraction of the Pds1 protein remains (Figure 3A) (Cohen-Fix et al., 1996; Charles et al., 1998; Shirayama et al., 1998). Similarly, cdc14-1, dbf2-2, and tem1-3 mutants all arrest with mitotic levels of Clb2 and low levels of Pds1 (Figure 3A), supporting the notion that the late mitotic mutants are defective specifically in the destruction of mitotic cyclins.

To directly measure the stability of Clb2 and Pds1, we constructed cdc15-2 mutant strains containing an integrated copy of CLB2 or PDS1 under the control of the GAL promoter. Each protein was fused to a single copy of an HA epitope tag at its carboxyl terminus. The half-lives of both proteins at various
points in the cell cycle were determined by inducing their expression with galactose and then repressing transcription and translation with dextrose and cycloheximide, respectively. Clb2HA and Pds1HA were competent for destruction, as both were highly unstable in a G1 arrest (Figure 3B). The rapid degradation of both proteins in G1 was dependent on APC function (our unpublished data). In cdc15-2 cells arrested in metaphase with the microtubule-depolymerizing drug nocodazole, Pds1 and Clb2 proteins were both stable (Figure 3B). In contrast, the majority of the Pds1 protein was rapidly degraded at the mutant arrest point (Figure 3B), although a significant fraction of the protein remained stable. This pool of stable Pds1 was larger than that observed in our studies of endogenous Pds1 (Figure 3A), suggesting that it represents an artifact of Pds1 overproduction in late mitotic cells.

Cyclin Ubiquitination by the APC Is Defective in Late Mitotic Mutants
To determine whether decreased Clb2 destruction in the late mitotic mutants is due to a defect in the cyclin-specific proteolysis machinery, we measured the cyclin–ubiquitin ligase activity of the APC in vitro. We used a recently described assay (Charles et al., 1998) in which the APC is immunoprecipitated from yeast extracts with antibodies against an epitope-tagged APC subunit, in this case Cdc27HA expressed on a plasmid under the control of its own promoter (Lamb et al., 1994). The immunoprecipitated APC is incubated with purified yeast E1 (Uba1), E2 (Ubc4), bovine ubiquitin, ATP, and 125I-labeled amino terminus of sea urchin cyclin B1 (Glotzer et al., 1991; Holloway et al., 1993). The conjugation of ubiquitin to the cyclin amino terminus is assessed by PAGE of reaction products.

Mutant strains were arrested in late anaphase by shifting asynchronous cultures to 37°C until 80–95%...
of the cells were arrested as large budded cells. The
APC isolated from the late mitotic mutants at 37°C
had negligible cyclin–ubiquitin ligase activity, except
in the case of cdc14-1 mutants, which reproducibly
contained a small amount of activity (Figure 4A). The
level of APC activity measured in vitro was reflective
of the amount of Clb2 protein and Clb2-associated
kinase activity (Figure 4, B and C, respectively). Thus,
the late mitotic proteins are required for activation of
the APC toward cyclin.

When mutant cells were arrested in G1 with α-factor
and then shifted to the restrictive temperature in the
continued presence of α-factor, the APC activity from
cdc15-2, cdc14-1, dbf2-2, and tem1-3 cells was equivalent
to that of wild-type cells arrested in α-factor (Figure 4A).
cdc5-1 cells displayed low APC activity in G1, probably
because this mutation results in a severe defect in APC
activation even at the permissive temperature (Charles et
al., 1998). We conclude that the late mitotic gene pro-
ducts are required for initiation but not maintenance of
APC activity toward cyclin.

All of the late mitotic mutants arrest with negligible
levels of the Cdk inhibitor Sic1 (Figure 4D). This is
consistent with previous evidence that Cdc28-depen-
dent kinase activity inhibits Swi5-dependent SIC1
transcription and also inhibits Sic1 stabilization (Moll
et al., 1991; Donovan et al., 1994; Toyn et al., 1996;
Verma et al., 1997).

### CDC15 Encodes a Protein Kinase Whose Activity Is
Not Regulated in the Cell Cycle

If the products of the late mitotic genes are activators
of the APC, their activity might be expected to in-

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**Figure 4.** The late mitotic mutants arrest with low APC activity toward cyclin. (A) Wild-type and mutant strains were transformed with a plasmid carrying CDC27HA under the control of its own promoter. Cells were grown to midlog phase at 23°C, cultures were divided, and half were shifted to 37°C for 4 h. The second set of cultures was arrested for 3.5 h at 23°C with 1 μg/ml α-factor and then shifted to 37°C in the presence of α-factor for an additional hour. The Cdc27HA subunit of the APC was immunoprecipitated from 300 μg of cell lysate with 12CA5, and conjugation of ubiquitin to the 125I-labeled amino terminus of sea urchin cyclin B1 was assessed as described in MATERIALS AND METHODS. Ubiquitin conjugates were observed at ~8-kDa intervals above the unconjugated cyclin B1 fragment. The asterisk indicates a nonspecific background band observed in the presence of cyclin substrate alone (far left lane). Note that APC activity in wild-type asynchronous cells is normally lower than that in G1-arrested cells (Charles et al., 1998); in this experiment, the high activity in asynchronous cells is due to the relatively high protein levels in these samples (see anti-Cdc28 Western blot in B). (B) Lysates (~35 μg) from the experiment in panel (A) were subjected to Western blotting with polyclonal antibodies against Clb2 (top) and Cdc28 (bottom). (C) Histone H1 kinase activity was measured in anti-Clb2 immunoprecipitates from 100 μg of cell lysate. (D) Cell lysates (100 μg) were subjected to immunoblotting with affinity-purified polyclonal antibodies against Sic1.
crease in mitosis. Indeed, the expression of \textit{CDC5}, \textit{CDC14}, and \textit{DBF2} is known to peak during mitosis (Johnston \textit{et al.}, 1990; Wan \textit{et al.}, 1992; Kitada \textit{et al.}, 1993); in addition, the levels and kinase activities of the Cdc5 and Dbf2 proteins rise during mitosis and decline as cells enter G1 (Toyn and Johnston, 1994; Hardy and Pautz, 1996; Charles \textit{et al.}, 1998; Shirayama \textit{et al.}, 1998). Studies of Cdc15 protein levels or activity during the cell cycle have not been reported.

\textit{CDC15} is predicted to encode a 110-kDa protein kinase (Schweitzer and Philippsen, 1991). To verify this prediction, we constructed a version of Cdc15 with three copies of the HA epitope tag at its carboxyl terminus and either expressed the gene from its own promoter on a 2\mu plasmid or replaced the endogenous gene with the epitope-tagged copy. Cells expressing Cdc15HA3 but not those expressing untagged Cdc15 contained a 110-kDa protein that was recognized by the anti-HA monoclonal antibody 12CA5 (Figure 5A). Immunoprecipitates from cells expressing Cdc15HA3 contained an associated kinase activity that phosphorylated MBP in vitro (Figure 5B). Kinase activity was abolished by a point mutation at a conserved lysine in the ATP binding site of the Cdc15 kinase domain (K54L; Figure 5B). In addition to phosphorylating MBP, Cdc15HA3 also phosphorylated itself (Figure 5B and our unpublished data).

To analyze Cdc15 protein levels across the cell cycle, cells in which the endogenous \textit{CDC15} was replaced with \textit{CDC15HA3} were arrested in G1 with mating pheromone and then released. Whereas Clb2 protein levels oscillated as cells progressed through the cell cycle, levels of Cdc15 protein remained constant (Figure 6A). We also measured Cdc15-associated kinase activity across the cell cycle, using a strain expressing Cdc15HA3 from its own promoter on a multicopy plasmid. As before, Cdc15 protein levels did not fluctuate as cells were released from a G1 arrest and allowed to proceed through the cell cycle (Figure 6B). Furthermore, neither Cdc15 autophosphorylation nor Cdc15-associated MBP kinase activity appeared to change across the cell cycle (Figure 6B).

\textbf{DISCUSSION}

Several lines of genetic evidence, presented here and in previous work, reveal extensive overlaps in the functions of Cdc15, Cdc5, Cdc14, Dbf2, and Tem1 (Kitada \textit{et al.}, 1993; Donovan \textit{et al.}, 1994; Shirayama \textit{et al.}, 1994b, 1996). First, mutants in these genes arrest at the restrictive temperature with remarkably similar phenotypes, including large buds, extended spindles, separated DNA masses, high levels of Clb2, low levels of Fds1 and Sic1, and low cyclin-directed APC activity. Second, the temperature-sensitive growth defect in many late mitotic mutants can be suppressed by overexpression of other genes in the family. Third, the growth defect in all of the mutants is enhanced by \textit{CLB2} overexpression and suppressed in all but one mutant by overexpression of \textit{SIC1}, \textit{SPO12}, and truncated \textit{YAK1}. Finally, we have found an extensive array of synthetic lethal interactions in strains bearing two late mitotic mutations. These results are all consistent with the possibility that the late mitotic genes promote overlapping functions required for the exit from mitosis.

The functions of the late mitotic genes appear to converge on the cyclin destruction machinery. All five of the genes we studied are required for the activation of cyclin–ubiquitin ligase activity of the APC in late
mitosis, whereas none is required for the maintenance of that activity in G1. We suspect that the products of the late mitotic genes directly promote cyclin-specific APC activation, rather than controlling it indirectly by promoting an essential mitotic process whose completion is required to allow cyclin destruction. The latter possibility does not seem consistent with the ability of \textit{SIC1} overexpression to suppress the growth defects in these mutants. Good evidence for a direct regulatory role exists for Cdc5, whose overproduction at any cell cycle stage triggers APC activation (Charles et al., 1998; Shirayama et al., 1998); in addition, the mammalian homologue of Cdc5, Plk1, is able to directly phosphorylate and activate the APC (Kotani et al., 1998).

Previous work showed that overexpression of genes that antagonize the cAMP pathway suppresses the growth defect in the \textit{cdc15-2} mutant (Spevak et al., 1993). Similarly, we found that many of the late mitotic mutants are suppressed by overexpression of truncated \textit{YAK1}, which may, like full-length \textit{YAK1}, oppose the actions of PKA (Garrett and Broach, 1989; Garrett et al., 1991; Hartley et al., 1994; Ward and Garrett, 1994). Considering recent evidence that PKA acts as an inhibitor of the APC in vitro (Kotani et al., 1998), it might be predicted that inhibition of the PKA pathway by \textit{YAK1} could increase APC activity and thereby allow late mitotic mutants to exit mitosis.

The late mitotic mutants are defective primarily in the degradation of cyclin and not that of Pds1, suggesting that these genes activate the Hct1-dependent pathway that is thought to specify the ubiquitination of late mitotic substrates such as Clb2, Ase1, and Cdc5 (Schwab et al., 1997; Visintin et al., 1997; Charles et al., 1998; Shirayama et al., 1998). The destruction of the majority of Pds1 in \textit{cdc15-2}, \textit{cdc5-1}, \textit{cdc14-1}, \textit{dbf2-2}, and \textit{tem1-3} is consistent with the fact that these mutants complete chromosome segregation. Interestingly, late mitotic mutants arrested in anaphase still contain a small amount of stable Pds1 protein, which may represent an inactive pool of the protein whose destruction is not required for chromosome segregation.

The products of the late mitotic genes may also contribute to Cdc28 inactivation by mechanisms other than cyclin destruction. Recent studies suggest that cyclin destruction is not essential for mitotic exit under some conditions (Minshull et al., 1996; Toyn et al., 1998).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Cdc15 protein levels and kinase activity are constant during the cell cycle. (A) A \textit{cdc15::CDC15HA3} strain (SLJ23) was arrested for 3 h at 30°C with 1 \mu g/ml a-factor, released from the arrest, and allowed to grow at 30°C. Cells were harvested at the indicated times, and lysates (100 \mu g) were analyzed by Western blotting with 12CA5 (top) or anti-Clb2 antibodies (bottom). (B) Wild-type cells carrying \textit{CDC15HA3} on a 2 \mu plasmid were arrested for 3 h at 30°C with 1 \mu g/ml a-factor, released from the arrest, and allowed to progress through the cell cycle at 30°C. Cells were harvested at the indicated times, and lysates (35 \mu g) were analyzed by Western blotting with 12CA5 (top) or anti-Clb2 antibodies (second from top). Cdc15HA3 was immunoprecipitated from 250 \mu g of lysate and tested for its ability to phosphorylate itself (third from top) or MBP (bottom).}
\end{figure}
1996; Schwab et al.  

Figure 7. Model of regulatory pathways governing Cdc28 activity in late mitosis. Late mitotic gene products stimulate mitotic cyclin destruction and may also induce increased levels of Sic1 (see DISCUSSION). This model also accommodates evidence that Cdc28 inhibits APC activity (Amon, 1997) and also inhibits SIC1 transcription and Sic1 stability (Moll et al., 1991; Toyn et al., 1996; Verma et al., 1997), resulting in a feedback system that triggers rapid and complete Cdc28 inactivation when Cdc28 activity is reduced to some threshold. For simplicity, this diagram does not include an additional feedback loop suggested by the observation that Cdc28–Clb complexes stimulate CLB transcription (Amon et al., 1993).

1996; Schwab et al., 1997; Visintin et al., 1997; Jin et al., 1998). Cells lacking HCT1 are able to exit mitosis despite a severe defect in cyclin destruction, possibly because Cdc28 is inactivated in these cells by the inhibitor Sic1 (Schwab et al., 1997; Visintin et al., 1997). The fact that the late mitotic genes are essential for mitotic exit implies that they may have functions in addition to the activation of cyclin destruction. For example, they may stimulate the synthesis or stabilization of Sic1 (Figure 7).

In light of previous evidence that APC-dependent proteolysis is inhibited by Cdc28 activity (Amon, 1997), it is conceivable that late mitotic gene products act entirely through the up-regulation of Sic1, which would lead indirectly to APC activation. This seems unlikely, however, given the fact that the late mitotic genes are essential for viability and SIC1 is not, and given the biochemical evidence that at least one late mitotic gene product, Cdc5, acts directly on the APC (Kotani et al., 1998).

The reversal of Cdc28 action in late mitosis cannot be accomplished solely by Cdc28 inactivation: dephosphorylation of its substrates is presumably required. Thus, defects in the dephosphorylation of Cdc28 substrates would also be expected to result in a late mitotic arrest. Interestingly, Cdc14 is homologous to protein phosphatases and possesses phosphatase activity in vitro (Wan et al., 1992; Taylor et al., 1997), raising the possibility that it is responsible for dephosphorylating Cdc28 substrates. Interestingly, the cdc14-1 mutant displayed unique behaviors in our experiments that are consistent with this possibility: the cdc14-1 mutant defect was not rescued effectively by any of the suppressors, and over-expressed CDC14 was the most effective suppressor of the other mutants.

To understand how the products of the late mitotic genes fit into the complex pathways that trigger Cdc28 inactivation after chromosome segregation, we will need a better understanding of the regulation of these proteins. Production of three of the late mitotic gene products (Cdc5, Cdc14, and Dbf2) is increased during mitosis at the time when APC activation occurs, but the mechanisms underlying this regulation remain obscure (Johnston et al., 1990; Wan et al., 1992; Kitada et al., 1993; Toyn and Johnston, 1994; Hardy and Pautz, 1996; Charles et al., 1998; Shirayama et al., 1998). We found that bulk Cdc15 protein levels and activity do not appear to be regulated during the cell cycle, but this does not exclude cell cycle-dependent changes in Cdc15 localization or accessibility of Cdc15 substrates. Alternatively, constant Cdc15 activity may act through a regulated component of the pathway (such as Cdc5) to specifically activate cyclin proteolysis at the end of mitosis.

The five genes studied in the present work are members of a growing family of genes with overlapping functions in the completion of mitosis. Additional genes in this family include LTE1, which interacts genetically with CDC15 and TEM1 and encodes a putative guanine nucleotide exchange factor (Shirayama et al., 1994a,b, 1996). MOB1 encodes a protein that physically associates with Dbf2 and is required for the completion of anaphase; mob1 mutants display genetic interactions with DBF2, CDC15, CDC5, and LTE1 (Komarnitsky et al., 1998; Luca and Winey, 1998). Dbf2 also interacts physically with the CCR4 transcription complex and might thereby exert effects on gene expression in late mitosis (Liu et al., 1997). The existence of this complex network of late mitotic regulatory proteins implies that progression from anaphase to G1 is a key regulatory transition in the cell cycle. It seems likely that the late mitotic regulators serve as components in signaling pathways that monitor mitotic events and promote Cdc28 inactivation and mitotic exit only upon successful completion of anaphase and preparation for cytokinesis.
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REFERENCES


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cycle mutant gene *dbf4* encodes a protein kinase and is identified as CDC5. Mol. Cell. Biol. 13, 4445–4457.


