Ddb1 Is Required for the Proteolysis of the *Schizosaccharomyces pombe* Replication Inhibitor Spd1 during S Phase and after DNA Damage*

Recently we showed that the *Schizosaccharomyces pombe* ddb1 gene plays a role in S phase progression. A mutant *S. pombe* strain lacking expression of the *ddb1* gene exhibited slow replication through both early and late regions causing a slow S phase phenotype. We attributed the phenotypes in the *ddb1* strain to an increased activity of the replication checkpoint kinase Cds1. However, the basis for a high basal Cds1 activity in the *ddb1* strain was not clear. It was shown that Ddb1 associates with the Cop9/signalosome. Moreover, the phenotypes of the *ddb1* strain are remarkably similar to the *Δcsn1* (or *Δcsn2*) strain that lacks expression of the Csn1 (or Csn2) subunit of the Cop9/signalosome. Cop9/signalosome cooperates with Pcu4 to induce proteolysis of Spd1, which inhibits DNA replication by inhibiting ribonucleotide reductase. Therefore, we investigated whether Ddb1 is required for the proteolysis of Spd1. Here we show that a *S. pombe* strain lacking expression of Ddb1 fails to induce proteolysis of Spd1 in S phase and after DNA damage. Moreover, deletion of the *spd1* gene attenuates the Cds1 kinase activity in cells lacking the expression of *ddb1*, suggesting that an accumulation of Spd1 results in the increase of Cds1 activity in the *ddb1* strain. In addition, the double mutant lacking *spd1* and *ddb1* no longer exhibits the growth defects and DNA damage sensitivity observed in the *Δddb1* strain. Our results establish an essential role of Ddb1 in the proteolysis of Spd1. In addition, the observation provides evidence for a functional link between Ddb1 and the Cop9/signalosome.

The *Schizosaccharomyces pombe* Ddb1 protein is homologous to the DDB1 subunit of the mammalian damaged DNA-binding protein DDB, which contains an additional subunit DDB2 (1). The mammalian DDB has been implicated in global genomic repair (1–3). The DDB2 subunit, which is not found in *S. pombe* or other lower organisms, is critical for recognition of damaged DNA and the global genomic repair functions of DDB (4). The *S. pombe* Ddb1 protein has been shown to be required for normal cell growth, progression through the S phase, and proper chromosome segregation (5, 6). Although it is not clear whether those functions are conserved in the mammalian DDB1 protein, both mammalian and yeast Ddb1 have been shown to associate with Cop9/signalosome, a large complex with homology to the 19 S lid complex of the proteasome (7, 8). The interaction of the mammalian DDB with the signalosome has been linked to repair, whereas the interaction of the yeast Ddb1 with signalosome has not been characterized.

Cop9/signalosome was identified as a regulator of photomorphogenesis in plants (for review, see Ref. 9). It was shown to control the levels of the plant transcription factors Hy5 and Hy21 through subcellular localization and proteolysis (10). The plant signalosome complex associates with the E2 conjugating enzyme Cop1 and E3 ubiquitin ligase that are involved in the proteolysis of the Hy5 and Hy21 transcription factors depending upon the availability of light. Signalosome has been characterized also from mammals and fission yeast (11, 12). Like the plant signalosome, the mammalian and the yeast signalosomes are involved in controlling proteolysis mediated by the cullin family of the E3 ubiquitin ligases. All cullins are modified by neddylation (NEDD8 conjugation), which is believed to be required for their ubiquitin ligase activity (13–15). Signalosome possesses a neddylation activity that is able to remove the NEDD8 conjugation from the cullins (16). The cullin deneddylation function of the signalosome was confirmed using both biochemical and genetic approaches (8, 16–18). For example, it was shown that the fission yeast mutant lacking expression of any of the signalosome subunits Csn1, Csn2, Csn3, Csn4, and Csn5 exhibited accumulation of the neddylated form of Pcu1 and Pcu3 (16, 17). Surprisingly, however, only the *Δcsn1* and the *Δcsn2* mutants exhibited a phenotype, suggesting the existence of additional functions of those two subunits of the *S. pombe* signalosome (11).

Recent studies on the function of the *S. pombe* Csn1 and Csn2 subunits revealed their role in positively regulating the activity of the ribonucleotide reductase (RNR) through a proteolysis of the inhibitor Spd1 (8). Spd1 retains the Suc22 subunit of RNR in the nucleus. The periodic proteolysis of Spd1 in early S phase releases Suc22, which associates with Cdc22 in the cytoplasm to reconstitute the active RNR that is required for the biosynthesis of dNTPs (8). It was shown that the Csn1 subunit of signalosome cooperated with Pcu4 to enhance the proteolysis of Spd1 (8). The proteolysis of Spd1 occurs also during DNA damage requiring the activation of the checkpoint pathway (8). It was shown that the checkpoint-dependent proteolysis of Spd1 involved Csn1 because the *S. pombe* strains lacking this gene failed to induce proteolysis of Spd1 after ionizing radiation (8). These observations established a positive role of the *S. pombe* signalosome in the ubiquitin-proteasome-mediated proteolysis of Spd1. Moreover, it was shown that

---

* This work was supported by Grant CA88863 from the NCI, National Institutes of Health (to P. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ”advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Genetics, University of Illinois at Chicago, 900 S. Ashland Ave., M/C 696, Chicago, IL 60607. Tel.: 312-413-0255; Fax: 312-355-3847; E-mail: Pradip@uic.edu.

‡ The abbreviations used are: DDB, damaged DNA-binding protein; FACS, fluorescence-activated cell sorter; GST, glutathione S-transferase; HU, hydroxyurea; MOPS, 4-morpholinepropanesulfonic acid; RNR, ribonucleotide reductase; MBP, myelin basic protein.
mutation of Spd1 caused a reversal of many of the growth-related phenotypes of the S. pombe strains lacking expression of csn1 (8, 11).

Our recent studies on a S. pombe strain lacking expression of Ddb1 exhibited a remarkable overlap of the phenotypes that were described for the strains lacking expression of the Csn1 or Csn2 subunits of the signalosome. As in the case of the Δcsn1 or Δcsn2 strain, the Δddb1 strain exhibited slow growth and extended S phase. We showed that there was a delay in the progression of DNA synthesis in the Δddb1 strain (6). Moreover, the Δddb1 strain was hypersensitive to DNA damage in S phase and failed to recover from hydroxyurea (HU) block (6). Further, as in the case of the Δcsn1 strain, the Δddb1 strain exhibited a high level of the active Cds1 kinase. Because of these overlaps in the phenotypes, we considered the possibility that Ddb1 might be involved in the same pathway with signalosome to regulate Spd1 during S phase and after DNA damage. Here we show that Ddb1 is an essential component in the proteolysis of Spd1. The S. pombe strain lacking expression of Ddb1 fails to induce proteolysis of Spd1 during the cell cycle and after DNA damage or replicative stress. Moreover, mutation in the Spd1 gene reverses many of the defects that were observed in the Δddb1 strain. The observations provide evidence for a functional link between Ddb1 and signalosome in the cell cycle- and checkpoint-regulated proteolysis of the replication inhibitor Spd1.

MATERIALS AND METHODS

Yeast Cultures—Cells were cultured in yeast extract plus supplements (YES) medium at 32 °C. Temperature-sensitive strains were cultured at room temperature (22 °C) and synchronized by a shift to 35 °C for 4 h. Genetic crosses were performed on EMG-glutamate plates. The double mutant strains were constructed by random spore mating (YE5S) medium at 32 °C. The cell cycle- and checkpoint-regulated proteolysis of the replication inhibitor Spd1.

S Phase Proteolysis of Spd1 Requires Ddb1—We showed that the S. pombe strain lacking expression of ddb1 exhibited a defective S phase, including slow replication, which could be explained by the increased Cds1 kinase activity in that strain (6). Interestingly, the signalosome mutant Δcsn1, which failed to cause proteolysis of Spd1 in S phase, also exhibited an extended S phase and increased Cds1 activity (8, 11). Therefore, we investigated whether Ddb1 is required for the proteolysis of Spd1. The proteolysis of Spd1 is critical for the biosynthesis of dNTPs, which is essential for DNA synthesis. A deficiency in Spd1 proteolysis is expected to slow down S phase progression and cause replicative stress, which would explain many of the phenotypes (including the increased Cds1 activity) observed in the Δddb1 strain (6). We compared the steady-state levels of Spd1 in the Δddb1 strain with that in the wild type strain in Western blot assays using a specific antiserum against Spd1. The results of that analysis clearly indicated a significant accumulation of Spd1 in the Δddb1 strain compared with the wild type (Fig. 1A).

The proteolysis of Spd1 occurs in S phase during each cycle of division to allow for an increase in the RNR activity (8). To analyze the cell cycle proteolysis of Spd1, we compared the cdc25-22 strain with the Δdmb1 cdc25-22 strain (19). The cdc25-22 encodes a temperature-sensitive allele of cdc25, which

**Table I**

| Strain | Genotype | Source |
|--------|----------|--------|
| ARC 556 | h + ade6-M216 ura4-D18 his3-leu1-32              | Paul Nurse |
| FY 319 | h + ade6-M216 ura4-D18 leu1-32 cdc25-22       | Susan Forsburg |
| TB 13-1 | h + ade6-M216 ura4-D18 leu1-32                  | Our stock    |
| TB 16  | h + ade6-M216 ura4-D18 leu1-32 cdc25-22 ddb1::kan' | Our stock    |
| KP 38  | h - ade6-704 ura4-D18 leu1-32 spd1::ura4      | Antony Carr  |
| TB 31-1 | h + spd1-GST                                    | Paul Nurse   |
| TB 36–21 | h + ddb1::kan' spd1::GST                      | This study   |

**RESULTS**

Pulse-Chase—Cells were grown into log phase in YE5S, span down, and resuspended in YE5S without methionine at 2 × 10⁶ cells/ml. After a 1-h incubation, Tran35S-label (ICN) was added to 20 μCi/ml, and the cell pellets were labeled for 1 h. Cells were washed with water and resuspended in YE5S supplemented with 1 mg/ml cold methionine and 10 mM HU. At different time points, cells were harvested, washed with STOP buffer, and lysed in NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Triton X-100) with protease inhibitors. 1 mg of the extract was incubated with glutathione-Sepharose beads for 2 h at 4 °C, and the bound Spd1-GST was washed three times with 1 ml of NETN buffer and then eluted with 100 mM glutathione in NETN. The eluted Spd1-GST was mixed with SDS loading buffer and resolved by SDS-PAGE. The gel was dried and the radioactive bands visualized by autoradiography and quantified by PhosphorImager.

In Vivo Ubiquitination—Log phase cells expressing Spd1-GST along with a control untagged strain were grown in YE5S in the presence of 10 mM HU for 3 h. Cell extraction and GSH bead binding were performed exactly as for the pulse-chase assay except that 5 μM N-ethylmaleimide was added to the lysis buffer, and Spd1-GST was eluted with SDS-loading dye. 25 μg of total protein of the control and Spd1-GST strains and 4 μg of total protein for the Δdmb1Δpd1-GST strain were used to normalize the amount of Spd1-GST. The Spd1-GST-ubiquitin conjugates were visualized by Western blotting.

Cds1 Kinase Assay—1-mg protein extracts were incubated with 0.5 μl of anti-Cds1 rabbit antibody prebound to 20 μl of protein G beads for 1 h at 4 °C with constant mixing. The kinase reaction was performed as described previously (6). FACs—Preparation of cell ghosts and flow cytometry were done as described previously (6).

**TABLE I**

| Strain | Genotype | Source |
|--------|----------|--------|
| ARC 556 | h + ade6-M216 ura4-D18 his3-leu1-32 | Paul Nurse |
| FY 319 | h + ade6-M216 ura4-D18 leu1-32 cdc25-22 | Susan Forsburg |
| TB 13-1 | h + ade6-M216 ura4-D18 leu1-32 | Our stock    |
| TB 16  | h + ade6-M216 ura4-D18 leu1-32 cdc25-22 ddb1::kan' | Our stock    |
| KP 38  | h - ade6-704 ura4-D18 leu1-32 spd1::ura4 | Antony Carr  |
| TB 31-1 | h + spd1-GST | Paul Nurse   |
| TB 36–21 | h + ddb1::kan' spd1::GST | This study   |
The proteolysis of Spd1 in S phase is compromised in Δddb1 cells. A. 0.5-mg extracts from the wild type (wt) or Δddb1 cells were assayed for the steady-state levels of Spd1 by Western blotting with anti-Spd1 antibody. The Δspd1 strain was used as an antibody specificity control, and Cds1 was assayed to control for loading. B, the cdc25-22 and cdc25-22 Δddb1 cells were synchronized in G2 by arresting at 35 °C for 3 h and subsequent release at 22 °C. The numbers on top represent the time after the release; the numbers at the bottom indicate the percentage of septated cells at each time point. The peak of septation is underlined. Equal amounts of the extracts (0.5 mg) were analyzed by Western blots. The blots were probed with Spd1 antibody or Cds1 antibody.

Fig. 2. Spd1 proteolysis induced by HU requires Ddb1. Δddb1 or Δddb1 strains expressing untagged Spd1 (A) or a GST-tagged Spd1 from its endogenous locus (B) were analyzed for Spd1 (or Spd1-GST) in asynchronous cells or cells synchronized in S phase by a 3-h treatment with 10 mM HU. 0.5-mg extracts were analyzed by Western blots using Spd1 antibody or Cds1 antibody.

Because it was shown that Spd1 is degraded through the ubiquitin-proteasome pathway (8), we sought to investigate whether the Δddb1 strain is deficient in ubiquitinating Spd1-GST. To enrich the cell population for S phase cells where Spd1 is normally degraded, we looked at the ubiquitination of Spd1-GST. To confirm further a deficiency in the proteolysis of Spd1 in the Δddb1 strain, we determined the decay rate of Spd1-GST in the wild type and in the Δddb1 strain after a treatment with HU. The cells were pulse labeled with [35S]methionine for 1 h before the addition of HU. The pulse-labeled cells were transferred to medium containing unlabeled methionine (chase) and 10 mM HU. At different time intervals, cells were harvested, and the cell lysates were bound to GSH beads to purify the Spd1-GST protein. After an extensive wash, the proteins bound to the GSH beads were eluted and subjected to SDS-PAGE followed by autoradiography (Fig. 3A). The band intensities were quantified by PhosphorImager and plotted against time of chase (Fig. 3B). As expected, the addition of HU caused a much faster decay of Spd1-GST in the wild type background relative to that observed in the Δddb1 strain treated with HU (Fig. 3B). The half-life of Spd1-GST in the Δddb1 strain was greater than 3 h in the presence of HU, whereas in the wild type background the half-life was less than 2 h. These observations further confirm the notion that the proteolysis of Spd1 is defective in the Δddb1 strain.

Because it was shown that Spd1 is degraded through the ubiquitin-proteasome pathway (8), we sought to investigate whether the Δddb1 strain is deficient in ubiquitinating Spd1-GST. To enrich the cell population for S phase cells where Spd1 is normally degraded, we looked at the ubiquitination of Spd1-GST in asynchronous cells or cells synchronized in S phase by a 3-h treatment with 10 mM HU. 0.5-mg extracts were analyzed by Western blots using Spd1 antibody or Cds1 antibody.

Because it was shown that Spd1 is degraded through the ubiquitin-proteasome pathway (8), we sought to investigate whether the Δddb1 strain is deficient in ubiquitinating Spd1-GST. To enrich the cell population for S phase cells where Spd1 is normally degraded, we looked at the ubiquitination of Spd1-GST in asynchronous cells or cells synchronized in S phase by a 3-h treatment with 10 mM HU. 0.5-mg extracts were analyzed by Western blots using Spd1 antibody or Cds1 antibody.

Because it was shown that Spd1 is degraded through the ubiquitin-proteasome pathway (8), we sought to investigate whether the Δddb1 strain is deficient in ubiquitinating Spd1-GST. To enrich the cell population for S phase cells where Spd1 is normally degraded, we looked at the ubiquitination of Spd1-GST in asynchronous cells or cells synchronized in S phase by a 3-h treatment with 10 mM HU. 0.5-mg extracts were analyzed by Western blots using Spd1 antibody or Cds1 antibody.

Because it was shown that Spd1 is degraded through the ubiquitin-proteasome pathway (8), we sought to investigate whether the Δddb1 strain is deficient in ubiquitinating Spd1-GST. To enrich the cell population for S phase cells where Spd1 is normally degraded, we looked at the ubiquitination of Spd1-GST in asynchronous cells or cells synchronized in S phase by a 3-h treatment with 10 mM HU. 0.5-mg extracts were analyzed by Western blots using Spd1 antibody or Cds1 antibody.

Because it was shown that Spd1 is degraded through the ubiquitin-proteasome pathway (8), we sought to investigate whether the Δddb1 strain is deficient in ubiquitinating Spd1-GST. To enrich the cell population for S phase cells where Spd1 is normally degraded, we looked at the ubiquitination of Spd1-GST in asynchronous cells or cells synchronized in S phase by a 3-h treatment with 10 mM HU. 0.5-mg extracts were analyzed by Western blots using Spd1 antibody or Cds1 antibody.
in Fig 3C, polyubiquitinated Spd1-GST was detected from cells with wild type background but not the Δddb1 background. The results are congruent with the notion that the Δddb1 strain is deficient in inducing the ubiquitination of Spd1.

Deletion of the Spd1 Gene Reduces the Constitutively High Cds1 Activity in Δddb1 Strain—We showed that the Δddb1 strain exhibited a constitutively high basal activity of the replication checkpoint kinase Cds1 (6). The high basal Cds1 activity was linked to many if not all growth-related defects and DNA damage sensitivity in the Δddb1 strain because mutation in the cds1 gene reversed the defects, and the double mutant Δddb1 Δcds1 behaved like the wild type strain. It was, however, not clear why the Δddb1 strain exhibited a high basal level of the Cds1 activity. Interestingly, the Δcsn1 strain also exhibited a high basal Cds1 activity, and deletion of the spd1 gene reduced the Cds1 activity to a level observed for the wild type cells. Therefore, we sought to investigate whether a deletion of the spd1 gene would reduce the Cds1 activity in Δddb1 cells. We constructed a double mutant, Δddb1 Δcds1, Δddb1 Δspd1, lacking the expression of both dd1 and spd1 genes. The double mutant was compared with the single mutants and with the wild type strain for the basal Cds1 activity. To assay for the Cds1 activity, cell extracts were immunoprecipitated with an antibody against Cds1. The immunoprecipitates were collected on protein A-Sepharose beads. The beads containing the immunopurified Cds1 were used to measure the activity of Cds1. In parallel, aliquots of the extracts were subjected to Western blot assays to compare the protein level of Cds1 from the various extracts (Fig. 4). The kinase activity of Cds1 was measured following a procedure described previously using myelin basic protein and [γ-32P]ATP as substrates. The reaction product was analyzed by SDS-PAGE followed by autoradiography. The phosphorylated MBP was quantified by PhosphorImager. Consistent with our previous results, the Δddb1 strain exhibited a significantly high level of the activated Cds1. In addition, we observed that the double mutant lacking expression of both Δddb1 and Δspd1 exhibited a much lower level of active Cds1. The level of active Cds1 in the double mutant was comparable with the Δ SPD1 single mutant strain (Fig. 4). These observations are consistent with the notion that the high Cds1 activity in Δddb1 strain is a result of the deficiency in the proteolysis of Spd1.

Mutation in the spd1 Gene Reversed the Growth and Size Abnormalities in the Δddb1 Strain—The Δddb1 cells exhibited several growth-related defects, including elongated cell phenotype, increased doubling time, small colony size, and slow S phase progression. We attributed those defects in the Δddb1 cells to the high basal Cds1 activity. Because the spd1 mutation attenuated the Cds1 activity in the Δddb1 cells, we predicted that the growth-related defects in the Δddb1 strain would not be observed in the Δddb1 Δspd1 double mutant. The Δddb1 mutant displays slow growth, which is reflected by smaller colony size compared with the wild type cells. If Spd1 is the cause of this cell cycle delay, deletion of spd1 is expected to increase the growth rate. Consistent with that notion, we observed that the double Δddb1 Δspd1 mutant exhibited normal growth rate and formed large colonies similar to the wild type strain (Fig. 5A).

FACS analysis of the asynchronously growing cultures of the Δddb1 cells revealed a heterogeneous distribution of DNA content (compare panels wt and Δddb1 in Fig. 5B). The heterogeneity was not a result of variations from cell size because the FACS analyses were carried out after cell wall digestion and detergent extraction, which efficiently eliminated the cytosolic background signals. The heterogeneous DNA content distribution is very similar to the “slow S phase” phenotype described for the Δcsn1 mutant (8, 11). The slow S phase phenotype is clearly in agreement with the slow replication observed in the Δddb1 cells (6). Because Spd1 is an inhibitor of S phase and DNA replication, we sought to determine whether mutation in the spd1 gene reverses the heterogeneous DNA content phenotype. Asynchronously growing log phase cultures of the single
pared with the wild type or broader distribution for both forward and side scattering compared to cell size. The results of a deficiency in the proteolysis of Spd1. The wild type and the Δddb1 cells were subjected to UV irradiation (450 J/m2). At different times after the UV irradiation, cells were harvested and 0.4-mg extracts were subjected to Western blot analysis for Spd1 and Cds1 (loading control). UV irradiation caused a significant reduction in the steady-state level of Spd1 in the wild type strain, but it had little effect on the steady-state level of Spd1 in the Δddb1 cells (Fig. 6A). To investigate whether a deficiency in Spd1 proteolysis is the cause of UV sensitivity, we compared the UV sensitivities of the various strains. We predicted that the double mutant lacking Δddb1 and Δspd1 would exhibit reduced sensitivity to UV compared with the Δddb1 strain. To compare the sensitivity to UV, triplicate samples of cells were treated with increasing dose of UV irradiation (0, 50, 100, and 200 J/m2). After irradiation, the cells on plates were incubated at 33 °C for 3 days. The sensitivity to UV was measured by counting the colonies formed after the 3-day incubation period. An average percentage of survival for each strain is plotted (Fig. 6B). The Δddb1 cells were sensitive and exhibited a lower percentage of survival after UV irradiation; but the sensitivity of the double mutant Δddb1 Δspd1 was comparable with the wild type cells, which is congruent with the notion that the UV sensitivity of the Δddb1 cells is a result of deficiency in Spd1 proteolysis. Taken together, the observations further reinforce the notion that the analyzed defects in Δddb1 strain resulted from a lack of proteolysis of Spd1.

**DISCUSSION**

The results presented here are significant in several ways. First, we show that Ddb1 is essential for the proteolysis of the replication inhibitor Spd1, and the growth defects in the Δddb1 strain are the consequences of the failure to degrade Spd1. Second, we show that the function of Ddb1 is linked to the UV damage response pathway. In addition, our observations provide clear evidence for a functional link between Ddb1 and Cop9/signalosome in inducing the proteolysis of Spd1 during the cell cycle progression and after DNA damage.

Our analyses on Spd1 were prompted by the observation that signalosome associates with Ddb1, as well as by the phenotypic similarities between the signalosome mutant Δcsn1 and the Δddb1 strains (5, 6, 8, 11). These strains exhibit the slow S phase or heterogeneous DNA content phenotype, sensitivity to DNA-damaging agents, and increased Cds1 activity. We showed that the defects in the Δddb1 strain could be relieved by a deletion of the Cds1 gene (6). However, it was not clear what was responsible for the higher basal level of the Cds1 kinase activity in Δddb1 cells. The observation that deletion of Spd1 brings down the Cds1 activity to a level observed in the wild type strain indicates that the primary defect in the Δddb1 cell might be in the regulation of Spd1. Spd1 is an inhibitor of RNR, which is critical for DNA synthesis. Spd1 sequesters a subunit of RNR, Suc22, in the nucleus, preventing its association with the other subunit Cdc22 in the cytoplasm (8). When active RNR is important, cellular mechanisms induce proteolysis of Spd1 (8). A lack of proteolysis of Spd1 in S phase will result in a failure to increase the activity of RNR, which is essential for the biosynthesis of dNTPs. Therefore, in the absence of Spd1 proteolysis, the cells will fail to increase the level of dNTPs. The low levels of dNTPs will cause stalling of the replication forks leading to the activation of the replication checkpoint kinase Cds1. The increased Cds1 activity will further slow down progression through S phase, which explains the slow S phase phenotype observed in both Δddb1 and Δcsn1 strains. Moreover, overexpression of Spd1 is known to cause a checkpoint-independent G2/S block and a checkpoint-mediated, replication-independent G2 block (20, 21). In addition, accumulation of Spd1 is likely to cause slow S phase progression because of both Cds1 activation and lack of dNTPs. Thus, it is possible that the accumulation of Spd1 observed in the Δddb1 mutant results in delays at all three stages of the cell cycle.
Saccharomyces cerevisiae, dNTP pools increase 7-fold after DNA damage (22). That increase is critical for survival because higher dNTP levels are required for an efficient translesion DNA synthesis. Because Spd1 proteolysis is important for survival after DNA damage, the deficiency in Spd1 proteolysis will also explain the sensitivity of the Δddb1 mutant cells to DNA damage.

The level of the Spd1 protein decreases during S phase and after DNA damage through proteolysis by the ubiquitin-proteasome pathway (8). It was shown that the Cop9/signalosome and the cullin family member Pcu4 are essential for the proteolysis of Spd1 through the ubiquitin-proteasome pathway. Mutant strains of S. pombe lacking expression of the signalosome subunit Csn1 or the cullin Pcu4 failed to induce proteolysis of Spd1 (8). Also, it was shown that Pcu4 could exist in a complex with the signalosome. Moreover, the same study identified Ddb1 as a signalosome-associated protein. Therefore, it is interesting that Ddb1 is essential for the proteolysis of Spd1. In mammalian cells, the counter part of Ddb1 (DDB1) binds to the counter part of Pcu4 (Cul-4A) (23). We showed that the mammalian DDB1-Cul-4A could induce proteolysis of the DDB2 protein, which binds to DDB1 (24). It is, therefore, possible that DDB1 functions as an adaptor that links Cul-4A to DDB2 in mammalian cells. However, we failed to detect a direct association between Spd1 and Ddb1 in the extracts of S. pombe (data not shown). It was proposed that Ddb1 localizes the signalosome to the chromatin (8). On the other hand, it is possible that Ddb1 is required for an assembly of the signalosome-Pcu4 complex that leads to the ubiquitination of Spd1. Also, it is not clear what governs the timing of proteolysis of Spd1. Ddb1 is a constitutively expressed protein. Moreover, the interaction between signalosome and Ddb1 was detected in the extracts of asynchronously growing cells in the absence of any DNA-damaging agents. Thus, although we have identified Ddb1 as an integral component of the machinery that ubiquitinates Spd1, the signal that marks Spd1 for ubiquitination and proteolysis in S phase or after DNA damage remains elusive.

The mammalian RNR subunits R1 and R2 are activated in early S phase through transcription and new synthesis (25–27). The transcription of RNR is activated by the E2F family of transcription factors. It is noteworthy that the mammalian DDB could function as a transcriptional coactivator for E2F1 (28). Therefore, it is possible that the mammalian DDB1 activates RNR by stimulating its expression. A transcription function was described also for the plant DDB1 protein. It was
shown that the plant DDB1 could associate with Det1, a protein required for the transcription of the photomorphogenic genes (29). But, the fact that the mammalian DDB1 associates with the signalosome in conjunction with Cul-4A points to a conserved function of DDB1. The R2 subunit of the mammalian RNR is degraded by ubiquitin-proteasome pathway during the cell cycle, and this degradation is delayed in S phase or upon DNA damage (30). It will be interesting to determine whether DDB1 is involved in the degradation of R2. An Spd1 homolog with the signalosome in conjunction with Cul-4A points to a protein required for the transcription of the photomorphogenic genes (29). But, the fact that the mammalian DDB1 associates with siRNA against DDB1 in mammalian cells indicated a further analysis to identify a conserved function of the DDB1-signalosome interaction in the proteolysis of replication inhibitors will be exciting.

Acknowledgments—We thank Paul Nurse, Antony Carr, and Susan Forsburg for strains; Paul Nurse for Spd1 antiserum; and Teresa Wang for Cds1 antiserum.

REFERENCES

1. Hwang, B. J., and Chu, G. (1993) Biochemistry 32, 1657–1666
2. Abramis, M., Levine, A. S., and Protic, M. (1991) J. Biol. Chem. 266, 22439–22500
3. Chu, G., and Chang, E. (1988) Science 242, 564–567
4. Oztin, V. R., Kuraoka, I., Nardos, T., McLennan, M., Eker, A. P. M., Stefanini, M., Levine, A. S.; and Wood, R. D. (1998) Mol. Cell. Biol. 18, 3182–3190
5. Zolezzi, F., Fuss, J., Uzawa, S., and Linn, S. (2002) J. Biol. Chem. 277, 41183–41191
6. Bondar, T., Mirkin, E. V., Ucker, D. S., Walden, W. E., Mirkin, S. M., and Raychaudhuri, P. (2003) J. Biol. Chem. 278, 37006–37014
7. Greisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Suito, M., Drapkin, R., Kisselev, A. P., Tanaka, K., and Nakatani, Y. (2003) Cell 113, 357–367
8. Liu, C., Powell, K. A., Mundt, K., Wu, L., Carr, A. M., and Caspari, T. (2003) Genes Dev. 9, 1130–1140
9. Cope, G. A., and Deshaies, R. J. (2003) Cell 114, 663–671
10. Osterlund, M. T., Ang, L. H., and Deng, X. W. (1999) Trends Cell Biol. 9, 113–118
11. Mundt, K. E., Porte, J., Murray, J. M., Brikos, C., Christensen, P. U., Caspari, T., Hagan, I. M., Miller, J. B., Simanis, V., Hofmann, K., and Carr, A. M. (1999) Curr. Biol. 9, 1427–1430
12. Seeger, M., Kraft, R., Ferrell, K., Beh-Chotschir, D., Dumdey, R., Schade, R., Gordon, C., Naumann, M., and Dubiel, W. (1998) FASEB J. 12, 469–478
13. Lammer, D., Mathias, N., Laplaza, J. M., Jiang, W., Liu, Y., Callis, J., Goehl, M., and Estelle, M. (1998) Genes Dev. 12, 914–926
14. Liakopoulos, D., Doenges, G., Matuschewski, K., and Jentsch, S. (1998) EMBO J. 17, 2208–2214
15. Kawakami, T., Chiba, T., Suzuki, T., Iwai, K., Yamanaka, K., Minato, N., Suzuki, H., Shimbara, N., Hidaka, Y., and Osaka, F. (2001) EMBO J. 20, 4003–4012
16. Lyapina, S., Cope, G., Shevchenko, A., Serino, G., Tsuge, T., Zhou, C., Wolf, D. A., Wei, N., and Deshaies, R. J. (2001) Science 292, 1382–1385
17. Zhou, C., Seibert, V., Geyer, R., Rhee, E., Lyapina, S., Cope, G., Deshaies, R. J., and Wolf, D. A. (2001) BMC Biochemistry http://www.biomedcentral.com/2001/27/12
18. Yang, X., Menon, S., Lykke-Andersen, K., Tsuge, T., Di, X., Wang, X., Rodriguez-Suarez, R. J., Zhang, H., and Wei, N. (2002) Curr. Biol. 12, 667–672
19. Russell, P., and Nurse, P. (1986) Cell 45, 145–153
20. Borge, A., and Nurse, P. (2000) J. Cell Sci. 113, 4341–4350
21. Woollard, A., Basi, G., and Nurse, P. (1996) EMBO J. 15, 4603–4612
22. Chabes, A., Georgieva, B., Domkin, V., Zhao, X., Rothstein, R., and Thelander, L. (2003) Cell 112, 391–401
23. Shiyunov, P., Nag, A., and Raychaudhuri, P. (1999) J. Biol. Chem. 274, 35309–35312
24. Nag, A., Bondar, T., Shiv, S., and Raychaudhuri, P. (2001) Mol. Cell. Biol. 21, 6738–6747
25. Chaboute, M. E., Clement, B., and Philippi, G. (2002) J. Biol. Chem. 277, 17845–17851
26. DeGregori, J., Kowalik, T., and Nevin, J. R. (1995) Mol. Cell. Biol. 15, 4215–4224
27. Bjorklund, S., Skog, S., Tribukait, B., and Thelander, L. (1999) Biochemistry 29, 5452–5458
28. Hayes, S., Shiyunov, P., Chen, X., and Raychaudhuri, P. (1998) Mol. Cell. Biol. 18, 240–249
29. Schroeder, D. F., Gahrts, M., Maxwell, B. B., Cook, R. K., Kan, J. M., Alonso, J. M., Ecker, J. R., and Chory, J. (2002) Curr. Biol. 12, 1462–1472
30. Chabes, A., and Thelander, L. (2000) J. Biol. Chem. 275, 17747–17753
31. Tomoda, K., Kubota, Y., Arata, Y., Morii, S., Maeda, M., Tanaka, T., Yoshida, M., Yoneda-Kato, N., and Kato, J. Y. (2002) J. Biol. Chem. 277, 2302–2310
