**Schizosaccharomyces pombe** Ddb1 Is functionally Linked to the Replication Checkpoint Pathway*

Received for publication, March 24, 2003, and in revised form, June 24, 2003
Published, JBC Papers in Press, July 10, 2003, DOI 10.1074/jbc.M303003200

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Schizosaccharomyces pombe Ddb1 is homologous to the mammalian DDB1 protein, which has been implicated in damaged-DNA recognition and global genomic repair. However, a recent study suggested that the *S. pombe* Ddb1 is involved in cell division and chromosomal segregation. Here, we provide evidence that the *S. pombe* Ddb1 is functionally linked to the replication checkpoint control gene *cds1*. We show that the *S. pombe* strain lacking *ddb1* has slow growth due to delayed replication progression. Flow cytometric analysis shows an extensive heterogeneity in DNA content. Furthermore, the *Δddb1* strain is hypersensitive to UV irradiation in S phase and is unable to tolerate a prolonged replication block imposed by hydroxyurea. Interestingly, the *Δddb1* strain exhibits a high level of the Cds1 kinase activity during passage through S phase. Moreover, mutation of the *cds1* gene relieves the defects observed in *Δddb1* strain. The results suggest that many of the defects observed in *Δddb1* cells are linked to an aberrant activation of Cds1, and that Ddb1 is functionally linked to Cds1.

The UV-damaged DNA-binding protein DDB in mammals consists of two subunits, DDB1 and DDB2 (1). DDB has high affinity for UV-induced DNA lesions, cisplatin-modified DNA, and bent DNA (2, 3). A damage sensor function of DDB has been proposed (4). A number of studies indicated a role for DDB in nucleotide excision repair; however, DDB plays a stimulatory but nonessential role in that process (1, 2, 5). The DDB2 gene is mutated in a subset of patients with xeroderma pigmentosum (XP-E), a hereditary disease manifested by sun sensitivity and high susceptibility to skin cancer (4). No mutation in the DDB1 gene has been identified in XP patients. The mammalian DDB1 has been also implicated in other pathways. For example, it was shown to participate in E2F1-activated transcription (6, 7). DDB1 is important in the life cycle of the hepatitis B virus and parameyxovirus (8–11). It was shown that the HBx protein encoded by hepatitis B virus and the V protein encoded by the parameyxoviruses associate with DDB1 and that these associations are critical for establishment of infection. Interestingly, DDB1, but not DDB2, is conserved in lower eukaryotes, including flies, worms, fission yeast, and slime mold (12).

The *Schizosaccharomyces pombe* and human DDB1 genes share 46% sequence homology, with three domains of 66, 58, and 35% homology that are conserved among other species (12). Markov’s model analysis of DDB1 structure predicts a number of β-propeller blades comprised of WD-like repeats (13). The position and number of these repeats are remarkably conserved in *S. pombe* Ddb1 (15 repeats in yeast versus 17 in humans), suggesting that the overall structure and function of the two proteins might be similar. Recently, Zolezzi et al. (14) isolated a cDNA clone of the putative *S. pombe* orthologue of mammalian DDB1 and characterized the phenotype of an *S. pombe* strain deficient for *ddb1*. Their *Δddb1* strain, although viable, displays an elongated cell phenotype, abnormal nuclear morphology, and chromosome segregation defects. Interestingly, that study failed to detect any significant involvement of *ddb1* in nucleotide excision repair and suggested that the function of yeast Ddb1 is linked to chromosome segregation and cell division. However, a link between *ddb1* and the DNA damage checkpoint pathways was not investigated.

Several DNA damage checkpoint genes have been characterized in *S. pombe*. The rad3 gene encodes a member of the phosphatidylinositol 3-kinase family and activates distinct checkpoint pathways mediated by the Chk1 and the Cds1 kinases during DNA damage and replication stress, respectively (for review, see Refs. 15 and 16). Cds1 and Chk1 inhibit Cdc2 by inhibiting the activator phosphatase Cdc25 (17, 18) and/or by activating the inhibitory kinases (19, 20). By inhibiting Cdc2, these checkpoint kinases block cell cycle progression. Cds1 also is involved in recovery from replication stress (21–25). *S. pombe* cells lacking Cds1 (∆cds1) very rapidly lose viability in the presence of hydroxyurea (HU) despite being able to delay mitosis until replication is complete (23). It has been proposed that Cds1 can somehow stabilize stalled forks, preventing their collapse or regression. Analysis of the replication intermediates in HU-treated Cds1 mutant by two-dimensional gel electrophoresis revealed an increased X-line signal, reflecting a higher level of unresolved recombination intermediates (24). Aberrant DNA structures were observed by electron microscopy in HU-treated *S. cerevisiae* cells when Rad53, the Cds1 homologue, was inactivated (25).

In response to replication stress caused by either depletion of
DNA was transferred to the Hybond-NX membrane (Amersham Biosciences) by standard capillary transfer and immobilized by baking at 80 °C in a oven vacuum. The probes were generated by PCR of the genomic DNA with the following primer pairs: TGATTCCTGAGGATGCGCCGCTCTGTTGCACTATTAGGG (rDNA probe); TGTTGAACGAGGGTTGGCGC; TGTAAAAAGATGGTGGGTGCGGAC (ARS27 probe). The probe was labeled using RediPrime II Random Prime labeling system (Amersham Biosciences) and hybridized to the membrane using the manufacturer's protocol. The membrane then was exposed to Kodak Biomax-AR film. When needed, the membrane was stripped by washing in 0.05 SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate), 0.5% SDS at 65 °C and re-probed with a different probe.

**Survival Assays**—For UV sensitivity experiments, equal aliquots of cells were plated on YESS agar and irradiated with 50–200 J/m² UV-C at a rate of 30 J/m²/s. For hydroxyurea sensitivity, exponentially growing cells were incubated in YESS containing 10–20 mM hydroxyurea for various time periods, and equal volume aliquots were plated onto YESS agar. Colonies were counted after incubation at 32 °C for 3 days (or at room temperature for 5 days for cdc25-22 strains). Survival is expressed as a percentage of colonies observed on the mock-treated plates. Each plot represents an average of at least two experiments.

**Protein Extracts and Western Blotting**—Cells were harvested at 1 x 10⁶ cells/ml with ice-cold STOP buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaVO₄, pH 8.0). Cell pellets were resuspended in lysis buffer (250 mM NaCl, 50 mM Tris-HCl, pH 7.5, 80 mM β-glycerophosphate, 15 mM p-nitrophenylphosphate, 0.1% Nonidet P-40, 5 mM EDTA, 1 mM dithiothreitol, 0.1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 40 μg/ml aprotinin) and broken open by vigorous vortexing with glass beads at 4 °C for 10 min. Beads with the beads were washed twice with the lysis buffer and the lysate was cleared by centrifugation at 1500 rpm for 2 min, and the supernatant (low spin) was centrifuged at 14,000 rpm for 10 min, yielding high spin supernatant and insoluble pellet. The pellet was solubilized in the same buffer plus 2% Nonidet P-40 and sonicated briefly; the recovered extracts were centrifuged at 14,000 × g for 15 min at 4 °C. Protein concentrations in the extracts were quantitated by the bicinchoninic acid method. Extracts were boiled in SDS-loading buffer and separated on SDS-PAGE. Proteins were transferred to nitrocellulose membrane. After blocking with 5% dry milk in phosphate-buffered saline containing 0.1% Nonidet P-40, the membrane was probed with anti-Cds1 antibody (a kind gift of T. Wang) followed by donkey anti-rabbit horserasid peroxidase-conjugated antibody. The signal was visualized by ECL (Amersham Biosciences).

**Cds1 Kinase Assay**—High spin supernatant and pellet fractions (1 mg) 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 beads were washed 4 times with 600 μl of lysis buffer and once with 1 ml of kinase buffer (40 mM Hepes, 10 mM MgCl₂, 5 mM magnesium acetate, 0.1 mM EDTA, 2 mM dithiothreitol, pH 7.6). The kinase reaction was performed at 30 °C for 15 min with 5 μg of soluble basic protein (Sigma), 5 μCi of [γ-³²P]ATP, and 100 μM unlabeled ATP in 20 μl of the kinase buffer. Reactions were stopped by boiling in SDS loading buffer, and products were analyzed by 12% SDS-PAGE followed by autoradiography. In parallel, 50 μg of the extract was analyzed by anti-Cds1 Western blotting as a control of equal starting material.

**Flow Cytometry and DAPI Staining**—Cells were fixed in 70% ethanol. Preparation of cell ghosts for flow cytometry was done as described (32) with the following modifications. 1 x 10⁶ fixed cells were washed in 50 mM potassium phosphate, pH 7.5, 10 mM β-mercaptoethanol, resuspended in the same buffer containing 10 units of zymolyase (Zymo Research), and incubated at 37 °C for 45 min. The protoplasts were collected by centrifugation, resuspended in 300 μl of cold 0.6 M KCl, overlaid onto 1 ml of cold 0.6 M KCl, 10% glycerol, and pelleted at 1000 × g for 10 min at 4 °C. Pellets were treated with 1 ml of 0.1 M KCl containing 0.1% Triton X-100 for 5 min on ice, washed with 1 ml of Tris-EDTA and treated with 0.1 mg/ml RNase A plus 4 μg/ml propidium iodide for 2 h at 37 °C. Aliquots were analyzed by fluorescent microscopy to visualize the propidium iodide signal and displayed no detectable cytosolic staining. DNA content was measured by the FACScalibur system, and the data were processed using WinMDI 2.9 software. For UV irradiation, ethanol-fixed cells were rehydrated with a slide glass, overlaid with 3 μl of mounting media (15% vino1 205 polyvinyl alcohol, 33% glycerol in 0.1 M NaCl, 0.1 M Tris-HCl, pH 8.5) containing 1 μg/ml DAPI, and covered with a coverslip. Cells were viewed under the fluorescence microscope. Images were captured by digital camera and Spot Advanced software.
**RESULTS**

**Growth Defects in Δddb1 Strain**—To investigate the function of ddb1, we constructed an S. pombe strain in which the complete open reading frame of the ddb1 gene was replaced by the kan" gene by homologous recombination. The construct was confirmed by PCR, Southern blot analysis, and DNA sequencing (data not shown). Our Δddb1 strain behaved similarly to the ddb1-deficient strain described by Zolezzi et al. (14). Zolezzi et al. (14) report that the average cell size of the Δddb1 mutant was larger than the wild type cells. In addition to an elongated-cell phenotype, we found that Δddb1 cells grow more slowly, as judged by increased doubling time (3 h 15 min for Δddb1 versus 2 h for the wild type at 32 °C in rich medium, Fig. 1A) and by the smaller colony size (not shown, see Fig. 6A).

FACS analysis of asynchronously growing log phase cultures revealed a more heterogeneous distribution of DNA content in Δddb1 cells (Fig. 1B). The heterogeneity is unlikely to be caused by variations in cell size since the FACS analysis was carried out after cell wall digestion and detergent extraction, which efficiently eliminated cytosolic background signal (32). Although it may be due in part to defective chromosomal segregation in the Δddb1 strain, as reported by Zolezzi et al. (14), an aberrant S phase progression also could explain the heterogeneous DNA distribution. A large number of cells specifically displayed DNA content intermediate between 1N(G1) and 2N(G2), which may represent slowly replicating cells.

To determine the timing of the cell cycle delay in Δddb1 cells, we studied cdc10-V50 Δddb1 temperature sensitive strain. The cdc10-V50 allele confers a temperature-dependent (35 °C) block in G2. Cells were grown at 35 °C for 4.5 h, and after a shift to 25 °C, progression through the cell cycle was monitored by DAPI staining (Fig. 1C) and FACS (data not shown). Although DNA synthesis initiated with the similar timing in both strains (not shown), mitosis occurred after 1-h delay in cdc10-V50 Δddb1 cells relative to the cdc10-V50 cells (Fig. 1C). Thus, the delay in the cdc10-V50 Δddb1 cells coincided with S to M transition, suggesting either G2 or S phase defect.

**Replication Defect in the Δddb1 Strain**—To investigate whether the slow growth phenotype of the Δddb1 strain was due to a defect in DNA replication, we assayed for the replication progression by two dimensional gel electrophoresis and Southern blot hybridization. To obtain a synchronized culture progression by two dimensional gel electrophoresis and due to a defect in DNA replication, we assayed for the replication intermediates isolated from cdc25-22Δddb1 and cdc25-22 cells were subjected to two-dimensional agarose gel electrophoresis to separate the replication intermediates from the non-replicating DNA followed by Southern hybridization (see “Materials and Methods”). The Southern blots were hybridized with two different probes; an rDNA probe was used to assay the early replicating ribosomal repeat, and the ARS727 probe was used to detect the single copy, late-replicating ARS 727 region (24).

The hybridization with the rDNA probe revealed trace amounts of replicating DNA at 40 and 60 min after the release, most likely representing incomplete synchronization (Fig. 2B). In the cdc25-22 cells, replication of the rDNA region started at 80 min and peaked at 100 min after release. At 120 min the signal dropped almost to the initial level. In contrast, the replication intermediates isolated from cdc25-22 Δddb1 cells were detected from 100 up to 180 min, with a peak at 120 min. Thus, replication of the rDNA region required at least 80 min in the cdc25-22 Δddb1 cells compared with only 20 min in the cdc25-22 cells. A similar result was obtained with the ARS727 probe that measured synthesis from the late-replicating region (Fig. 2C). In cdc25-22 cells replication intermediates corresponding to the ARS727 were detected predominantly at 100 and 120 min after the shift to permissive temperature. But in the cdc25-22 Δddb1 cells, replication started at 100 min, and the intermediates were detectable as late as at 180 min after release. Thus, the replication intermediates from both early and late-replicating zones were detectable for a much longer time in the cdc25-22 Δddb1 cells compared with cells expressing Ddb1. It is likely that this defect in replication is responsible for the slow-growth phenotype of the Δddb1 cells.

**S. pombe Lacking ddb1 Is Hypersensitive to UV Irradiation in S Phase**—Exponentially growing Δddb1 cells are moderately sensitive to UV irradiation (Ref. 14 and Fig. 3A, bottom chart). However, we observed that when growth-arrested by glucose starvation, the Δddb1 strain did not exhibit any significant difference in sensitivity to UV irradiation compared with the parental strain (Fig. 3A, top chart). This difference between growth-arrested and exponentially growing cells suggested the possibility that UV sensitivity in the Δddb1 strain might be phase-specific. To investigate this possibility, we compared the survival rates of the cdc25-22Δddb1 and cdc25-22 strains UV-irradiated throughout the cell cycle progression. The arrested cells were released by a shift to permissive temperature, and at different times after the release, cells were plated on YE5S and immediately UV-irradiated at 100 J/m². In parallel, cell cycle progression was monitored by determining mitotic and septation indices of the DAPI-stained cells. Both cdc25-22 and cdc25-22 Δddb1 cells were in S phase about 100–120 min after shifting to permissive temperature (Fig. 3B). Interestingly, although the parental strain was significantly resistant to UV irradiation in S phase, the ddb1 cells exhibited hypersensitivity to UV irradiation in S phase. Although Δddb1 cells were 2–7-fold more sensitive to UV during late S, G2, and M phases.
compared with the wild type (0–60 and 180–240 time points),
they were 240-fold more sensitive at the peak of S phase.
Because our cell cycle analysis did not allow discrimination
between cells in G1 and S phase, we could not exclude a possi-
bility that \( \Delta \text{ddb1} \) cells are hypersensitive to UV in G1 phase.
Therefore, we tested UV sensitivity of the \( \text{cdc10-V50} \) and
\( \text{cdc10-V50} \Delta \text{ddb1} \) cells after synchronization in G1 by incuba-
tion at 35 °C for 4.5 h followed by a shift to 25 °C. Replicative intermediates
were isolated at various times after the shift to 25 °C, digested with EcoRI, and
resolved by two-dimensional gel electrophoresis. The membrane was hybridized
with the rDNA probe to assay for an early replicating zone (B) and a late-replicating
zone (ARS727 probe, (C)). The genomic loci are represented by open boxes, and
the probes are represented by black bars. E, EcoRI sites.

\[ \text{cdc10-V50} \Delta \text{ddb1} \]
in S phase after treatment with 5 mM HU, and then they resume growth. We observed that the ΔΔdb1 cells fail to form colonies on medium containing 5 mM HU (data not shown). To investigate the timing of the decrease in viability of the ΔΔdb1 cells in the presence of HU, we treated the parental and the ΔΔdb1 cells with 10 mM HU for various time periods in suspension culture and then plated the cells in rich medium without HU. Aliquots were stained with DAPI, and nuclear morphology was analyzed. The survival of the ΔDb1 strain was only slightly diminished during the first 6 h of incubation (Fig. 4A and data not shown). ΔDb1 cells became elongated, similar to the wild type cells, indicating intact replication checkpoint function (Fig. 4D, 6- and 12-h time points). However, ΔDb1 cell viability decreased dramatically during the next 3 h, resulting in 85% loss of viability by 9 h. Thus, a lethal event occurred in the majority of ΔDb1 cells by this time. The lethality was further investigated by FACS analysis and DAPI staining after treatment with HU (Fig. 4, B–D). Both wild type and ΔDb1 cells arrested with G1 DNA content within the first 3 h of treatment with 20 mM HU. As noted above, the ΔDb1 cells always exhibit a broader peak for DNA content compared with the parental strain. Moreover, there was no apparent deficiency in overcoming the HU block and resuming DNA synthesis after 3–6 h of HU treatment (see shift in DNA content from the 3- to 6-h time point in Fig. 4B). However, the cellular DNA content increased and became even more heterogeneous by 12 h in the ΔDb1 cells, indicating over-replication and/or unequal segregation of nuclear material into daughter cells during mitosis. DAPI staining of the cells taken from the same culture revealed morphological abnormalities that correlated with broadened FACS profile and decreased viability. Nuclear displacement, DNA fragmentation, and enucleated cells characteristic of aberrant mitosis comprised about 40% of the ΔDb1 cells at this time point (Fig. 4C). Taken together, these data indicate that ΔDb1 cells are deficient in tolerating a prolonged replication stress induced by hydroxyurea, presumably because aberrant DNA replication leads to irreparable damage in the absence of Db1.

ΔDb1 Cells Contain Activated Cds1—To investigate the basis for aberrant S phase and S phase-specific lethality of the ΔDb1 strain upon UV- or HU-induced stress, we sought to analyze the activity of the S phase specific checkpoint kinase Cds1. Lysates from the wild-type and the ΔDb1 cells were fractionated to obtain a supernatant and a pellet fraction. The pellet fraction was further extracted with buffer containing a higher concentration of detergent (pellet fraction in Fig. 5A). The supernatant and the solubilized pellet fractions were immunoprecipitated with Cds1-specific antibody. The immunoprecipitates were subjected to kinase assays using [γ-32P]ATP and myelin basic protein (MBP) as substrates. We observed that the Cds1 activity was elevated in the extracts of asynchronously growing ΔDb1 cells (Fig. 5A). Because Cds1 is an S phase-specific kinase, we measured Cds1 activity in the extracts of cdc25-22 cells synchronously progressing through the cell cycle. The cell cycle progression after release from the G2 block was monitored by looking at the binucleated cells and by assaying for the Mik1 protein, a marker for S phase (not shown). Extracts, prepared at the indicated times after release,
were analyzed for Cds1 kinase activity, as described under "Materials and Methods." The Cds1 kinase activity in the cdc25-22 and cdc25-22/ddb1 cells was essentially the same at 60 min after the shift to the permissive temperature. A remarkable increase of Cds1 kinase activity became evident in cdc25-22/ddb1 cells but not in cdc25-22 cells at S phase (150 min, Fig. 5B). Thus, it appears that the lack of Ddb1 caused a replication stress, leading to the activation of Cds1 in this strain.

Deletion of cds1 Largely Reverses the Defects in cdc25-22/ddb1 Cells—To further study the role of the replication checkpoint activation in cdc25-22 cells, we constructed double mutants of ddb1 and the checkpoint effector kinase cds1. Quite unexpectedly, we observed almost complete reversal of the cdc25-22 defects in the Δddb1Δcds1 strain. First, the Δddb1Δcds1 mutant formed larger colonies on rich medium plates, of a size similar to the wild type or the Δcds1 strains, indicating that the growth rate was increased compared with the Δddb1 strain (Fig. 6A). FACS analysis revealed that the putative "slow S phase" subpopulation (the left shoulder of the peak, cells with less than 2N DNA) characteristic of the Δddb1 strain was eliminated by deletion of cds1 (Fig. 6B). This finding is consistent with the well studied role of Cds1 in slowing down the progression of S phase and our observation that Cds1 is activated in Δddb1 cells.

We then compared sensitivity to UV irradiation of the asynchronously growing Δddb1Δcds1 cells to that of single Δddb1 and Δcds1 mutants. Deletion of cds1 significantly reduced the sensitivity of ddb1 mutant to the UV irradiation (Fig. 7A). Given that the double mutant was not more sensitive to UV than either of the single mutants, we conclude that these two proteins are involved in the same pathway during UV response. Because both Δcds1 (26) and Δddb1 (this study) strains are deficient in recovery from replication stress, we asked if Ddb1 and Cds1 function in the same pathway upon replication stress imposed by HU treatment. The Δcds1 strain was substantially more sensitive to transient incubation in 20 mM HU than the Δddb1 strain (Fig. 7B). However, the deletion of Δddb1 did not cause additive sensitivity in the Δcds1 background, again suggesting that Ddb1 and Cds1 function in the same pathway.

Δddb1 Cells Are Hypersensitive to Cds1 Overexpression—Because deletion of cds1 ameliorated several defects found in the Δddb1 mutant, we speculated that Cds1 activity might be toxic in the Δddb1 background. To test this, we studied the effect of Cds1 overexpression on Δddb1 cells. A plasmid ex-
pressing Cds1-GST from a thiamine-repressible nmt1 promoter was introduced in the Δddb1 or parental strain. Remarkably, the Δddb1 strain underwent extreme elongation and severe growth retardation even under the conditions where Cds1-GST expression was repressed by the addition of thiamine. Parental cells exhibited very little elongation and no significant growth delay under these conditions. (Fig. 8, EMMS – leu – thiamine, and data not shown). When Cds1 expression was induced, Δddb1 cells where unable to grow (Fig. 8, EMMS – leu), whereas growth of the wild type cells was only slightly inhibited. This effect was observed with four randomly picked Cds1-GST-transformed clones of each genotype.

**DISCUSSION**

The results presented here establish a functional interplay between Ddb1 and the replication checkpoint pathway in *S. pombe*. Moreover, the results also provide an insight into the function of *ddb1* in *S. pombe*. The presence of activated Cds1 in Δddb1 cells suggests that Ddb1 directly or indirectly controls activation of Cds1. We did not see a stable interaction between Cds1 and Ddb1 (data not shown); therefore, we think it is an indirect control. The replication checkpoint protein Cds1 is activated by stalled replication forks and by agents (such as hydroxyurea) that cause replication stress. Therefore, the high level of activated Cds1 in Δddb1 cells during the passage through S phase (Fig. 5) suggests a role of Ddb1 in blocking appearance of stalled replication forks or attenuating replication stress normally encountered during replication. Cds1 is known to play a protective role during replication stress by inducing a reversible cell cycle arrest at S phase (26). In the Δddb1 strain, the HU-induced arrest in S phase is irreversible, suggesting that the protective function of Cds1 during replication stress involves Ddb1. The mechanism of recovery from prolonged replication stress is not fully understood; however, it has been proposed that stabilization of replication complexes is required for productive restart. In the absence of stabilization, replication forks regress or collapse, leading to irreparable structures (24, 25). Thus, it is possible that Ddb1 is involved in stabilization of replication forks and that Δddb1 cells are impaired in the replication restart after a prolonged stress. Such replication defects can result in abnormal mitosis. For exam-
ple, in a situation when two converging forks collapse, replication (and, hence, segregation) of DNA in between the collapsed forks would be impaired. Therefore, it is likely that the aberrant mitosis observed in a large fraction of the \(H9004\) \(ddb1\) cells at 12 h of incubation in HU was a consequence of abnormal replication. The same mitotic abnormalities were observed at a lower frequency in the \(H9004\) \(ddb1\) strain without any treatment (Ref. 14 and Fig. 4), which may be a consequence of a replication defect. Thus, it is possible that HU simply amplifies the defects observed in the untreated \(H9004\) \(ddb1\) strain.

A role for Ddb1 in DNA replication would explain the observation that the \(ddb1\) strain is specifically hypersensitive to UV irradiation during S phase. One explanation could be that the \(ddb1\) strain is deficient in nucleotide excision repair. In G2, this deficiency is masked by recombinational repair, but in S phase no template for recombination is available for unreplicated fragments, resulting in increased sensitivity to DNA damage. On the other hand, this model would predict even higher sensitivity to irradiation during G1, which was not observed. An alternative model of the S phase hypersensitivity to UV is that, as replication forks encounter UV-induced lesions and stall, the integrity of replication complexes is lost in the \(ddb1\) cells. This model is attractive because it is consistent with the sensitivity of the \(ddb1\) strain to MMS (14) and the intrinsic S to M transition defect found in these cells (Fig. 1).

Additional support for our model implicating Ddb1 in replication complex integrity is provided by the functional interaction of Cds1 and Ddb1. We found that deletion of \(cde1\) largely reverses abnormalities of the \(ddb1\) strain, suggesting that the defects in \(ddb1\) cells were mainly caused by Cds1. Because we failed to detect a physical interaction between the endogenous Ddb1 and Cds1 proteins, we suspect that the downstream targets of Cds1 (replication proteins and the Cdc2-regulatory proteins) are important in the functional interplay between Cds1 and Ddb1. We speculate that, in the absence of Ddb1, the Cds1 substrates behave in an anomalous fashion, leading to the phenotypes seen in the \(ddb1\) strain. It is noteworthy that mutation in \(cde1\) has been shown to reverse defects observed in strains with mutations in the replication factor genes, such as Dfp1 and Hsk1. Dfp1 and Hsk1, the two subunits of the Cdc7 replication initiation kinase, are essential for origin firing and apparently have an additional function in the intra-S phase checkpoint, as they are required for recovery from replication stress (29, 34, 35). The temperature-sensitive phenotype of an Hsk1 mutant is partially rescued by deletion of \(cde1\) (35). Moreover, sensitivity to MMS in a strain expressing truncated Dfp1 is partially suppressed by a \(cde1\) deletion (36). Based on these similarities with replication factor genes, we suggest that Ddb1 plays a role in maintaining the integrity of the replication complexes in a way that is linked to Cds1.
Acknowledgments—We thank Paul Nurse, Susan Forsburg for strains, Paul Russell for strains and Cds1 expression plasmid, and Teresa Wang for Cds1 antibody.

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