Proteolysis of the yeast G1 cyclins is triggered by their Cdc28-dependent phosphorylation. Phosphorylated Cln1 and Cln2 are ubiquitinated by the SCF-Grr1 complex and then degraded by the 26 S proteasome. In this study, we identified a cak1 allele in a genetic screen for mutants that stabilize the yeast G1 cyclins. Further characterization showed that Cln2HA was hypophosphorylated, unable to bind Cdc28, and stabilized in cak1 mutants at the restrictive temperature. Hypophosphorylation of Cln2HA could thus explain its stabilization.

To test this possibility, we expressed a Cak1-independent mutant of Cdc28 (Cdc28-43244) in cak1 mutants and found that Cln2HA phosphorylation was restored, but surprisingly, the phospho-Cln2HA was stabilized. When bound to Cdc28-43244, Cln2HA was recognized and polyubiquitinated by SCF-Grr1. The Cdc28-43244 mutant thus reveals an unexpected complexity in the degradation of polyubiquitinated Cln2HA by the proteasome.

Cell cycle progression is driven by the orderly activation of cyclin-dependent kinases (Cdks).1 Cdk activity requires association with cyclins and Cdk phosphorylation on a conserved threonine in a region called the T-loop by a distinct Cdk-activating kinase (CAK) (1). The concentration of cyclins oscillates during the cell cycle and is determined by successive rounds of transcription followed by ubiquitin-mediated proteolysis by the proteasome (2). Cyclin destruction results in abrupt inactivation of the associated Cdk.

In Saccharomyces cerevisiae, one major Cdk, Cdc28, regulates the cell cycle, and Cak1/Civ1 is the activating kinase that phosphorylates Cdc28 (3–5). Activation of Cdc28 by the G1 cyclins Cln1, Cln2, and Cln3 regulates the G1/S transition (6). Upon Cdc28 binding, the G1-phase cyclins are phosphorylated by the Cdc28 kinase and very rapidly degraded by ubiquitin-dependent proteolytic pathways (7–9). The primary signal that triggers G1 cyclin degradation is their phosphorylation (Refs. 8 and 10–12 and for a review see Ref. 13). Mutation of multiple Cdc28 phosphorylation sites eliminates most Cln2 phosphorylation and results in enhanced Cln2 stability (10). Once G1 cyclins are phosphorylated, their degradation requires their polyubiquitination by a ubiquitin-conjugating enzyme Cdc34 (7, 14) in combination with an SCF ubiquitin-ligase complex composed of at least Skp1, Cdc53, Rbx1/Hrt1, and the F box protein Grr1 (SCF-Grr1) (8, 9, 15–18). The F box protein Grr1 is the substrate specificity factor that recognizes phospho-Cln1/2 (9). Phospho-Cln1 ubiquitination was recently reconstituted in vitro using complexes of SCF-Grr1 immunopurified from insect cells infected with baculoviruses expressing individual subunits and supplemented with purified Cdc34, ubiquitin-activating enzyme, ubiquitin, and ATP (19). The polyubiquitinated G1 cyclins are then degraded by the 26 S proteasome.

Little is known about the requirements for efficient degradation of polyubiquitinated substrates by the 26 S proteasome. The 26 S proteasome consists of a 20 S protease core and a 19 S regulatory cap that recognizes polyubiquitinated substrates. Although the subunits of the 19 S particle have been identified (20), their precise functions in substrate recognition and presentation to the 20 S core particle are not known. The only known proteasomal subunit that binds multiubiquitin chains is Rpn10 (21), but it is not essential for the degradation of many ubiquitinated proteins (22, 23). It was recently shown that Ubr1 and Ufd4, the ubiquitin-ligase complex components of the N-end rule and ubiquitin fusion degradation proteolytic pathways, directly interact with specific proteins of the 26 S proteasome (24). This interaction might facilitate proteasomal recognition of proteins polyubiquitinated by these ubiquitin-ligase complex activities. Moreover, stabilization of ubiquitin fusion degradation substrates in cdc48 ATPase mutants was suggested to be due to defects in post-ubiquitin degradation of these proteins (25). Finally, a genetic, physical, and functional interaction between Cdc28, Cks1, and components of the 19 S regulatory subunit of the proteasome has been described (26). Cks1 is a small, highly conserved protein with poorly defined functions that binds the C-terminal lobe of Cdks (27). The Cdc28–1N mutant is defective in Cks1 binding (27). Stabilization of ubiquitinated Clb2 in cdc28-1N and in cks1 mutants suggested that Cks1 may have a role in controlling proteolysis of M-phase targets (26).

In this study, we found that Cdc28-activating phosphorylation was required for Cln2-Cdc28 complex formation and Cln2 degradation. Through the study of a CAK-independent mutant of Cdc28, we also found that Cln2 phosphorylation and ubiquitination was not sufficient for G1 cyclin degradation by the 26 S proteasome and that Cks1, previously shown to be required for mitotic proteolysis, may also be involved in the proteolysis of the G1 cyclins.

**EXPERIMENTAL PROCEDURES**

*Yeast Strains and General Methods*—The genotypes of the yeast strains used in this study are listed in Table I. Yeast media, growth...
conditions, and genetic and molecular techniques were as previously described (28, 29). Yeast strain EC3195 was made by transforming strain GF3195 with the marker-swaps plasmid pTTH digested with EcoRI/Xhol (30).

Plasmids—Table II shows the plasmids used in this study. Plasmids pTRP250, pHIS250 and pCLN2MYC were constructed as follows. For pTRP250, pCM185 (31) was cut with BamHI and HpaI to allow homologous DNA recombination in yeast with the pCM250 plasmid carrying the CLN2HA gene. The amplified fragment of 4.6 kb was gel-purified. The 3'-end of the amplified fragment was 5'-phosphorylated and purified using a Wizard SV Miniprep System (Promega). The pCM250 plasmid was opened with the downstream stop codon for the CLN2 gene. The amplified DNA fragment was then transformed into the yeast strain BMA64-1A with pCM250 to allow for homologous DNA recombination. Transformants were selected for growth on medium without uracil and histidine.

Western Blot Analyses—Protein extracts were made from 15-ml aliquots of exponentially growing cells (see figure legends for strains). Cells were pelleted, transferred to an Eppendorf tube, and boiled for 2 min in 50 ml of 0.5 M urea. An equal volume of glass beads was added, and the cells were lysed by vortexing 3 min at room temperature. Then 50 ml of lysis buffer (2% SDS, 100 mM Tris-HCl, pH 6.8) were added, and the cells were lysed by vortexing for 3 more min. Extracts were then clarified by centrifuging the samples for 15 at 13,000 rpm. Equal amounts (10–20 mg) of total cell extracts were loaded onto 10% SDS-polyacrylamide gels. Proteins were then transferred to Immobilon P membranes, incubated with 5% non-fat dry milk, and then with primary antibodies diluted 1:2000 in Tris-buffered saline containing 0.1% Tween 20 (TBS/T). After washing, membranes were further incubated with secondary antibodies conjugated to horseradish peroxidase (1:10,000 dilution in TBS/T). Bands were then visualized by chemiluminescence. Chemiluminescent quantification of Western blots was carried out using a FluorChem 8000 digital imaging system from Alpha Innotech Inc.

Comimonoprecipitation of CLN2HA, CLN2MYC, CDC28HA, and GST-GRR1—Log phase yeast cells were harvested (0.7 g wet weight), washed once with cold water, and resuspended in 0.7 ml of cold extraction buffer (50 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 8) containing protease inhibitors (2 mg/ml each of pepstatin, leupeptin, aprotinin, chymostatin, and 1 mM phenylmethylsulfonyl fluoride) and phosphatase inhibitors (10 mM sodium orthovanadate, 15 mM p-NO2-phenyl phosphate, 50 mM b-glycerophosphate). Cells were broken with an Eaton press, and the extract was centrifuged at 4 °C for 25 min at 40,000 rpm. Crude extracts (0.5–6 mg) were incubated for 1 h at 4 °C either with 40 ml of magnetic Dyna-beads containing 2 ml of 12CA5 anti-HA or 9E10 anti-Myc antibodies or with 50 ml of glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech). After washing with extraction buffer, proteins bound to the beads were resuspended in 30 ml of SDS-PAGE sample buffer, heated at 80 °C for 4 min and loaded into SDS-PAGE.

Detection of Cln2-Ubiquitin Conjugates—We essentially used the protocol described by Willems et al. (8), except that the pH of buffer G was raised to pH 8.0. Yeast strains carrying plasmids pUB223 and pCM250 were grown in selective medium to early log phase, and the CUP1 promoter was induced by the addition of 250 mM CuSO4. After 6 h, cells were harvested and broken in buffer G (6 M guanidinium hydrochloride, 0.1 M NaH2PO4, 0.1 M NaCl, and 0.1 M Tris-HCl, adjusted to pH 8.0) with the Eaton press. After centrifugation for 25 min at 40,000 rpm, crude extracts (0.5 mg) were incubated with Ni-NTA agarose resin (Qiagen) for 1 h at room temperature. The beads were washed three times with buffer G and three times with buffer C (100 mM Tris-HCl, pH 8.0, 500 mM NaCl). Bound proteins were eluted by addition of buffer E (100 mM Tris-HCl, pH 6.8, 1% SDS, 100 mM dithiothreitol, 100 mM EDTA) and run on a 6% Tricine gel.

Histone H1 Kinase Assay—Cell extracts and immunoprecipitation were performed as described above. Immune complex kinase assays were carried out as described previously (3).

RESULTS

G1 Cyclins Are Stabilized and Hypophosphorylated in cak1 Mutants—To better understand the molecular mechanisms of G1 cyclin proteolysis, a genetic screen for mutants that stabilize a Cln1-β-galactosidase fusion protein was developed. This screen successfully identified Grr1 as being required for Cln1/2 degradation (33). Here, we characterize another mutant isolated in this screen that stabilized Cln1-β-galactosidase, had an elongated cell shape at 24 °C, and was temperature-sensitive for growth at 37 °C. This phenotype was exploited to clone the corresponding wild-type gene by complementation of the temperature-sensitive growth defect with a yeast genomic DNA library. We identified the mutated gene as the CAC1/CIV1 gene encoding the Cdc28-activating kinase (3–5). Since two different cak1 mutants (civ1-2 and civ1-4) had already been extensively characterized in our laboratory (3), we pursued our study of the Cln1-dependent stabilization of G1 cyclins in these mutants. We determined the half-life of epitope-tagged Cln2 by promoter shut-off experiments with a tacycline-repressible CLN2HA gene (31, 34). The half-life of Cln2HA in the wild-type strain was less than 10 min at 37 °C, whereas in civ1-2 and civ1-4 mutants grown at the restrictive temperature, Cln2HA exhibited a half-life of 40 and 20 min, respectively (Fig. 1A). Furthermore, we observed that Cln2HA migrates as a series of phosphorylated forms (10) in the wild
type and the cak1 mutants at the permissive temperature, whereas it migrates as a single, hypophosphorylated band in the cak1 mutants at the restrictive temperature (Fig. 1, A and B). Since G1 cyclin phosphorylation is required for their rapid degradation (10), the stabilization of Cln2HA observed in the cak1 mutants might be a direct consequence of their hypophosphorylation.

Cak1 Phosphorylation of Cdc28 Is Necessary for Cln2-Cdc28 Complex Formation and Activity—Cln2 phosphorylation is dependent on both Cdc28 activity and on the ability of Cln2 to bind Cdc28 (7, 10). Cak1 activates Cdc28 by phosphorylation of Thr-169 (3–5). We examined whether phosphorylation of Cdc28 by Cak1 was required for Cln2HA-Cdc28 kinase activity and the formation of a Cln2HA-Cdc28 complex. Cln2HA was immunoprecipitated from wild type and cak1 mutants grown at the restrictive temperature, and the associated H1-kinase activity in vitro, as well as the quantity of associated Cdc28, was determined. As shown in Fig. 2, H1 kinase activity and associated Cdc28 could be immunoprecipitated with Cln2HA only from the wild type strain (1st lane), demonstrating that Cln2HA-Cdc28 complex formation and activity requires Thr-169 phosphorylation. It is thus likely that Cln2HA hypophosphorylation and stabilization in the cak1 mutants at 37 °C is due to inactivation of Cdc28 by dephosphorylation of Thr-169. The residual instability of Cln2HA seen in the cak1 mutants (Fig. 1A) may be due to a yet unidentified proteolytic pathway that has been proposed to degrade slowly hypophosphorylated Cln2 that is not bound to Cdc28 (10).

Phosphorylation of Cln2 Is Not Sufficient for Its Degradation—Although CAK1 is essential in wild type strains, Cross and Levine (38) identified mutants of a non-phosphorylatable version of Cdc28 (Cdc28-T169E) that allow viability in the absence of Cak1. We tested whether one of these mutants (Cdc28-43244, mutated at T18S, L61I, H78R, K83R, K96E, A125E, T169E, and A234V) could rescue the growth defect of our cak1 mutants. Cdc28-43244 was able to support growth of the civ1-2 and civ1-4 mutants at 33 °C but, surprisingly, not at 37 °C (Fig. 3). Cdc28-43244 is not itself thermolabile as it allowed growth of a cdc28Δ CAK1− strain at 37 °C (data not shown). The inability of Cdc28-43244 to support growth of the cak1 mutants at 37 °C thus indicates that Cak1 has an essential role at 37 °C that is unrelated to Cdc28 activation (35). We next analyzed the phosphorylation status and stability of Cln2HA in CDC28-43244/cdc28Δ cells in the presence or absence of CAK1. An immunoblot analysis (Fig. 4A) showed that Cdc28-43244 supported G1 cyclin phosphorylation at the same rate as wild type Cdc28, regardless of the presence or absence of Cak1. However, unexpectedly, the phosphorylated Cln2HA was significantly stabilized in CDC28-43244 cells (Fig. 4B). Quantification of three independent experiments revealed that the half-life of Cln2HA at 30 °C in the wild type strain (27 ± 5.3 min) was clearly increased in both the CDC28-43244 (72 ± 9.5 min) and the CDC28-43244/cak1Δ strains (71 ± 3.5 min). This effect was even stronger at 37 °C since Cln2HA decay was much faster for the wild type (Fig. 1 and data not shown) but not for the mutant strains (data not shown). In contrast, mRNA...
levels were not significantly different between the three strains during the promoter shut-off experiment, although doxycycline repression was much faster at 37 °C compared with 30 °C for all three strains (data not shown). The shorter half-life of Cln2HA at 37 compared with 30 °C in the wild type can thus be explained by the more rapid decay of its mRNA at 37 °C.

Phosphorylated Cln2-Cdc28-43244 Binds to Grr1 and Is Ubiquitinated in Vivo—The stabilization of phosphorylated Cln2HA could be due to a number of different mechanisms. Cln2 is phosphorylated by wild type Cdc28 on multiple Ser-Pro and Thr-Pro phosphorylation sites (10). Cdc28-43244 might phosphorylate Cln2HA at non-physiological phosphorylation sites, thus affecting its recognition by the SCF-Grr1 ubiquitin ligase complex. To test this hypothesis, a CLN2HA mutant (referred to as CLN2HA<sup>PTIS</sup>), in which all seven Cdc28 putative phosphorylation sites were mutated to alanine (10), was introduced into two different wild type (W303 and GF312-17C backgrounds) and isogenic CDC28-43244/cak1Δ mutant strains (1834-2A and GF3254, respectively). Cln2HA migrated as a series of lower mobility phosphorylated forms in both CDC28<sup>+/H11001</sup> and CDC28-43244 backgrounds, whereas Cln2HA<sup>PTIS</sup> migrated as a higher mobility hypophosphorylated form in both CDC28<sup>+/H11001</sup> and CDC28-43244 backgrounds (Fig. 5A, compare 1st to 3rd, 2nd to 4th, 5th to 7th, and 6th to 8th lanes). The absence of lower mobility phosphorylated forms of Cln2HA<sup>PTIS</sup> in the CDC28-43244 strains strongly suggests that the mutant Cdc28-43244 kinase phosphorylates Cln2HA on the same Ser-Pro and Thr-Pro sites as the wild type Cdc28 kinase.

Further possible explanations for stabilization of phosphorylated Cln2HA could be that phospho-Cln2HA is not recognized by the SCF-Grr1 or that it is recognized by the latter and polyubiquitinated but not degraded by the 26 S proteasome. To distinguish between these two possibilities, we analyzed the interaction of a GST-tagged Grr1 (16) with Cln2HA and Cdc28. Wild type, CDC28-43244/CAK1, and CDC28-43244/cak1Δ strains were transformed with plasmids expressing GST-Grr1 from the GAL1 promoter and Cln2HA from the tetracycline-repressible promoter (Fig. 5B, lanes 2–4 and 6–8). As a control, the wild type strain was transformed with plasmids carrying pGAL1-GST and pTet-CLN2HA (Fig. 5B, lanes 1 and 5). GST-Grr1 was bound to glutathione-Sepharose beads and any asso-

Fig. 2. Cln2-Cdc28 complex formation and activity requires phosphorylation at Thr-169. A wild type (GF312-17C) and cak1 mutant strains civ1-2 (CMY975) and civ1-4 (GF2351) transformed with pCM250 expressing CLN2HA were grown to log phase at 24 °C and then shifted to 37 °C for 6 h. Total extracts were prepared; Cln2HA was immunoprecipitated with anti-HA antibodies, and the quantity of associated Cdc28 and histone H1 kinase activity was determined as described under “Experimental Procedures.”
ciated Cln2HA, and Cdc28 was revealed with anti-HA and anti-Cdc28 antibodies after SDS-PAGE and immunoblotting. As shown in Fig. 5B, GST-Grr1 bound Cln2HA as well as Cdc28 not only from the wild type strain (lane 6) but also from the strains expressing Cdc28-43244 (lanes 7 and 8), thus demonstrating that the observed defect in Cln2HA degradation is not
due to a loss of interaction between the phospho-Cln2HA-Cdc28-43244 mutant complexes and GST-Grr1. Interestingly, when normalized to the amount of GST-Grr1 precipitated from the wild type extracts, about 2-fold more Cln2HA was bound to GST-Grr1 in Cdc28-43244 extracts compared with Cln2HA bound to GST-Grr1 in the wild type extract. This result suggests that GST-Grr1 may have a higher affinity for phospho-Cln2HA bound to Cdc28-43244 compared with wild type Cdc28.

We next determined whether Cln2HA bound to Cdc28-43244 was ubiquitinated. Polyubiquitinated proteins are generally very difficult to visualize in vivo, because the polyubiquitin chains are hydrolyzed very rapidly by abundant ubiquitin isopeptidase activities (36). To enhance our ability to detect in vivo Cln2HA-ubiquitin conjugates, we used cells expressing a polyhistidine- and MYC-tagged K48R mutant ubiquitin (UbiH(RES-MYC-RA)) (8). UbiH(RES-MYC-RA) can be incorporated into ubiquitin chains, but the K48R substitution prevents further polymerization of ubiquitin from the Lys-48, and the G76A substitution inhibits hydrolysis by ubiquitin isopeptidases. The polyhistidine and Myc tags facilitate purification and detection of low-abundance polyubiquitinated proteins. Cln2HA and UbiH(RES-MYC-RA) were coexpressed in wild type and Cdc28-43244 cells, and denatured protein extracts were prepared. UbiH(RES-MYC-RA) protein conjugates were purified on a Ni-NTA resin, and bound proteins were analyzed by SDS-PAGE and immunoblotting. As detected with an anti-HA monoclonal antibody, lower mobility forms of Cln2HA were retained on the Ni-NTA matrix, indicating the presence of Cln2HA-UbiH(RES-MYC-RA) conjugates in the wild type as well as in the Cdc28-43244 cell lysate (Fig. 5C, lanes 2 and 3). Taken together, these data show that (i) Cdc28-43244 is not affected in its ability to bind and phosphorylate Cln2HA, and (ii) stable, phosphorylated Cln2HA/Cdc28-43244 is bound by the SCF-Grr1 and ubiquitinated.

Cdc28-43244 Bound to Cln2 Loses Its Ability to Interact with Cks1—Cks1/Suc1 proteins are highly conserved, small proteins that bind Cdk s and that have been attributed diverse but poorly characterized functions. Recently, Cks1 was shown to be required for proteolysis of ubiquitinated Clb2 in yeast (26). We thus examined the interaction of Cks1 with Cdc28-43244 in the presence and absence of Cak1. Cdc28HA and Cdc28-43244HA were immunoprecipitated with anti-HA antibodies, and coprecipitation of Cks1 was monitored with anti-Cks1 antibodies after SDS-PAGE and immunoblotting. In both cases, and also in the absence of Cak1, associated Cks1 could be detected (Fig. 6A). To determine specifically whether Cks1 interacts with Cln2-13MYC-Cdc28-43244, we carried out immunoprecipitation experiments of Cln2-13MYC with anti-MYC antibodies in wild type, Cdc28-43244, and Cdc28-43244/cak1Δ strains. Surprisingly, we found that Cks1 interacted much less effectively with Cln2-13MYC-Cdc28-43244 mutant complexes than with their wt type counterparts (Fig. 6B, compare 3rd and 4th lanes to 2nd lane). Cks1 was recently shown to be required for the full activity of Cin-Cdc28 complexes (37). The apparent inability of Cks1 to bind Cln2-13MYC-Cdc28-43244 mutant complexes may thus explain the weak kinase activity associated with this complex (38). These results suggest that Cks1 has different affinities for free and cyclin-bound Cdc28-43244 and that it might play a role in G1 cyclin degradation.

**DISCUSSION**

We identified a cak1 mutant in a genetic screen for mutants stabilizing the yeast G1 cyclins. Cak1 is required for Cdc28 activity (3, 4), and rapid proteolysis of the G1 cyclins is dependent on their phosphorylation by Cdc28 (10). The inactivation of Cdc28 in the cak1 mutants can thus explain the observed hypophosphorylation and stabilization of Cln2HA. Furthermore, we found that Cln2HA does not form a stable complex with Cdc28 in the absence of activating phosphorylation. Expression of a CAK-independent mutant of Cdc28 restored Cln2HA phosphorylation but not its rapid degradation. This unexpected uncoupling of Cln2HA phosphorylation and proteolysis led us to study in greater detail the molecular mechanisms by which phosphorylated Cln2HA is recognized by the SCF-Grr1 ubiquitin ligase complex, ubiquitinated, and addressed to the proteasome for degradation.

**Cak1 Is Required for Cin2-Cdc28 Complex Formation and Activity**—We found through immunoprecipitation experiments that Cdc28-activating phosphorylation by Cak1 is necessary for stable Cln2HA binding to Cdc28 in vivo and subsequent activation of Cdc28. Ross et al. (39) have recently shown that Cak1 phosphorylation is also required for Cdc28-Cln2 complex formation in vivo and for stable binding of Cin2 to Cdc2 in vitro. Although many cyclin/Cdk interactions do not require Cdk-activating phosphorylation, stable binding of cyclin A to Cdc2 does require activating phosphorylation (40, 41). A better understanding of why activating phosphorylation is required for stable binding of some specific cyclin/Cdk combinations will require the determination of the crystal structure of these complexes.

Based on the interpretation of some genetic experiments, it was suggested that Cak1 and Thr-169 phosphorylation of Cdc28 is not required for G1 cyclins to activate Cdc28 (42, 43). Our results and those of Ross et al. (39), showing that Cak1 is required for Cin2-Cdc28 activity, indicate that these genetic experiments were misinterpreted. Chun and Goebl (45) noticed that cak1 disruption spores had multibudded terminal morphologies after germination that were similar to those of cdc34 mutants. These latter mutants are multibudded because they express Cin/Cdc28 activity in the absence of any Clb/Cdc28 activity. Chun et al. (43) then concluded that Cak1 was necessary for Clb/Cdc28 activity but not Cin/Cdc28 activity. However, as we now show directly that Cak1 is necessary for Cin/Cdc28 activity, a more likely explanation is that inhibition of
Cln2HA to Cdc28-Glu-169 is simply due to the inability of this phosphorylation of Cdc28 on Thr-169 to block Cln2HA and Cdc28-Ala169 mutants, whereas Cln2 could inter- 
g1 cyclin degradation. Like Cln2, phosphorylation of p27-Kip1 for mitotic proteolysis (26). In particular, a subunit from a putative receptor on the proteasome and Cks1 might have the recycling possibility, suggested by Kaiser

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Cln2HA to Cdc28-Glu-169 is simply due to the inability of this phosphorylation of Cdc28 on Thr-169 to block Cln2HA and Cdc28-Ala169 mutants, whereas Cln2 could inter- 
g1 cyclin degradation. Like Cln2, phosphorylation of p27-Kip1 for mitotic proteolysis (26). In particular, a subunit from a putative receptor on the proteasome and Cks1 might have the recycling possibility, suggested by Kaiser et al. (26), is that Cks1 may function as a recycling factor for Cdc28; after cyclin proteolysis, Cks1 bound to Cdc28 may be required for release of the kinase subunit from a putative receptor on the proteasome and allow new cyclin-Cdc28 complexes to bind. Indeed, a yet unknown factor could be necessary for the release of ubiquitinated G1 cyclins from the SCF-Grr1, and Cks1 might have the recycling role previously described. The ubiquitination of G1 cyclins bound to Cdc28 by SCF-Grr1 has recently been achieved in vitro (19). A further in vitro analysis of factors required for the degradation of ubiquitinated G1 cyclin-Cdc28 complexes by the 26 S proteasome may reveal novel mechanisms required for proteolysis after ubiquitination.

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G1 Cyclin Ubiquitination without Proteolysis

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