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The *Schizosaccharomyces pombe* S-Phase Checkpoint Differentiates Between Different Types of DNA Damage

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ABSTRACT

We have identified an S-phase DNA damage checkpoint in *Schizosaccharomyces pombe*. This checkpoint is dependent on Rad3, the *S. pombe* homolog of the mammalian ATM/ATR checkpoint proteins, and Cds1. Cds1 had previously been believed to be involved only in the replication checkpoint. The requirement of Cds1 in the DNA damage checkpoint suggests that Cds1 may be a general target of S-phase checkpoints. Unlike other checkpoints, the *S. pombe* S-phase DNA damage checkpoint discriminates between different types of damage. UV-irradiation, which causes base modification that can be repaired during G1 and S-phase, invokes the checkpoint, while γ-irradiation, which causes double-stranded breaks that cannot be repaired by a haploid cell if induced before replication, does not invoke the checkpoint. Because the same genes are required to respond to UV- and γ-irradiation during G2, this discrimination may represent an active suppression of the γ response during S-phase.

CELL cycle delay is a general response to DNA damage. Such a delay is considered a checkpoint if the arrest is an active response that can be overridden by a mutation or by drug treatment (Hartwell and Weinert 1989). Three major DNA damage checkpoints have been studied in eukaryotic cells: The G1 DNA damage checkpoint arrests cells at Start (the yeast equivalent of the mammalian restriction point) before commitment to a new mitotic cell cycle; the S-phase DNA damage checkpoint delays the progression of replication; and the G2 DNA damage checkpoint prevents cells from proceeding through mitosis (reviewed in Elledge 1996). Of these, the G1 and G2 DNA damage checkpoints are the best understood (Furnari *et al.* 1997; Hansen and Oren 1997; Peng *et al.* 1997; Sanchez *et al.* 1997; Sidorova and Breeden 1997).

The mechanism of the G2 DNA damage checkpoint is conserved between mammals and the fission yeast *Schizosaccharomyces pombe* (Ford *et al.* 1994; Furnari *et al.* 1997; Peng *et al.* 1997; Sanchez *et al.* 1997; Savitsky *et al.* 1995). In *S. pombe* the G2 DNA damage checkpoint has been genetically dissected into three parts. The first part is comprised of six known proteins, the products of the "checkpoint *rad* genes." These proteins, Rad1, Rad3, Rad9, Rad17, Rad26, and Hus1, are required for the G2 DNA damage checkpoint, as well as the S-M replication checkpoint that prevents mitosis until the completion of S-phase (al-Khodairy and Carr 1992; al-Khodairy *et al.* 1994; Enoch *et al.* 1992). The S-M replication checkpoint is distinct from the S-phase DNA damage checkpoint. The former prevents mitosis in response to unreplicated DNA, while the latter slows replication in response to damaged DNA. Several of the checkpoint *rad* gene products are homologous to other proteins involved in DNA metabolism. For instance, Rad1 is similar to the *Ustilago maydis* Rec1 exonuclease (Thelen *et al.* 1994), and Rad3 is similar to DNA-PK, a protein kinase that binds, and is activated by, broken DNA ends (Hartley *et al.* 1995). Thus, the checkpoint *rad* gene products may be involved directly in the recognition of DNA damage.

The next part of the G2 DNA damage checkpoint consists of the Chk1 protein kinase, the Rad24 14-3-3 protein, and Crb2 (al-Khodairy *et al.* 1994; Ford *et al.* 1994; Saka *et al.* 1997; Walworth *et al.* 1993). These proteins are required for the G2 DNA damage checkpoint but not for the S-M replication checkpoint. Furthermore, both Crb2 and Chk1 are phosphorylated in response to DNA damage in a checkpoint *rad* gene dependent manner (Saka *et al.* 1997; Walworth and Bernards 1996). They are therefore believed to transmit the DNA damage signal, but not the S-M replication signal, from the checkpoint *rad* gene products to the third part of the checkpoint, the basic cell cycle machinery that regulates mitosis. Specifically, Chk1 binds to and phosphorylates the Cdc25 mitotic inducer, thus providing a link between the G2 DNA damage checkpoint pathway and cell cycle regulation (Furnari *et al.* 1997).

The ultimate target of the G2 DNA damage checkpoint is the activity of Cdc2. This checkpoint arrests cells in G2 by preventing the dephosphorylation and activation of Cdc2 that is required for mitosis (Rhind *et al.* 1997). The activation of Cdc2 at the G2/M bound-

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ary is regulated by inhibitory phosphorylation on tyrosine-15 (Gould and Nurse 1989). This phosphorylation is catalyzed by the Wee1 and Mik1 protein kinases (Featherstone and Russell 1991; Lee *et al.* 1994; Lundgren *et al.* 1991; Parker *et al.* 1992; Russell and Nurse 1987) and removed by the Cdc25 phosphatase (Millar *et al.* 1991; Russell and Nurse 1986). The checkpoint acts through the Chk1-dependent inhibition of Cdc25 to prevent mitosis in response to DNA damage (Furnari *et al.* 1997).

All three parts of the *S. pombe* G2 DNA damage-checkpoint pathway are conserved in mammalian cells. ATM, the mammalian homolog of Rad3, is required for the G1, S-phase, and G2 DNA damage checkpoints (Savitsky *et al.* 1995). While it is unclear how ATM invokes the S-phase checkpoint, it is believed to interact with mammalian Chk1 in the G2 checkpoint. Furthermore, the mammalian G2 DNA damage checkpoint appears to include the mammalian homologs of Chk1 and Rad24 and to involve the phosphorylation of Cdc25 by Chk1 (Peng *et al.* 1997; Sanchez *et al.* 1997). Finally, in vertebrates, the timing of mitosis is controlled by the inhibitory phosphorylation of Cdc2 on tyrosine-15, as well as on theronine-14 (Krek and Nigg 1991; McGowan and Russell 1993; Norbury *et al.* 1991), and, as in *S. pombe*, this phosphorylation is targeted by the G2 DNA damage checkpoint (Blasina *et al.* 1997; Jin *et al.* 1996).

It appears that many of the upstream checkpoint proteins can elicit various checkpoints, presumably through different downstream targets. For instance, in *Saccharomyces cerevisiae*, Rad9p, Rad24p, and Rad53p are required for the G1, S-phase, and G2 DNA damage checkpoints (Allen *et al.* 1994; Paulovich and Hartwell 1995; Siede *et al.* 1994); likewise, in mammals, ATM is required for all three checkpoints (Beamish *et al.* 1994; Painter and Young 1980; Savitsky *et al.* 1995); and in *S. pombe* the checkpoint rad genes are required for both the G2 DNA damage and S-M replication checkpoints (al-Khodairy and Carr 1992; al-Khodairy *et al.* 1994; Enoch *et al.* 1992). In contrast to the progress being made on understanding the downstream targets of these proteins in the G1 and G2 DNA damage checkpoint (Kastan *et al.* 1992; Sidorova and Breeden 1997; Weinert 1997), little is known about how they elicit the S-phase checkpoint. We have undertaken a study of the G1 and S-phase checkpoints in *S. pombe* with the goal of elucidating the different targets of the conserved upstream checkpoint genes at different points in the cell cycle.

MATERIALS AND METHODS

Growth and manipulation of *S. pombe* strains: General methods for studying fission yeast were performed as described (Moreno *et al.* 1991). The following strains were used: PR109 (*h⁻ leu1-32 ura4-D18*), NR1592 [*h⁻ leu1-32 ura4-D18 ade6-704 chk1::ura4⁺*; also known as *rad27::ura4⁺*; al-Khodairy *et al.* (1994)], NR1626 [*h⁻ leu1-32 ura4-D18 cdc10-V50*; Marks *et al.* (1992)] NR1826 [*h⁻ leu1-32 ura4-D18 ade6-704 rad3::ura4⁺*; the kinase domain deletion from Bentley *et al.* (1996)], NB2117 (*h⁻ leu1-32 ura4-D18 cds1::ura4⁺*; Boddy *et al.* (1988)], and NR2192 (*h⁻ leu1-32 ura4-D18 cdc10-V50 cds1::ura4⁺*). Unless otherwise stated, all strains were grown in yeast extract with supplements (YES) medium, a rich yeast extract based medium, at 25°. Synthetic proline (SP) medium is a modification of Edinburgh Minimal Medium 2(EMM2), a defined minimal medium, with 1.2 mg/ml proline as the sole nitrogen source and supplemented with 75 μg/ml each of adenine, histidine, leucine, and uracil (Fantes and Nurse 1977). Synchronous cultures were prepared by centrifugal elutriation with a Beckman JE-5.0 elutriation rotor (Creanor and Mitchison 1979). For synchronization of SP cultures, cells were grown in SP medium for at least three generations and then elutriated into YES. The percentage of cells having passed mitosis was determined microscopically as the number of cells having begun or finished septation, divided by the total number of cells. For the asynchronous hydroxyurea (HU) block experiment, cells were grown to mid-log phase and then shifted to 12 mm HU/YES for 4 hr. For the synchronous HU block/UV-irradiation experiments, small cells in early G2 were collected by elutriation into 12 mm HU/YES. Cells were allowed to complete one cell division and arrest in the following S-phase, which is concurrent with cytokinesis (Nasmyth *et al.* 1979). When all cells in the culture had divided, and thus just accumulated in S-phase, they were released from the HU block and immediately irradiated. The time of irradiation was designated 0 min. We estimate that cells spent between 10 and 50 min arrested at the HU block.

Irradiation: Cells were γ-irradiated while suspended in YES medium with 3.3 Gy/min from a ^{137}Cs source at room temperature, approximately 23°. For UV-irradiation, cells were filtered onto Metricel 45 μm membrane (Gelman Sciences) at 1 OD unit/cm^2, irradiated at 254 nm with a Stratalinker (Stratagene) and immediately resuspended in liquid. For the kill curves in Figure 4B, cells were plated at 300 CFU/plate and then irradiated. G1 cells were synchronized at the *cdc10* execution point by a 4-hr incubation at 35°. Asynchronous log phase cells, 80% of which are in G2 (Nasmyth *et al.* 1979), were used to represent G2 cells.

FACS analysis: Cells were prepared for fluorescence activated cell scanning (FACS) analysis by overnight fixation in 70% EtOH at 4°, followed by a 10-min incubation at room temperature in 0.1 N HCl, 0.5% Triton X-100, and then 2 hr at 37° in 250 μg/ml RNase A in 1× phosphate buffered saline (PBS). Cells were resuspended in 2.5 μg/ml propidium iodide in 1× PBS at approximately 2 × 10^6 cells/ml, and analyzed on a Becton-Dickinson FACSort. For the quantitation of the percentage of cells in S-phase presented in Figure 3B, the cells were prepared as above but stained with 1 μm Sytox Green (Molecular Probes), which gives lower background and better peak definition than propidium iodide staining, allowing for more accurate identification of cells in S-phase. The data were quantitated with the ModFit LT FACS data curve fitting software package (Becton-Dickinson), which fits Gaussian peaks at 1C and 2C and counts the data points between the 1C and 2C curves as S-phase cells.

RESULTS

γ-Irradiation during G1 does not significantly delay passage through Start or S-phase: We initially examined the *S. pombe* G1 DNA damage checkpoint. Because *S. pombe* cells have a very short G1 under normal vegeta-

tive condition, we used nitrogen limiting growth conditions that cause cells to grow more slowly and with an elongated G1 (Fantes and Nurse 1977). Synchronized G1 cells were obtained by growing cultures in SP, a synthetic medium with proline as a nitrogen source, and separating the small G1 cells by elutriation. For the experiments presented in Figures 1 and 2, we used *cdc10-V50* cells. Cdc10 is a transcription factor required for passage through Start (Lowndes *et al.* 1992; Nurse and Bissett 1981). The temperature-sensitive *cdc10-V50* mutation allowed us to confirm that the G1 cells we produced were pre-Start. When the cells were shifted to 35° immediately after elutriation they arrested before S-phase, demonstrating that they had not passed the *cdc10* execution point, the definition of Start in *S. pombe* (data not shown). Experiments with wild-type cells gave similar results (data not shown).

Pre-Start G1 cells were irradiated with 400 Gy of γ-radiation and followed through S-phase and mitosis. This treatment does not cause a significant delay in the entry into S-phase (Figure 1A and B), or progression through S-phase (Figure 1C). There is a slight, perhaps 10 min, delay in entry into S-phase seen in Figure 1A and a slight slowing of progression through S-phase seen in the higher percentage of cells with S-phase DNA content in the irradiated samples at 3.5 and 4 hr in Figure 1C. These are minor effects and, for reasons discussed below, we believe that they are not checkpoint related.

While γ-irradiation in G1 does not activate an S-phase checkpoint, γ-irradiation in G1 does cause a strong G2 checkpoint. Cells irradiated with 400 Gy during G2 delay mitosis for about 2 hr after the end of the irradiation (Figure 1B) and retain full viability (Figure 1D). In contrast, cells irradiated during G1 delay mitosis for at least 6 hr after irradiation. These cells then enter mitosis with slower kinetics and many do not divide properly (Figure 1B and data not shown). Furthermore, cells irradiated during G1 are sensitive to γ-irradiation and show high inviability at relatively low doses (Figure 1D). These effects are presumably due to the fact that double-strand breaks caused by γ-irradiation during G1 cannot be repaired because of the lack of a homologous chromatid and are thus lethal, but they are recognized as damage in G2 and invoke the G2 DNA damage checkpoint.

UV-irradiation during G1 delays passage through S-phase: One possible explanation for the lack of a G1 or S-phase checkpoint in response to γ-radiation is that such DNA damage checkpoints exist, but, because the γ-ray-induced damage is irreparable, they are not activated. We therefore examined the response to UV-irradiation. UV-irradiation causes single-strand damage, which is thought to be repaired by nucleotide excision mechanisms that do not require a homologous chromatid (Lindahl 1982) and should therefore be reparable in G1. Using the same experimental strategy described above, we irradiated pre-Start G1 cells with 200 J/m^2 of 254 nm UV radiation. Cells UV-irradiated during G1 show a 1- to 2-hr delay of S-phase (Figure 2). Furthermore, these cells show a 2- to 3-hr delay in mitosis. Thus, some forms of DNA damage during G1 are capable of eliciting an S-phase checkpoint.

UV-, but not γ-, irradiation at a G1 block delays passage through S-phase: To confirm our results by an independent method with cells grown in rich media, we examined the radiation response of cells arrested in G1 by a mutation in *cdc10*. As above, γ-irradiated cells show no significant delay in entry to or passage through S-phase, while UV-irradiated cells show a distinct S-phase checkpoint (Figure 3A). For the UV-irradiation, we irradiated with a range of doses from 0 to 200 J/m^2. Figure 3A shows the FACS data for 0 and 200 J/m^2. Figure 3B shows the quantitation of the percentage of cells in S-phase for all four doses. This quantitation shows that the entry into S-phase is slightly delayed in a dose-dependent manner and that progression through S-phase is greatly slowed, again in a dose-dependent manner. At the higher doses, as many as half of the cells do not complete S-phase within the course of the experiment. Given the relatively low resolution of FACS analysis, we cannot determine if the delay of entry into S-phase represents a G1 delay, with unfired replication origins, or a delay at the beginning of S-phase, with fired origins but arrested replication forks.

***rad3* and *cds1* are required for the S-phase checkpoint:** We next investigated the requirement for various known checkpoint genes in the UV-induced S-phase DNA damage checkpoint. This was complicated by the fact that it is difficult to block checkpoint mutants in G1. Cells doubly mutant for *cdc10* and any of the six checkpoint *rad* genes or *chk1* fail to arrest properly in G1 at the *cdc10* execution point and instead proceed through mitosis without replicating (Carr *et al.* 1995). In addition, cells mutant for *rad1* or *rad3* are unable to grow in SP medium (N. Rhind, unpublished results). This is presumably related to the fact that mutations in the checkpoint *rad* genes are synthetically lethal with *wee1* mutations (al-Khodairy and Carr 1992), and that *wee1* activity is reduced in nitrogen limited media, such as SP (Fantes and Nurse 1977). Since all six checkpoint *rad* genes are synthetically lethal with *wee1*, we predict that they would all fail to grow in SP medium. So, instead of irradiating cells during G1, we irradiated them during an early S-phase HU block. Checkpoint *rad* gene mutants do not delay mitosis in response to HU arrest; thus, we could not arrest an asynchronous population with HU because some cells would divide before others had arrested. However, although the checkpoint *rad* gene mutants do not delay mitosis in response to HU arrest, they do grow to the normal size for mitosis before dividing, even in HU, and this takes about 3 hr after S-phase. Therefore, we synchronized cultures by elutriation so that the cells would enter S-phase at about

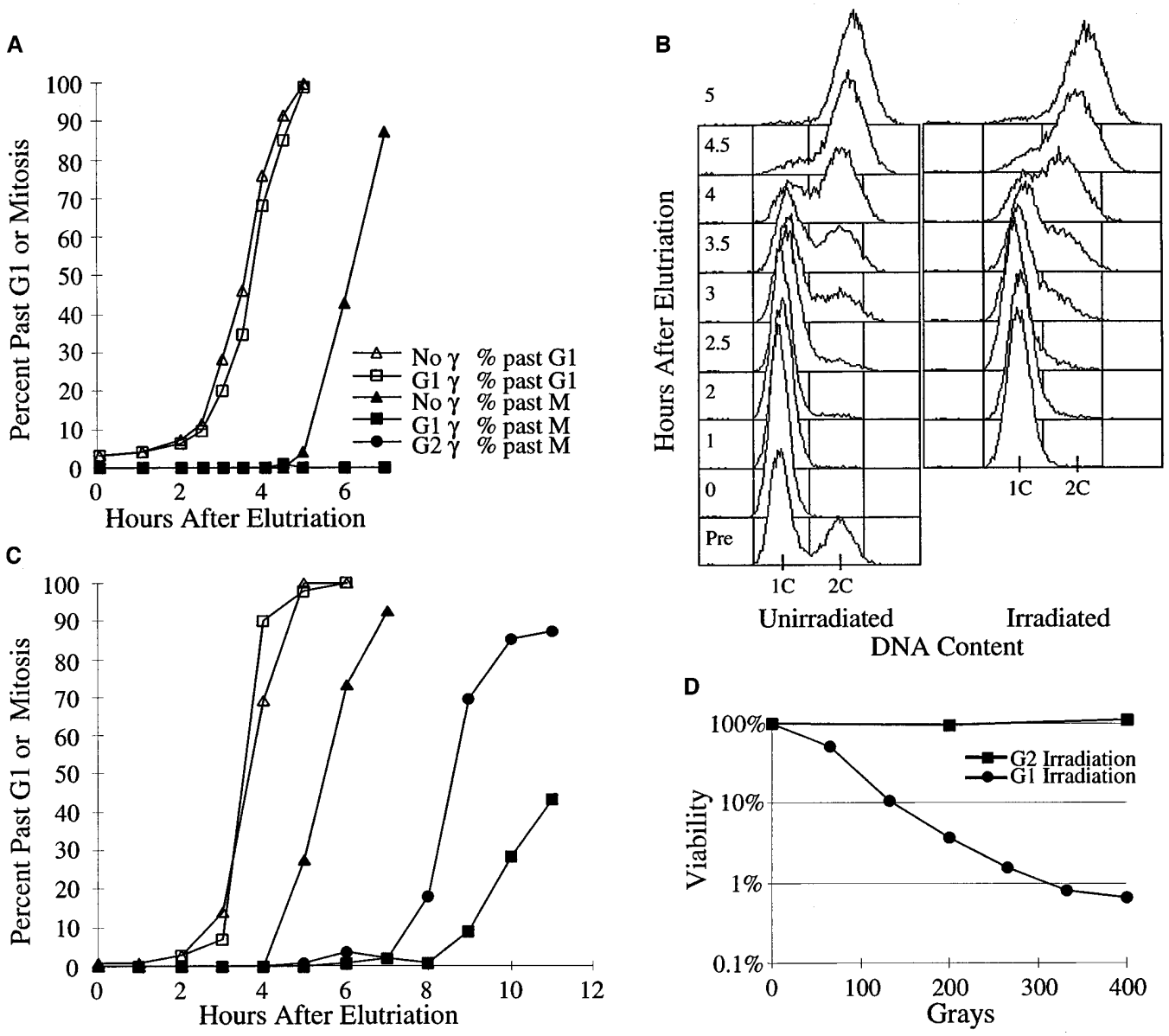

Figure 1.—γ-Irradiation during G1 does not cause a delay in replication. (A) NR1626 (*h⁻ leu1-32 ura4-D18 cdc10-V50*) cells were grown in SP medium, a poor nitrogen source, which greatly elongates G1 (Fantes and Nurse 1977). A synchronous population of pre-Start G1 cells was prepared by elutriation and half was irradiated with 400 Gy of γ-radiation between 0 and 2 hr. Progression through S-phase was monitored by FACS analysis and is plotted as percent cells having passed the beginning of replication. Progression through mitosis was monitored microscopically and is plotted as percent cells having passed the beginning of septation. Open triangles, percent of unirradiated cells having begun replication; open squares, percent of G1 irradiated cells having begun replication; closed triangles, percent of unirradiated cells having completed mitosis; closed squares, percent of G1 irradiated cells having completed mitosis. (B) A longer time course of a similar experiment shows that γ-irradiation during G1 blocks mitosis for longer than γ-irradiation during G2, presumably because replication of damaged DNA creates irreparable damage. The G1 irradiated cells never fully divide and many undergo an aberrant mitosis (data not shown). Cells were irradiated with 400 Gy either in G1, between 0 and 2 hr, or G2, between 4 and 6 hr. The symbols are the same as in A, and closed circles represent the percent of G2 irradiated cells having completed mitosis. (C) The FACS data used for the quantitation in A. Each individual histogram plots number of cells *vs.* amount of fluorescence. The numbers to the left indicate the times after elutriation at which the samples were taken. "Pre" is the asynchronous culture before elutriation. Cells in the right column were irradiated with 400 Gy in G1, between 0 and 2 hr. (D) Cells from the experiment in B were plated for viability after irradiation in G1 or G2.

the same time. Then, by adding HU, we could arrest all the cells in early S-phase with HU, irradiate them, and release them from the HU block while they were still relatively small. This way, we could examine their S-phase delay before they reached the size at which they would enter mitosis. This approach not only allowed us to look at the checkpoint gene requirement for this checkpoint, it also demonstrated that the cells do not have to be irradiated before the beginning of S-phase to invoke the checkpoint.

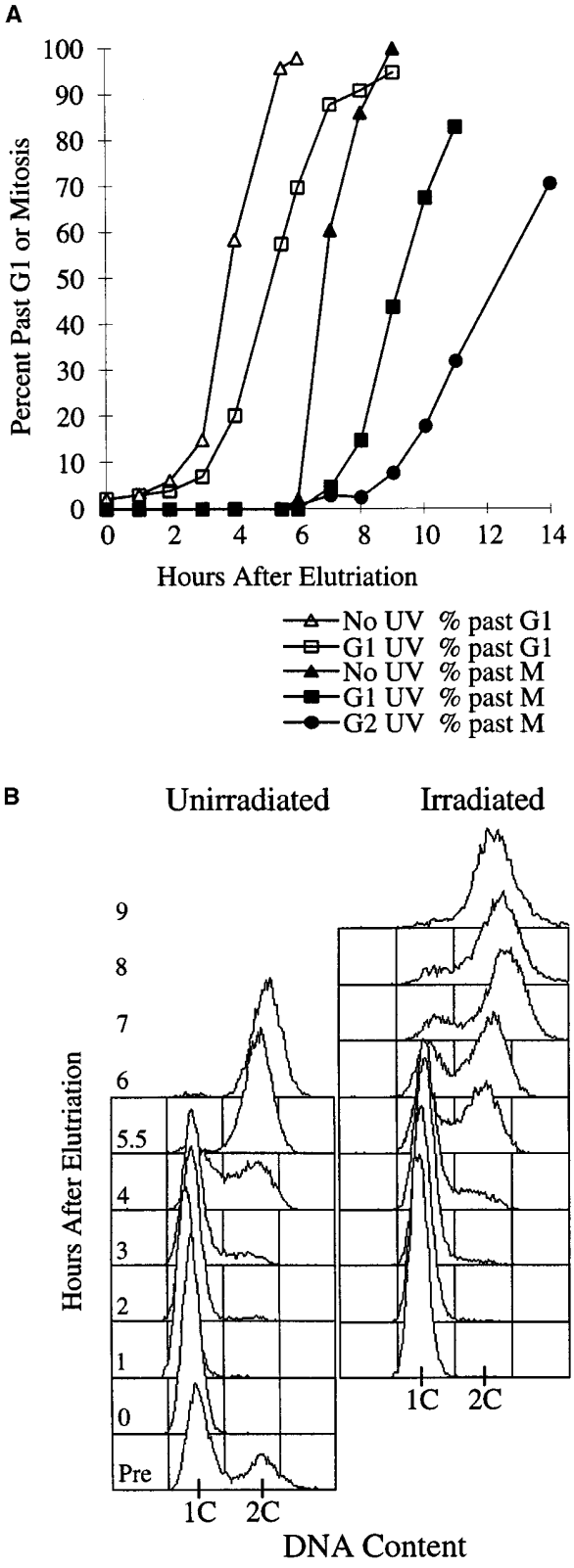

Figure 2.—UV irradiation during G1 causes a delay in replication. (A) Pre-Start G1 NR1626 (*h⁻ leu1-32 ura4-D18 cdc10-V50*) cells, prepared and analyzed as in Figure 1, were irradiated with 200 J/m² of 254 nm UV radiation at 1 hr after elutriation. The symbols are the same as in Figure 1. (B) The FACS data used for the quantitation in A.

Wild-type cells irradiated during early S-phase show a UV-induced S-phase delay (Figure 4A) similar to that seen when cells are irradiated in G1 (Figure 3A). This delay consists of both a delay in entry into bulk replication, which happens at 60 min in the untreated culture and 80 min in the irradiated culture, and progression though S-phase, which is completed by 80 min in the untreated culture and is not completed by 120 min in the irradiated culture. In contrast, a strain mutant for *rad3* does not delay S-phase after irradiation, and replicates with kinetics very similar to that of unirradiated cells (Figure 4A). The dependence on the *rad3* gene demonstrates that the S-phase delay is a checkpoint response, as opposed to a physical block to replication (Hartwell and Weinert 1989). The role of Rad3 in the S-phase checkpoint is consistent with Rad3 and its homologs, being involved in DNA damage recognition in a variety of different checkpoints. Likewise, the fact that *chk1* is not required is consistent with the observation that Chk1 interacts specifically with the mitotic control machinery (Furnari *et al.* 1997). The checkpoint is also dependent on *cds1*, a gene which has previously been implicated in the S-M replication checkpoint (Murakami and Okayama 1995; Murray *et al.* 1997; Saka *et al.* 1997).

While the *rad3* and *cds1* mutations greatly reduce the UV-induced S-phase delay, there remains a small but reproducible difference in the level of FACS signal at the later timepoints in Figure 4A. This slight reduction in FACS signal in response to irradiation is seen in all experiments, whether the cells were irradiated with γ or UV, and whether they were irradiated during G1 or S-phase (Figures 1A, 3A, 4A). Although it is possible that these data represent a very subtle checkpoint response that is independent of the known checkpoint pathways, other explanations seem more likely. One possibility is that physical damage caused by the radiation slightly impedes replication. Alternatively, it is possible that irradiation inhibits mitochondrial DNA replication, which would lower the background FACS signal.

The effect of eliminating the S-phase DNA damage checkpoint was investigated by arresting *cdc10* cells or *cdc10 cds1* double-mutant cells in G1 and by UV-irradiating them. Since *cds1* is not required for the G2 DNA damage checkpoint or for resistance to UV-irradiation during G2 (Murakami and Okayama 1995; Figure 4B), any difference in viability between *cdc10* cells or *cdc10 cds1* double-mutant cells would be attributable to the loss of the S-phase checkpoint. Although both strains are dramatically more sensitive to UV-irradiation during G1 than in G2, the elimination of the S-phase checkpoint by the mutation in *cds1* has no significant effect on the UV sensitivity of cells in G1 (Figure 4B).

DISCUSSION

We have investigated the checkpoint response of *S. pombe* to DNA damage caused during G1 or S-phase.

We find that some damage, that caused by UV-irradiation, invokes an S-phase delay, while other damage, that caused by γ-irradiation, does not. Since this S-phase delay requires the *rad3* and *cds1* genes, it meets the empirical definition of a checkpoint.

That one sort of DNA damage would invoke a checkpoint, while another would not, is unexpected. In previous studies of budding and fission yeast DNA damage checkpoints, DNA damage caused by UV- or γ-irradiation or by chemical alkylating agents have all invoked similar responses when directly compared (al-Khodairy and Carr 1992; al-Khodairy *et al.* 1994; Walworth and Bernards 1996). One example of UV- and γ-irradiation leading to different results is the induction of p53 in mouse prostate cells (Lu and Lane 1993). In response to γ-irradiation, the cells show a rapid and transient accumulation of p53, while UV-irradiation causes a slower, sustained expression of p53. However, since the difference seen is in the kinetics of p53 accumulation, it could reflect a difference in the kinetics of induction and repair of the two types of damage, as opposed to a difference in the checkpoint response.

One obvious difference between UV- and γ-radiation is the spectrum of damage each causes (Ramotar and Masson 1996). γ-Irradiation-induced DNA damage is predominantly double-strand breaks, which cannot be efficiently repaired by a haploid cell, such as *S. pombe*, during G1 because their repair requires a sister-chromatid recombination template. Conversely, UV-irradiation causes mainly base modifications that can be repaired without a template by nucleotide excision repair. Thus, one model to explain the different responses to the different sorts of damage in *S. pombe* postulates that double-strand breaks are recognized as irreparable damage, and thus the checkpoint signal is suppressed. In this case, the lack of delay in response to γ-irradiation would be an active process that can be thought of as instant adaptation. Another possibility is that double-strand breaks are simply not recognized as damage during G1 due to the lack or inactivity of some protein that is required to recognize double-strand breaks but not base modifications. At the moment, we cannot distinguish between these or other possible models. However, the S-phase checkpoint requires *rad3*, which is also required for recognition of double-strand breaks by the G2 DNA damage checkpoint (Jimenez *et al.* 1992) and which, as a DNA-PK homolog (Hartley *et al.* 1995), is hypothesized to recognize damaged DNA. So it seems likely that the basic S-phase DNA damage checkpoint machinery is shared with the G2 DNA damage checkpoint. Furthermore, *S. cerevisiae*, which has a similar S-phase checkpoint, responds to G1 γ-irradiation even when haploid (Siede *et al.* 1994), so the simple fact that the damage is irreparable cannot generally explain the lack of a checkpoint response. It is also possible that the important difference between UV and γ-radiation is that UV causes many more lesions and that the delay

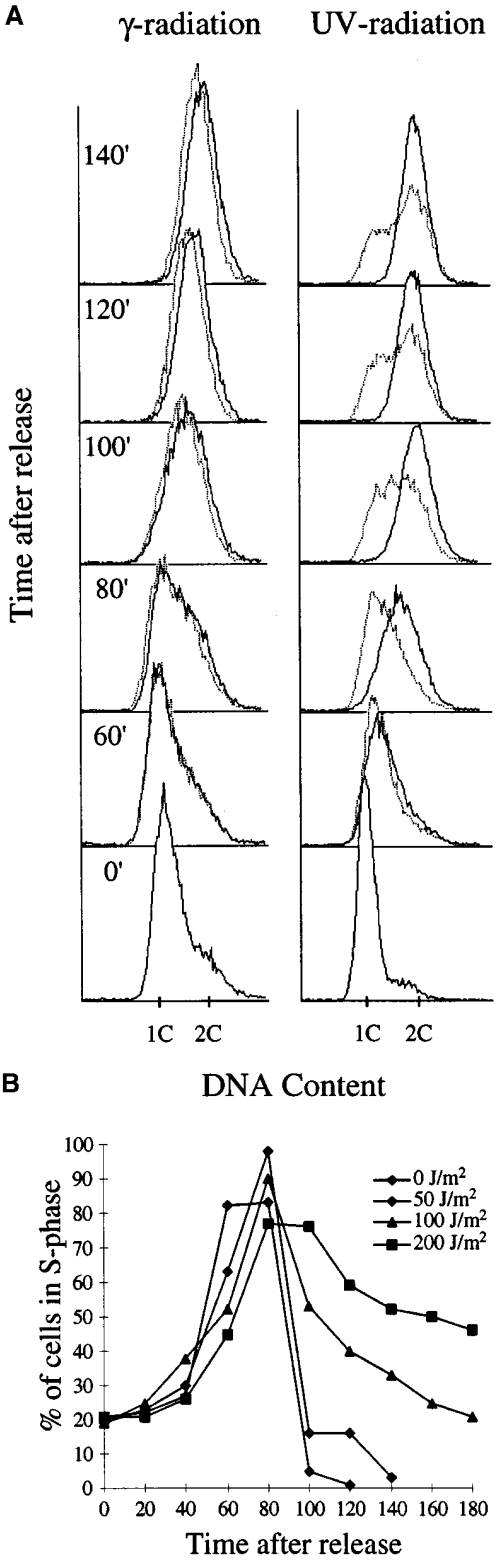

Figure 3.—UV, but not γ, irradiation at a G1 block causes a delay in replication. (A) NR1626 (*h⁻ leu1-32 ura4-D18 cdc10-V50*) cells were arrested in pre-Start G1 by a 4-hr incubation at 35° and irradiated (dashed line) with either 100 Gy of γ-radiation or 200 J/m² of UV radiation or mock irradiated (solid line) immediately before release. (B) Quantitation of the UV induced S-phase delay at different doses.

of S-phase we observe is dependent on the number of lesions induced. Although we cannot exclude this possibility, we do not favor it for two reasons. First, the doses used, 200 J/m^2 and 400 Gy, cause roughly the same length of delay of mitosis when administered during G2 (Figures 1 and 2). Second, the doses used are within the range that kills less that 20% of wild-type cells but greater than 99% of *rad3*Δ cells (Figure 1 and data not shown; see also al-Khodairy and Carr 1992). So all of the cells are receiving many potentially lethal lesions.

The requirement of *cds1* for the S-phase DNA damage checkpoint is surprising. Previously, *cds1* was thought to act only in response to replication arrest because it is not required for resistance to UV during G2 (Murakami and Okayama 1995). However, the role of *cds1* in the S-phase DNA damage checkpoint suggests that it may be a general target required for the inhibition of replication in response to a variety of checkpoint signals. Since HU inhibits nucleotide synthesis and thus prevents replication (Henderson and Paterson 1973), it is not possible to observe a *cds1* dependent S-phase delay in response to a HU block. But perhaps in a HU block, *cds1* is required to arrest replication before DNA polymerase actually runs out of nucleotides, and this arrest is required to allow efficient resumption of replication upon release from the HU block. This would explain the role of *cds1* in recovery from a HU block (Murray *et al.* 1997) and provide a uniform role for it in the two checkpoints. Such a model predicts that at low doses of HU, which slow but do not prevent replication, *cds1* should be required to regulate the rate of replication.

It is presumed that the purpose of DNA damage checkpoints is to allow time for DNA repair to occur before proceeding with the next cell cycle event. This has been shown to be true for G2 DNA damage checkpoints in budding yeast, fission yeast, and mammalian cells (al-Khodairy and Carr 1992; Blasina *et al.* 1997; Weinert and Hartwell 1988). However, there is no evidence that checkpoints that delay passage through G1 or S-phase in response to DNA damage lead to enhanced DNA damage resistance. On the contrary, in mouse fibroblast cells, the p53 dependent G1 checkpoint makes cells more sensitive to a range of DNA damaging treatments due to the checkpoint dependent apoptosis of damaged cells (Lowe *et al.* 1993). Moreover, failure of the mammalian S-phase checkpoint does not seem to correlate with sensitivity to G1 γ-irradiation (Zdzienicka 1996). Likewise, imposition of a G1 arrest in budding yeast does not increase resistance of *rad9* mutant cells to G1 UV or γ-irradiation (W. Siede, personal communication). Our results that the S-phase DNA damage checkpoint in *S. pombe* does not contribute significantly to G1 DNA damage resistance, while surprising, seems to support the idea that G1 and S-phase checkpoints are less important to cellular survival than the G2 checkpoint. In this context, it is worth noting that wild-type cells UV-irradiated in G1 delay mitosis as well as S-phase, implying that DNA damage remains even after the S-phase delay (Figure 2A). Thus, even the unperturbed S-phase checkpoint is unable to delay bulk replication until all DNA damage is repaired. Whether the damaged DNA is replicated (Kadyk and Hartwell 1993), or small regions of unreplicated, damaged DNA remains after the completion of bulk synthesis, is unknown. Likewise, in *S. cerevisiae*, S-phase

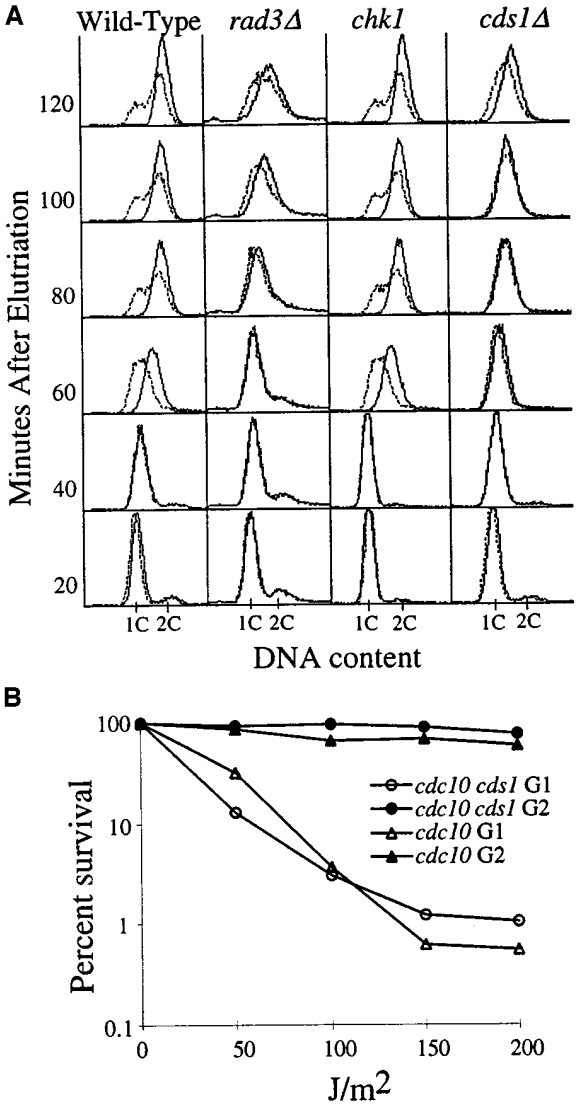

Figure 4.—*rad3*$^+$ and *cds1*$^+$ are required for the replication delay. (A) PR109 (*h*$^-$ *leu1-32 ura4-D18*), NR1826 (*h*$^-$ *leu1-32 ura4-D18 ade6-704 rad3::ura4*$^+$), NR1592 (*h*$^-$ *leu1-32 ura4-D18 ade6-704 chk1::ura4*$^+$) or NB2117 (*h*$^-$ *leu1-32 ura4-D18 cds1::ura4*$^+$) cells, blocked in HU and elutriated to obtain a synchronous S-phase population, were irradiated with 200 J/m^2 of UV radiation (dashed line) or mock irradiated (solid line), immediately after release. (B) The *cds1* dependent replication delay does not contribute to UV resistance. NR1626 (*h*$^-$ *leu1-32 ura4-D18 cdc10-V50*) and NR2192 (*h*$^-$ *leu1-32 ura4-D18 cdc10-V50 cdc1::ura4*$^+$) cells, arrested in pre-Start G1, were irradiated with various doses of UV radiation immediately after release and plated for viability. Asynchronous log phase cells, 80% of which are in G2, were used to represent G2 cells.

is slowed but not stopped by the presence of continuous, chemically induced DNA damage (Paulovich and Hartwell 1995). Consistent with this observation, wild-type cells are much more sensitive to UV damage in G1 than in G2 (Figure 4B). Perhaps not all UV-induced damage can be repaired by nucleotide excision repair, and recombination is more important in the repair of UV damage than previously thought. This idea is supported by the fact that mutants lacking Rhp54, a *S. pombe* recombinational repair protein homologous to *S. cerevisiae* Rad54p, are very sensitive to UV radiation (Muris *et al.* 1996).

The *S. pombe* S-phase DNA damage checkpoint appears to be quite similar to that seen in *S. cerevisiae.* In *S. cerevisiae*, there is a checkpoint that delays progression through S-phase in response to UV-irradiation, γ-irradiation, and alkylating DNA damage (Paulovich and Hartwell 1995; Siede *et al.* 1994). This checkpoint is dependent on Mec1p, the homolog of ATM and *S. pombe* Rad3; Rad24p, the homolog of *S. pombe* Rad17; and Rad53p, the homolog of *S. pombe* Cds1. These two checkpoints are, in turn, similar to the ATM-dependent S-phase checkpoint in mammalian cells, the failure of which is referred to as radio-resistant DNA synthesis (Painter and Young 1980). Given how little is known about the S-phase DNA damage checkpoint that prevents radio-resistant DNA synthesis in mammalian cells and about the high degree of conservation of the G2 DNA damage checkpoint between *S. pombe* and mammals, study of the *S. pombe* S-phase DNA damage checkpoint may provide new insights into the checkpoint controls required for mammalian genome stability.

The major difference between the DNA damage checkpoints of fission yeast on one hand and budding yeast and mammals on the other is that fission yeast seem to lack a pre-Start G1 DNA damage checkpoint. We cannot rule out the possibility that there is a G1 checkpoint in response to UV because cells irradiated in G1 do show a brief delay of entry into S-phase (Figure 3). The resolution provided by FACS does not allow us to determine if cells delay in G1, with unfired replication origins, or at the beginning of S-phase, with fired origins but arrested replication forks. However, the same delay is seen when the cells are irradiated in early S-phase, in an HU arrest (Figure 4), so a pre-Start G1 DNA damage checkpoint is not required for the effect. It has been previously speculated that haploid organisms should minimize the amount of time they spend in G1 so as to minimize their sensitivity to radiation (Nasmyth *et al.* 1991). This idea may be especially true for unicellular fungi; at least one class of fungicides, the bleomycins, cause double-strand DNA breaks (Berdy 1980). Consistent with this speculation, *S. pombe*, which is haploid, generally controls its cell cycle at the G2/M transition (Nurse and Fantes 1981). It may be that *S. pombe* has eliminated all G1 checkpoints, save for those required for mating, in order to further minimize the time it spends with a vulnerable 1C DNA content. Since G1/S checkpoints do not seem to be as important for resistance to damage, even presumably reparable damage, as G2 checkpoints, the elimination of the G1 DNA damage checkpoint may not have a dramatic effect on the fitness of the organism. This line of reasoning predicts that other haploid fungi, such as *Ustilago maydis*, should also lack G1 checkpoints.

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