Induction of a G₁-S checkpoint in fission yeast

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Entry into S phase is carefully regulated and, in most organisms, under the control of a G₁-S checkpoint. We have previously described a G₁-S checkpoint in fission yeast that delays formation of the prereplicative complex at chromosomal replication origins after exposure to UV light (UVC). This checkpoint absolutely depends on the Gcn2 kinase. Here, we explore the signal for activation of the Gcn2-dependent G₁-S checkpoint in fission yeast. If some form of DNA damage can activate the checkpoint, deficient DNA repair should affect the length of the checkpoint-induced delay. We find that the cell-cycle delay differs in repair-deficient mutants from that in wild-type cells. However, the duration of the delay depends not only on the repair capacity of the cells, but also on the nature of the repair deficiency. First, the delay is abolished in cells that are deficient in the early steps of repair. Second, the delay is prolonged in repair mutants that fail to complete repair after the incision stage. We conclude that the G₁-S delay depends on damage to the DNA and that the activating signal derives not from the initial DNA damage, but from a repair intermediate(s). Surprisingly, we find that activation of Gcn2 does not depend on the processing of DNA damage and that activated Gcn2 alone is not sufficient to delay entry into S phase in UVC-irradiated cells. Thus, the G₁-S delay depends on at least two different inputs.

Proliferating cells go through a repetitive series of events known as the cell cycle. The progression from one cell-cycle phase to the next is tightly regulated. Cell-cycle progression is monitored and inhibited by checkpoint mechanisms that preserve the correct order of events (1). Some checkpoints are activated by DNA-damaging agents (“DNA damage checkpoints”), and the accompanying cell-cycle delay allows time for damaged DNA to be repaired. Therefore, checkpoints prevent accumulation of mutations and genome instability, which may lead to cancer development (2). Progression through G₁ phase is under particularly tight control because during G₁, the cells can choose between alternative developmental pathways. Entry into S phase is carefully regulated and, in most organisms, under the control of a G₁-S checkpoint. The critical importance of this cell-cycle transition is underlined by the fact that most cancer cells are defective in the G₁-S checkpoint (3–5).

The cell cycle of the model organism Schizosaccharomyces pombe is regulated by checkpoints, such as the intra-S, the S-M, and the G₂-M checkpoint that monitor the DNA replication status and the presence of DNA damage (6–8). These checkpoints are largely conserved through evolution. We have described a G₁-S checkpoint in fission yeast that delays formation of the prereplicative complex (preRC) at chromosomal replication origins (9) and absolutely depends on the Gcn2 kinase (10), which is a regulator of translation. The only known mechanism of action of Gcn2 is phosphorylation of the initiation factor eIF2α, which inhibits initiation of translation when phosphorylated.

In all cases previously examined, Gcn2 activation (eIF2α phosphorylation) and delayed entry into S phase correlate, but a causal relationship has not been demonstrated (11). Certain DNA-damaging agents activate the Gcn2-dependent checkpoint, whereas others do not, arguing that it is not a general DNA-damage-inducible checkpoint. However, it does not exclude the possibility that some form(s) of DNA damage can trigger the checkpoint.

It is important to identify the molecular nature of the checkpoint trigger for at least two reasons: First, there is little information about such triggers for any checkpoint. Second, the most obvious possibility is DNA damage and DNA-damage checkpoints are the first barrier against cancer (12, 13). The best-studied checkpoint-activating signal is that for activation of homologs of the ATR kinase. A generally accepted view is that these checkpoint proteins can be activated by single-stranded DNA coated with replication protein A (RPA) (14). Generation of this signal requires damage processing by nucleotide excision repair (NER) (15–17) or by resection of single strands at sites of DNA double-strand breaks (18, 19).

Here, we explore the signal for activation of the Gcn2-dependent G₁-S checkpoint in fission yeast after exposure to UV light (UVC). If DNA damage can activate the checkpoint, we expected DNA-repair-deficient mutants to display a longer checkpoint-induced delay. We show here that the checkpoint delay correlates with the repair capacity of the cells in some repair mutants, strongly suggesting that the checkpoint signal derives from DNA damage. However, the activating signal is not generated in mutants deficient in the earliest stages of DNA repair, arguing that it derives not from the initial damage, but from a repair intermediate(s). Surprisingly, activation of Gcn2 can be uncoupled from the cell-cycle delay in certain DNA-repair mutants, leading us to conclude that although Gcn2 activation is necessary for the cell-cycle delay, it is not sufficient.

Results

Cell-Cycle Delay and DNA Repair in Wild-Type Cells. The dominating damage to DNA after exposure to UVC is formation of cyclobutane pyrimidine dimers (CPDs), and their removal is crucial for cell survival. To explore whether DNA damage and/or its repair is important for checkpoint activation, we measured the repair kinetics of radiation-induced CPDs in wild-type and mutant cells. To this end, we monitored the level of remaining repair, arguing that it derives not from the initial damage, but from a repair intermediate(s). Surprisingly, activation of Gcn2 can be uncoupled from the cell-cycle delay in certain DNA-repair mutants, leading us to conclude that although Gcn2 activation is necessary for the cell-cycle delay, it is not sufficient.

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from both strands (Fig. 1A). Approximately 60% of the dimers were removed during 60 min of incubation at 25 °C and approximately 80% was removed in 120 min, consistent with the efficient repair observed earlier (21).

We have shown that cells UVC-irradiated in G1 phase delay entry into S phase because of the Gcn2-dependent G1-S checkpoint and, subsequently, delay DNA replication, presumably when the replication forks reach DNA lesions. Three assays were used to discriminate between cells in G1 and S phase in the present work: the loading of the preRC onto chromatin and the presence of Rum1 and phosphorylated Cdc2 (22). The preRC formation is essential for the initiation of DNA replication and occurs in G1 phase. The frequency of cells displaying preRC formation, as measured by the presence of chromatin-bound Mcm7-GFP (preRC) at different timepoints after release into the cell cycle. Data for unirradiated control cells are plotted by using filled symbols and dashed lines; data for UV-irradiated cells are plotted using open symbols and solid lines. (C–E) Immunoblots of total cell extracts from UVC-irradiated wild-type cells, probed with antibody against Rum1 (C), phosphorylated Cdc2 (D), and phosphorylated eIF2α (E). The presence of tubulin was used as loading control.

Excision Repair in Fission Yeast. Fission yeast contains a classic NER pathway and, in addition, the UV-damaged DNA endonuclease-dependent excision repair (UVER) pathway (29). Like for most organisms, NER consists of two subpathways: the transcription-coupled repair (TCR), which removes damage from the transcribed strand of actively transcribed genes and the global genome repair (GGR), which removes damage from the genome overall. In most cell types, repair through the TCR pathway occurs more rapidly than through the GGR pathway (21, 29, 30). In S. pombe the UVER pathway apparently repairs CPDs equally well on transcribed and nontranscribed strands and, importantly, at a much higher rate than NER does (21, 29, 30).

Both NER and UVER start by recognition of the lesion, followed by endonuclease incision next to the lesion, excision, resynthesis and ligation (Fig. 2). In analogy to NER in human and budding yeast cells the XPA/Rad14 homolog Rhp14 has

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**Fig. 1.** CPD repair and cell-cycle progression in wild-type cells. (A) Quantitative analysis of the CPD repair in the ade6 locus (XhoI fragment) from Southern blots (Fig. S1A) of genomic DNA isolated at different times after UVC irradiation: transcribed (TS) and nontranscribed (NTS) strand. (B) Percentage of cells that contained chromatin-bound Mcm7-GFP (preRC) at different timepoints after release into the cell cycle. Data for unirradiated control cells are plotted by using filled symbols and dashed lines; data for UV-irradiated cells are plotted using open symbols and solid lines. (C–E) Immunoblots of total cell extracts from UVC-irradiated wild-type cells, probed with antibody against Rum1 (C), phosphorylated Cdc2 (D), and phosphorylated eIF2α (E). The presence of tubulin was used as loading control.

**Fig. 2.** Removal of UVC-induced DNA damage in fission yeast. The major steps of NER and UVER are shown, and the points of action of the genes used in this work are indicated.
a role in damage recognition, whereafter the endonucleases Rad16 and Rad13 make cuts on either side of the lesion (31–33). Then the oligonucleotide containing the lesion is removed by a helicase, followed by resynthesis and ligation. The UVER pathway (Fig. 2) is initiated by the Uve1 endonuclease (34, 35), whereafter a flap-structure containing the lesion is created by helicase activity and the flap is released by the endonuclease Rad2 (21). Finally, resynthesis and ligation complete the repair pathway.

No G1 Delay in the rhp14 uve1 Mutant. To test whether DNA damage is important for checkpoint activation, we measured CPD repair and cell-cycle delay in mutants with different capacities for DNA repair. Deletion of the genes encoding the initiating proteins Rhp14 (NER) and Uve1 (UVER) was expected to prevent excision repair altogether. As expected, no CPD removal was detected in the rhp14 uve1 mutant (Fig. S2). Remarkably, the UVC-induced G1 delay is strongly reduced and almost abolished, because preRC loading occurs with very little delay after irradiation (see Figs. 3A and C). Consistently, Rum1 levels are markedly reduced at the same early point in time and Cdc2 phosphorylation also occurs early and regardless of UVC exposure (Fig. 3B and C). We conclude that these cells enter S phase without activating a G1-S checkpoint. These results suggest that the cell-cycle delay depends on DNA damage and not on damage to other macromolecules. Furthermore, checkpoint activation requires either recognition of the DNA damage or a biochemical step downstream of recognition. Surprisingly, the remarkable effect on cell-cycle progression by removing both Rhp14 and Uve1 is not accompanied by a reduced duration of eIF2α phosphorylation (Fig. 3D). In the mutant lacking both repair proteins, eIF2α remains phosphorylated after the cells have entered S phase, and there seems to be no correlation among checkpoint induction, S-phase entry, and eIF2α phosphorylation.

Strongly Reduced G1 Delay in the rad16 rad13 uve1 Mutant. To address whether DNA damage recognition is sufficient to activate the checkpoint, we used a rad16 rad13 uve1 mutant where all incision activity is abolished, but where damage recognition by Rhp14 is retained. Like for the rhp14 uve1 mutant cells (above), preRC loading occurred with only a short delay, if any, after UVC exposure (see Figs. 4A and 6), indicating that checkpoint activation is impaired. Consistently, Rum1 is degraded and Cdc2 is phosphorylated with about the same timing in irradiated and unirradiated cells (Fig. 4B and C). These data strongly argue that full checkpoint activation requires damage processing at least up to the incision step and most likely beyond. In the rad16 rad13 uve1 cells, eIF2α is phosphorylated (Fig. 4D) with the same timing as in repair-proficient (Fig. 1E) and rhp14 uve1 cells (Fig. 3D). Therefore, in these mutant cells, Gcn2 activation occurs in the absence of DNA-damage processing and is uncoupled from the cell-cycle delay.

Fig. 3. Cell-cycle progression in the rhp14 uve1 mutant. (A) Percentage of cells that contained chromatin-bound pre-RC at different timepoints after release into the cell cycle. Symbols are as in Fig. 1B. (B–D) Immunoblots of total cell extracts from UVC-irradiated rhp14 uve1 cells, probed with antibody against Rum1 (B), phosphorylated Cdc2 (C), and phosphorylated eIF2α (D). The presence of tubulin was used as loading control. The gray line in D indicates that intervening timepoints have been removed.

Fig. 4. Cell-cycle progression in the rad16 rad13 uve1 mutant. (A) Percentage of cells that contained chromatin-bound pre-RC at different timepoints after release into the cell cycle. Data for unirradiated control cells are plotted by using filled symbols and dashed lines; data for UV-irradiated cells are plotted by using open symbols and solid lines. (B–D) Immunoblots of total cell extracts from UVC-irradiated rad16 rad13 uve1 cells, probed with antibody against Rum1 (B), phosphorylated Cdc2 (C), and phosphorylated eIF2α (D). The presence of tubulin was used as loading control.
Prolonged G1 Delay in the rad16 uve1 and rad13 uve1 Mutants. An equally repair-deficient double mutant as those above is rad16 uve1, in which no CPD removal could be detected for at least 4 h after UVC (Fig. S3). The irradiated mutant cells arrested in G1 for over 3 h, as seen by the delayed loading of the preRC (Figs. S4 and 6), by the continued presence of Rum1 and by the delayed Cdc2 phosphorylation (Fig. 5 B and C). Similarly, in the rad13 uve1 mutant, very little CPD removal could be detected (Fig. S4A). The rad13 uve1 cells entered S phase with a delay of 60–80 min as shown by a delayed preRC loading (Fig. S4B and Fig. 6) and a delayed Cdc2 phosphorylation (Fig. S4C). The long UVC-induced G1 delay in the rad16 uve1 and in the rad13 uve1 mutants compared with no delay in rad16 rad13 uve1 indicates that the delay depends on processing the damage at least up to and including the incision step.

Importantly, phosphorylation of eIF2α was maintained about as long as in wild-type cells in both these mutants (Fig. S4D) and, therefore, did not correlate with the length of the cell-cycle delay.

Deletion of gcn2 Reduces but Does Not Abolish the Prolonged G1 Delay. To explore the role of Gcn2 in the prolonged delay of the rad16 uve1 mutant, we measured the length of the G1 delay in the rad16 uve1 gcn2 mutant after exposure to UVC. PreRC loading in irradiated cells occurred 60 min later than in unirradiated cells (Figs. 5E and 6), consistent with the appearance of phosphorylated

Fig. 5. CPD repair and cell-cycle progression in the rad16 uve1 mutant. (A) Percentage of rad16 uve1 cells that contained chromatin-bound pre-RC at different timepoints after release into the cell cycle. Data for unirradiated control cells are plotted by using filled symbols and solid lines; data for UV-irradiated cells are plotted using open symbols and solid lines. (B–D) Immunoblots of total cell extracts from UVC-irradiated rad16 uve1 cells, probed with antibody against Rum1 (B), phosphorylated Cdc2 (C), and phosphorylated eIF2α (D). The presence of tubulin was used as loading control. (E) Percentage of rad16 uve1 gcn2 cells that contained chromatin-bound Mcm7-GFP (pre-RC) at different timepoints after release into the cell cycle. (F) Immunoblots of total cell extracts from UVC-irradiated rad16 uve1 gcn2 cells, probed with antibody against phosphorylated Cdc2. The presence of tubulin was used as loading control. (G) Percentage of rad16 uve gcn2 rad3 cells that contained chromatin-bound pre-RC at different timepoints after release into the cell cycle. (H) Immunoblots of total cell extracts from UVC-irradiated rad16 uve1 gcn2 rad3 cells, probed with antibody against phosphorylated Cdc2. The presence of tubulin was used as loading control.

Fig. 6. Cell-cycle progression after UVC irradiation in the investigated mutants. The percentage of cells containing chromatin-bound Mcm was determined as described in Materials and Methods in at least three independent experiments. The delay was determined as the time difference at reaching maximal preRC loading between irradiated and unirradiated cells. We have published that gcn2 cells have no UVC-induced delay (10), and a summary of these previously published data are shown here for comparison.
Cdc2 at 150 min after irradiation versus at 90 min in control cells (Fig. 5F). This delay is significantly shorter than that found in the otherwise isogenic *gen2*" cells (Figs. 5 A–C and 6). We conclude that the prolonged cell-cycle delay of the *rad16 uve1* mutant is reduced, but not completely abolished, in the absence of Gcn2. Therefore, a part of the prolonged delay is Gcn2-dependent.

The G1 Delay is Abolished in the *rad16 uve1 gen2 rad3* Mutant. The presence of a Gcn2-independent G1 delay in the *rad16 uve1* mutant suggests the existence of an additional mechanism that delays entry into S phase. To address the involvement of the classic checkpoint protein Rad3, we monitored S-phase entry after UVC irradiation of the *rad16 uve1 gen2* mutant cells. PreRC loading occurred with the same kinetics in control and irradiated cells (Fig. 5G), suggesting that the remaining delay observed in the *rad16 uve1 gen2* mutant is due to the activation of a Rad3-dependent pathway. Consistently, Cdc2 phosphorylation occurred at the same time in control and irradiated cells, at 90 min (Fig. 5H). In the irradiated cells, Cdc2 soon becomes dephosphorylated again, most likely because in the absence of Rad3 and the S-M checkpoint, the irradiated cells enter mitosis prematurely.

Discussion

Most organisms display checkpoints that arrest the cell-cycle progression after exposure to DNA-damaging agents, but the molecular signal(s) inducing the checkpoints are in most instances not known. Here, we have investigated the inducing signal for the G1-S checkpoint in fission yeast.

**G1-S Delay Requires Processing of UVC-Induced Lesions.** The *rhp14 uve1* double mutant displayed a strongly reduced UVC-inducible G1 delay compared with that observed in wild-type cells (Fig. 6). The most likely explanation is that in the absence of Rhp14 and Uv1, the UVC-induced DNA damage is not recognized, which, in turn, leads to a lack of checkpoint induction. Thus, our data implicate a link between ongoing DNA repair and checkpoint induction. An alternative explanation for the *rhp14 uve1* loss of the checkpoint is that the Rhp14 and/or Uv1 proteins themselves signal to activate the checkpoint. This explanation is unlikely because the checkpoint is also defective in the *rad16 rad13 uve1* mutant (Fig. 6) where Rhp14 is present. Our data strongly argue that one or more of the repair intermediates induce the checkpoint.

The presence of one endonuclease (either Rad13 or Rad16) resulted in checkpoint activation, strongly suggesting that incision is a critical step to generate the activating signal. Furthermore, because either endonuclease is able to activate the checkpoint, it appears that either of them can cleave independently of the other. This finding is in contrast to observations in human cells, where the 3′ incision depends on a prior 5′ incision (36). Interestingly, the presence of either the 5′ or 3′ NER-endonuclease resulted in cell-cycle delays of slightly different durations, suggesting that the length of the delay is determined by how the incision is processed. In the *rad16 uve1* mutant, Rad13 seems to be able to make an incision 3′ to the lesion. The damaged strand might be displaced by a helicase to generate single-stranded DNA required for activation of Rad3. However, the single-strand gap cannot be readily filled by DNA repair synthesis, because there is no 3′ OH group that can be extended. In the *rad13 uve1* mutant, Rad16 appears to make an incision 5′ to the lesion, which might initiate repair synthesis in the absence of the 3′ incision, as was shown for human cells (36). It is likely that this difference in processing of lesions is the reason for the longer delay in the *rad16 uve1* mutant than in the *rad13 uve1* cells.

**Activation of Gcn2 Is Necessary but Not Sufficient for the G1-S Checkpoint.** Here we have shown that Gcn2 activation, measured as eIF2α phosphorylation, does not always correlate with the observed cell-cycle delay. First, in two repair-deficient mutants (*rhp14 uve1* and *rad16 rad13 uve1*) the duration of eIF2α phosphorylation after UVC was as long as for wild-type cells, but the cell-cycle delay was considerably shorter or absent (Fig. 6). Second, in the repair-deficient *rad16 uve1* and *rad13 uve1* mutants the period of eIF2α phosphorylation was similar to that found in wild-type cells, whereas the duration of the cell-cycle delay was much longer (Fig. 6). This difference suggests that the signal for Gcn2 activation does not depend on DNA damage or its processing and indicates a different route of activation (Fig. 7). Furthermore, activation of Gcn2 is not sufficient to produce a G1 delay, although Gcn2 is absolutely required for the checkpoint both in wild-type cells (10) and in repair-deficient cells (Fig. 6). We conclude that the length of the G1 delay depends on at least two different inputs: activation of Gcn2 and processing of DNA damage. We suggest that Gcn2 is essential for the DNA-damage-dependent pathway (Fig. 7), possibly by maintaining efficient translation of some of the components.

Incomplete Repair of DNA Damage Can Induce a Rad3-Dependent G1 Delay. Surprisingly, the long delay in *rad16 uve1* cells was not completely abolished in the absence of Gcn2 (Fig. 6). The remaining delay is due to the activity of Rad3, because there was no delay in the *rad3 gen2 rad16 uve1* mutant (Fig. 6). G1-S checkpoints have been described in most organisms, but little is known about their initiating signals and the targets. UVC irradiation of *Saccharomyces cerevisiae* cells induces a checkpoint that arrests the cells in G1 and activates the ATR and Rad3 homolog Mec1 (37). Similarly, in human cells, an ATR-dependent checkpoint is activated by UVC in G1 (38). No Rad3-dependent G1-S checkpoint has been described in fission yeast. Here, we show that Rad3 can contribute to a G1 delay in a repair-deficient mutant and it remains to be seen whether it can also do so in wild-type cells.

We have shown that fission yeast cells delay entry into S phase after UVC irradiation by a Gcn2-dependent mechanism (10). Here, we demonstrate that this radiation-induced delay in G1 phase depends on processing of the DNA damage. The DNA repair must proceed at least past the incision step. This observation is similar to the findings that in *S. cerevisiae* and human cells the Mec1/ATR-dependent checkpoints require NER activity (15–17). Thus, a common strategy for generating the activating signal for the G1-S checkpoints appears to involve damage processing by the prevalent repair mechanisms. Furthermore, activation of Gcn2 does not depend on processing DNA damage and the inducing signal remains to be identified. We conclude that the G1-S delay depends on at least two different inputs.

**Materials and Methods**

**Yeast Strains and Cell Handling.** All of the strains are derivatives of the *S. pombe* L972 h+ strain. The strains used are listed in Table S1. Media and growth conditions were as described (39). The cells were grown in liquid

![Fig. 7.](image-url) Induction of a G1-S checkpoint in fission yeast. A model consistent with all our results. In the absence of Gcn2, the DNA-damage pathway cannot induce the delay. In DNA-repair mutants, the relevant repair intermediate cannot be generated regardless of the presence of Gcn2.
Edinburgh minimal medium (EMM) or yeast extract medium (YE), at 25 °C, to a cell concentration of 2-4 × 10^6/mL (OD_{600} of 0.1-0.2). The cells were synchronized in G1 phase by incubating cdc10-M17 cells at 36 °C for 4 h (EMM) or 3 h (YE) before release into the cell cycle at 25 °C.

**UVC Irradiation.** A dose of 1,100 J/m² of UVC irradiation (254 nm) was given at an incident dose rate of approximately 250 J/m² per min in a thin, stirred suspension in EMM medium, as described (9). G1-synchronized cells were irradiated immediately after the release from the cdc10 block. Samples for analysis of proteins or DNA were collected immediately after irradiation (time 0) and after further incubation at 25 °C.

**Immunoblots.** Immunoblotting was performed as described (11). The antibody used were as follows: anti-phospho-Cdc2 (Tyr15; Cell Signalling, 9115) at dilution 1:400; anti-Rml1, 1:500 (a kind gift form S. Moreno); anti-phosphorylated elf2α (Biosource, no. 44-7282) 1:2,000; and anti-α-tubulin (T-5168 Sigma), 1:30 000.

**PreRC Loading.** Extraction of unbound MCM proteins was performed as described (23). All experiments were performed at least three times. Representative results are shown in Figs. 1–6; the average of at least three experiments is shown in Fig. 6.


**CPD Analysis.** Genomic DNA was isolated from UVC-irradiated cells, as described (39). CPD analysis of the XhoI fragment containing the ade6 gene was done as described (20). Briefly, the genomic DNA was cut to completion with XhoI, cut at CPDs with T4-endonuclease V or mock treated, separated on alkaline agarose gels, blotted, and hybridized to strand-specific probes close to the XhoI site in the ade6 gene. The probes were made by primer extension using the EcoRI-XhoI DNA of ade6 as a template and primers 1069 (5′-TCTGCAATTTTCTGCACGAC-3′) and 1068 (3′-GCC-TCACAAGGAGAAAGTGATGAGT-5′) to generate probes that hybridize to the bottom and top strand, respectively. The bottom strand of ade6 corresponds to the transcribed strand (TS), the top strand to the nontranscribed strand (NTS). Afterward, the blots were stripped and hybridized with probes detecting the other strand and, in both cases, quantified by using Phosphomager. The average number of CPDs/restriction fragments were calculated by using the Poisson expression (40).

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Fig. S1. Measuring CPD repair. CPD repair was measured in the ade6 locus (Materials and Methods). Shown are Phospholmager scans of Southern blots using radioactive probes toward the nontranscribed strand (NTS) (Left) and the transcribed strand (TS) (Right) of the ade6 locus. Boxes and arrows indicate the position and orientation of the ade6 gene; XhoI indicates the restriction fragment used for CPD calculation. DNA in the even-numbered lanes was cut at CPDs with T4-EndonucleaseV (+T4), whereas the DNA in the odd-numbered lanes were not (-T4). (A) Lane 1 shows DNA size marker and lanes 3–12 shows genomic DNA (digested with Xhol), isolated from wild-type cells at intervals from 0 to 2 h after UVC-irradiation. (B) Lanes 1 and 2 show controls of unirradiated cells; lanes 3–14 show genomic DNA (digested with Xhol) isolated from a repair-deficient mutant (rad13 uve1) at intervals from 0 to 2.5 h after UVC irradiation. Although CPD removal was efficient in the wild-type strain (A), no repair was obvious in the mutant (B). Note that in the samples not treated with UV (lanes 1–2; B) neither unspecific nor specific T4 cuts were observed. Some damage sites in the promoter region (dots) appear to be relatively resistant to repair in wild-type cells. The reason for this phenomenon is not known.

Fig. S2. CPD repair a rhp14 uve1 mutant. Quantitative analysis of the CPD repair in the ade6 locus (Xhol fragment) from Southern blots of genomic DNA isolated at different times after UVC irradiation: transcribed (TS) and nontranscribed (NTS) strand.
Fig. S3. CPD repair in a rad16 uve1 mutant. Quantitative analysis of the CPD repair in the ade6 locus (XhoI fragment) from Southern blots of genomic DNA isolated at different times after UVC irradiation: transcribed (TS) and nontranscribed (NTS) strand.

Fig. S4. Cell-cycle progression and CPD repair in a rad13 uve1 mutant. (A) Quantitative analysis of the CPD repair in the ade6 locus (XhoI fragment) from Southern blots of genomic DNA isolated at different times after UVC irradiation: transcribed (TS) and nontranscribed (NTS) strand. (B) Percentage of rad13 uve1 cells that contained chromatin-bound pre-RC at different timepoints after release into the cell cycle. Data for unirradiated control cells are plotted using filled symbols and dashed lines; data for UV-irradiated cells are plotted using open symbols and solid lines. (C) Immunoblots of total cell extracts from UVC-irradiated rad13 uve1 cells, probed with antibody against phosphorylated Cdc2. The presence of tubulin was used as loading control. (D) Immunoblot of total cell extracts from UVC-irradiated rad13 uve1 cells, probed with antibody against phosphorylated eIF2α. The presence of tubulin was used as loading control.
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